The Canadian Council for Donation and Transplantation

Assessment and Management of Immunologic Risk in Transplantation:

A CCDT Consensus Forum

January 28-30, 2005

Montréal, Québec

Report and Recommendations

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Contents

Preface

The assessment and management of immunologic risk in organ transplantation fall within the scope of transplant physicians and surgeons and histocompatibility laboratories. Ongoing consultation and collaboration are required among leading practitioners to continually monitor, share, develop and update practices to optimize patient outcomes.

Currently, pre-transplant immunologic evaluation in Canada is not standardized, nor is it at a level that would allow for optimal patient risk assessment and organ allocation in all programs. As a consequence, transplants are at risk for early rejection and/or graft loss and many highly sensitized patients may be denied access to transplantation unnecessarily. This translates into costs that could be avoided or minimized.

The Consensus Forum on the Assessment and Management of Immunologic Risk in Transplantation was the culmination of a first-of-a-kind multidisciplinary effort sponsored by the Canadian Council for Donation and Transplantation (CCDT) and the Canadian Society for Transplantation (CST). The Forum set out to probe and develop consensus among leading experts around key questions pertaining to immunologic risk in transplantation. During the Forum, expert panelists and speakers presented the latest research on key topics related to the subject; conference delegates worked in groups and in plenary to arrive at consensus positions, considerations and recommendations designed to influence future practice.

This report and the recommendations it contains has important implications for the optimization of graft survival, organ utilization and organ allocation in Canada; it is meant to provide guidance to practitioners, stakeholders and policy makers in their efforts to improve patient and systemic outcomes.

The recommendations will also provide a basis for promoting greater consistency and standardization in the assessment and management of high risk transplant patients through enhanced lab practices leading ultimately to improved equity, affordability and patient outcomes.

Dr. David Hollomby

Chair, CCDT Transplant Committee

Foreword

The CCDT Forum, *Assessment and Management of Immunologic Risk in Transplantation*, was conceived as a vehicle to examine current practices, literature and new technologies for the assessment of HLA antibodies pre-transplant with the goal of being able to develop recommendations on best practices. The improved measurement of the immunologic risk profile of potential transplant recipients is the first step towards improving organ allocation, addressing the needs of sensitized individuals, optimizing the use of new drugs and generally working towards improved graft survival rates.

Underpinning the CCDT Forum were the following key premises:

- Transplant immunologic risk assessment and management is neither optimized nor standardized in Canada;
- Equity of organ allocation for patients at immunologic risk is neither optimized nor standardized across Canada;
- Addressing these deficits will lead to improvements in graft and patient survival, patient safety and resource utilization and allocation; and
- Development of standardized HLA laboratory practices across Canada in support of solid organ transplantation will provide the foundation on which to develop both national organ allocation registries and high risk treatment protocols.

Sponsored by the Canadian Council for Donation and Transplantation and the Canadian Society for Transplantation, the Forum was held in Montreal on January 28-30, 2005. There were 66 invited participants at the conference, including leading national and international experts in solid organ transplantation (heart, lung and kidney), laboratory medicine and health care administration.

Supported by extensive background research, the CCDT Forum featured presentations from leading experts, followed by facilitated group discussion by respective disciplines aimed at exploring and achieving consensus, including recommendations for practitioners and health care providers around key issues related to the assessment and management of immunologic risk.

This report summarizes the proceedings and recommendations from the CCDT Forum. By forging consensus and reducing uncertainty, it is hoped that the report will serve as an instrument for change and improvement, laying a foundation for enhanced lab practices and effective national strategies and solutions for treating the high risk patient.

Peter Nickerson

Chair, CCDT Forum

Acknowledgements

Any success and impact that the CCDT Forum will have is the result of a team effort. First of all, a special note of thanks goes to John Gelder, Kim Liss and Nancy Greene who worked tirelessly behind the scenes before, during and after the CCDT Forum to make it run smoothly and to ensure that we were able to produce the highest quality product.

The Steering Committee, Forum Recommendations Group and the Logistics Group who captured the vision, pushed to find consensus, and worked into the night to generate the best possible document – you have my deepest gratitude.

To David Hollomby, thank you for your belief in this project and for allowing us the privilege of putting on the first CCDT Consensus Forum on behalf of the Transplant Committee; it was a joy to work with you and your team.

Finally, hats off to all of the participants. You came ready to be fully engaged in the process and I believe as a result of your effort we now find ourselves on the brink of unprecedented change in the delivery of transplant care in Canada. My encouragement to all of you is to press on relentlessly to realize this future: at the end of the day it is our patients that will benefit the most from our efforts.

Peter Nickerson

Chair, CCDT Forum

Glossary and Definition of Terms

AHG-CDC crossmatch	An HLA crossmatch (see below) performed using cell death as the readout to indicate a positive test result. It is considered less sensitive than a Flow crossmatch.
AHG PRA	A PRA assessment (see below) using cell death as the readout to indicate a positive test result. It is considered less sensitive than an ELISA or Flow PRA assessment.
ASHI	American Society of Histocompatibility and Immunogenetics. This organization has developed standards in the U.S. for HLA tissue typing, crossmatching and HLA Ab specificity analysis. In addition, it is recognized in the U.S. as an accrediting body for histocompatibility laboratories.
CAT	Canadian Association of Transplantation: An association of health care professionals committed to facilitating and enhancing organ and tissue donation and the transplant process.
CCDT	Canadian Council for Donation and Transplantation
CDC crossmatch (or NIH CDC crossmatch)	An HLA crossmatch (see below) performed using cell death as the readout to indicate a positive test result. It is considered the least sensitive crossmatch method.
CDC PRA	A PRA assessment (see below) using cell death as the readout to indicate a positive test result. It is considered the least sensitive PRA method.
CSA	Canadian Standards Association: An organization which provides standards to the Standards Council of Canada for consideration as a National Standard of Canada.
CST	Canadian Society of Transplantation: A scientific organization of health care professionals associated with solid organ transplantation in Canada.
CORR	Canadian Organ Replacement Registry: A national information system that records, analyzes and reports the level of activity and outcomes of vital organ transplantation and renal dialysis activities. CORR is funded through the federal and provincial ministries of health through the Canadian Institute for Health Information (CIHI), which manages CORR.
ELISA PRA	A PRA assessment (see below) using colour change as the readout to indicate a positive test result. It is considered less sensitive than a Flow PRA assessment, but more sensitive than an AHG PRA assessment.
ESRD	End stage renal disease: a state requiring dialysis or kidney transplantation for survival.
Flow crossmatch	**A**n HLA crossmatch performed using cell surface fluorescence as the readout to indicate a positive test result. It is considered the most sensitive crossmatch test.

Flow PRA	A PRA assessment (see below) using surface fluorescence on microparticle beads coated with HLA molecules as the readout to indicate a positive test result. It is considered the most sensitive PRA assessment available at present.
Histocompatibility laboratory (or HLA laboratory or tissue typing laboratory)	A laboratory affiliated with one or more ODOs and one or more transplant centres that has the responsibility for the HLA tissue typing of donors and recipients and for performing crossmatch (i.e., histo-compatibility) testing to determine if the organ recipient has preformed antibodies directed at the donor HLA molecules. The presence of such preformed HLA antibodies directed at the donor represents an immune risk to the recipient for early rejection or graft loss.
HLA	Human leukocyte antigen: differences between donor and recipient HLA molecules stimulate the recipient immune system to reject the graft. This can be overcome with immunosuppressive medications (i.e., anti-rejection drugs).
HLA Ab	Human leukocyte antigen directed antibody: an antibody which is capable of causing early rejection or graft loss if directed at the donor HLA molecules.
HLA crossmatch (or T cell crossmatch or B cell crossmatch)	An evaluation for the presence of HLA Ab in the recipient's serum that is directed against the HLA molecules of the donor. The presence of donor specific HLA Ab is an immunologic risk factor for early rejection or graft loss. T cells are generally used as targets for Class I IgG donor specific antibodies, while B cells can be used to detect both Class I and Class II IgG donor specific antibodies.
Immunologic Risk	**T**his refers to a patient who has laboratory or clinical evidence of prior exposure to the organ donor HLA antigens (e.g., via blood transfusion, pregnancy or prior transplant). This risk is at present determined in the lab via PRA and HLA crossmatch assessments.
ODO	Organ Donation Organization: A group responsible for procuring donor organs for the purpose of transplantation
PRA	Panel reactive antibody: a measure of the degree to which a person has been sensitized (i.e., exposed and developed antibodies to foreign HLA molecules usually via blood transfusion, pregnancy or prior organ transplant) to the different HLA molecules that exist in the general population. The higher the % PRA the greater the degree of sensitization which is associated with a decreased likelihood that a deceased donor organ will be acceptable (i.e., a negative HLA crossmatch).
Sensitized Patient	A patient who has been exposed to foreign tissue antigens (HLA) and developed an immune response (i.e., HLA Ab) against the foreign HLA molecules.
Serologic Crossmatch	A CDC or an AHG-CDC crossmatch.
Solid Phase Assays	These are tests using purified HLA molecules as targets (ELISA, Flow based).
UNOS	United Network for Organ Sharing: his is the US based organization that is charged in the United States with deceased donor organ allocation on a national level.

Executive Summary

Overview

CCDT and CST held a consensus forum (Montréal, January 28–30, 2005) entitled, *Assessment and Management of Immunologic Risk in Transplantation*, which brought together clinical and laboratory specialists from transplant programs across Canada. The mandate was to review the literature, to listen to the current data presented by experts and to conduct an environmental scan of current Canadian practice. From this the participants developed consensus recommendations to be used to improve immunologic risk assessment and management in transplantation with the goal to:

- improve solid organ transplant outcomes;
- improve equity of access to organ transplants for highly sensitized patients;
- reduce the wait-list time for highly sensitized patients; and
- increase the number of organ donors.

Environmental Scan

A review of the Canadian Organ Replacement Registry (CORR) was undertaken and supplemented with a review of the Manitoba Renal Transplant Program database and the American United Network for Organ Sharing (UNOS) registry where gaps in CORR data existed. Further, the CSA Z900.2.3-03 standard for "Perfusable Organs for Transplantation" was reviewed, as was a survey of current histocompatibility laboratory practices in support of transplantation. The following items were highlighted:

- The annual expenditure to the health care provider for the end stage renal disease (ESRD) patient on hemodialysis is $104,277/year, whereas the annual expenditure (beyond the first year) for transplantation is $32,196/year.

- The incidence and prevalence of end stage renal disease (ESRD) in Canada continues to increase, while the proportion of ESRD patients treated with transplantation has decreased over time. Indeed, < 50% of prevalent ESRD patients aged 45-64 are treated with transplantation.

- Deceased organ donation per million population (DPMP) in 2003 was 13.5 as compared to the 2005 target of 25 DPMP put forward in 1999 by the National Coordinating Committee for Organ and Tissue Donation and Transplantation. The number of living kidney donors has increased to the point that it now exceeds the number of deceased donor transplants.

- Amongst ESRD patients, 30% of those on the wait-list have prior exposure to donor tissue antigens (HLA) from pregnancy, transfusions or prior transplants, resulting in preformed HLA antibodies (i.e., they are "sensitized"), which can lead to early rejection and graft loss.

- Sensitized ESRD patients, while making up 30% of the wait-list, receive < 5% of the kidney transplants in Canada. These patients have prolonged wait-times compared to non-sensitized ESRD patients due to the fact that kidneys are not shared between provinces – *local donor pools are often too small to find an acceptable kidney donor for this disadvantaged group.*

- The majority (78%) of highly sensitized patients in Manitoba and 83% of those on the UNOS registry (USA) waiting for a first kidney transplant are women, as sensitization commonly occurs through pregnancy. This inequity of access to kidney transplants for women likely exists on all wait-lists in Canada.

- Despite excellent short-term outcomes, 7.2% of all deceased donor transplants and 3.9% of all living donor transplants still fail during the first post-transplant year requiring the patient to return to dialysis. Further improvement in early graft survival would result in significant cost savings for the health care provider.

- Early graft loss commonly occurs due to undetected donor specific HLA antibodies. This suggests that newer, more sensitive, diagnostic technologies (e.g., flow-based) can be used to predict and prevent this occurrence. Proof of this concept comes from the Manitoba Transplant Program whose early graft survival has gone from 89.1% to 98.6% since the implementation of high resolution flow-based testing in 2000 – *improves utilization of a limited resource.*

- With the availability of high resolution flow-based testing as well as new therapeutic agents (e.g., IVIG, Thymoglobulin), a few Canadian programs have established a high risk living donor program for sensitized patients who previously would wait years on the wait-list for a deceased donor kidney – *adds new donors to the pool.*

- Emory University (Atlanta, USA) has implemented high resolution flow-based testing to identify "acceptable" mismatched tissue antigens (HLA) in their sensitized population. Using this approach, coupled with the UNOS National Registry to increase the available donor pool, 25 to 40% of the kidney transplants at Emory are now performed in sensitized patients (who make up 30% of their wait-list) – *the equity issue can be corrected.*

- A review of the CSA Z900.2.3-03 standards highlights that there is no minimum test method specified for histocompatibility laboratories supporting solid organ transplant programs. An environmental scan of Canadian histocompatibility laboratories and transplant programs revealed that the type and practice of testing provided in support of solid organ transplantation vary widely across Canada.

CCDT Clinical Consensus Forum Recommendations

While a number of recommendations were developed at the CCDT Consensus Forum, those identified as critical to moving kidney, heart and lung transplantation forward in Canada are highlighted below (for more detail see Section 5 and Appendix 8).

- Patients should be screened for HLA (Class I and II) antibodies while on the wait-list, optimally by flow-based techniques.
- If an HLA antibody is detected, then the specificity of the antibodies should be characterized, optimally by flow-based techniques.
- In kidney transplantation, a donor specific T cell and B cell crossmatch should be performed pre-transplant. If the potential recipient is known to be sensitized, then the final crossmatch should be via flow-based techniques.
- It was universally endorsed that Canada should establish a national high risk patient registry for sensitized kidney, heart and lung patients.
- For transplant centres to participate in a national high risk patient registry it was universally endorsed that histocompatibility laboratories supporting the transplant centre would have to upgrade to high resolution (flow-based) HLA Ab technologies.
- If histocompatibility testing is upgraded to high resolution (flow-based) HLA Ab technologies, then the possibility of a national paired living kidney donor exchange registry should be explored for both HLA and ABO incompatibility.

Clinical Benefits of High Resolution HLA Ab testing Technologies

The consensus that all transplant programs be supported with high resolution flow-based testing by HLA laboratories, once funded and implemented, will facilitate the following:

- Optimization of early graft survival of both living and deceased donor solid organ transplants.
- Development of a national high risk patient registry whose goal will be to allocate deceased donor organs to a disadvantaged group (one that makes up 30% of kidney deceased donor wait-lists but receives < 5% of the deceased donor kidneys in Canada).

While these latter two outcomes will not add new donors to the existing pool, the implementation of high resolution flow-based testing will allow for the following strategies, thereby adding new living kidney donors to the pool.

- Ability to develop a national paired living kidney donor exchange registry; and
- Ability to safely transplant in the face of a positive crossmatch and with new therapeutic approaches (e.g., IVIG) for living donor kidney transplants.

Economic Benefits of High Resolution HLA Ab Testing Technologies

An economic evaluation (Markov model) of the cost/benefit of universally implementing high resolution flow-based HLA Ab assessment was undertaken by Dr. Kevin McLaughlin for the CCDT. This analysis revealed the following:

- High resolution flow-based testing is associated with increased patient longevity, increased transplant longevity, additional discounted quality of life years/patient and reduced global health care costs to the health care provider. Therefore this is a dominant strategy.
- A sensitivity analysis found that the break-even point (cost/benefit) of high resolution flow-based HLA Ab testing is a 3% false negative rate for standard HLA Ab testing – generally the false negative rate is reported to be much higher at 10 to15%.

Recommendations to Stakeholders

Based on the environmental scan, the clinical consensus recommendations and the economic evaluation, the following recommendations are put forward:

- High resolution flow-based technologies are endorsed as the optimal standard of care in all histocompatibility laboratories supporting solid organ transplantation in Canada.
- Funding for high resolution flow-based technologies is to be provided by the provinces via the regional health authorities or hospitals. Beyond the up-front establishment costs, consideration should be given to linking budgets so that savings can be used to fund additional lab testing (i.e., cost savings from dialysis linked to cost increase in the lab testing budget).

Furthermore, the CCDT Consensus Forum has charged the CCDT with the following tasks, which are on the CCDT work plan for 2005–2007:

- CCDT is to explore the logistics and cost associated with establishing a national high risk patient registry to optimize equity of access to deceased donor organs for highly sensitized patients.
- CCDT is to explore the possibility of a national paired living kidney donor exchange registry for patients who have living donors that cannot donate to their relative because of a positive HLA crossmatch or ABO incompatibility.
- CCDT is to pass on the Clinical Forum Recommendations to the CSA Transplantation Committee for review and possible implementation as an amendment to the CSA Z900.2.3-03 standards for "Perfusable Organs for Transplantation."

Part I:

Problem Definition

1. Key Issues and Challenge

The following case from the Manitoba Renal Transplant Program (provided with patient consent) illustrates the overarching themes that led to the CCDT Consensus Forum: *Assessment and Management of Immunologic Risk in Transplantation*.

Case Study

In 1986, at age 30, Ms. X developed ESRD requiring hemodialysis. Because Ms. X had had children and had been transfused, she tested positive for HLA antibodies – she was "sensitized." Unfortunately, Ms. X had antibodies against several family members who wished to donate a kidney to her, as shown in a screening serologic HLA crossmatch (i.e., Ms. X had HLA antibodies specific for the potential donor's HLA molecules and there would have been a very high risk of immediate graft loss to acute rejection). Ms. X was listed for transplant on the deceased donor wait-list in Manitoba in 1986.

In 1988, a deceased donor kidney became available where the serologic HLA crossmatch between Ms. X and the donor was negative. Ms. X proceeded to be transplanted, but by day 4 post-transplant the kidney had undergone irreversible HLA antibody-mediated rejection and had to be removed. This type of rejection only occurs when a preformed HLA antibody, specific for the donor's HLA, is present but not picked up by the pre-transplant crossmatch. Ms. X remained on dialysis for 16 years, between 1988 and 2004, because all subsequent potential donors in Manitoba tested positive with Ms. X's sera – she was highly sensitized. In contrast, many patients coming onto the deceased donor wait-list between 1988 and 2004, who did not have HLA antibodies, were transplanted within one to two years.

In October 2004, a deceased donor who was a good tissue (HLA) match to Ms. X became available; however, the pre-transplant crossmatch, while negative by serologic methods, was still positive by more sensitive flow-based techniques. Because this was her best chance to be transplanted, it was decided, after informed consent, to proceed to transplant despite the higher risk (i.e., a positive flow crossmatch). This time, because her doctors knew about the existence of the anti-donor antibody prior to the transplant, Ms. X was pretreated with IVIG and Thymoglobulin, which have been shown to be effective in preventing acute graft rejection in the face of a positive flow crossmatch. To date Ms.X remains rejection free with excellent kidney function.

Challenge

Because of the significant advances in diagnostic methods over the last five years, HLA laboratories can now apply more sensitive tools to assess a patient's serum for HLA antibodies, accurately predict the specificity of these antibodies and assign a risk to the patient for a given donor. However, even with such tools, the small size of local donor pools still leaves patients like Ms. X, who are highly sensitized, at a disadvantage compared to most patients. The challenge of the CCDT Forum is to find national solutions for individuals like Ms. X who wait years to receive a transplant, and indeed often never do.

2. Outcomes and Allocation Equity for the Sensitized Patient

To outline the scope of the problems facing patients who have evidence of HLA antibodies in their sera prior to transplant (i.e., they are sensitized), a summary of a number of clinical databases is provided. Kidney transplantation is used as the "model organ" as the data on it are the most complete and definitive. However, it is likely that the findings can be extrapolated to patients waiting for heart and lung transplantation. This report was prepared for the CCDT Forum by Dr. John Gill of the Canadian Organ Replacement Registry (CORR), who provides a snapshot of the state of kidney transplantation in Canada as well as the impact of "sensitization" on organ allocation and outcomes for kidney transplant recipients. To examine the demographics of patients on a typical kidney transplant wait-list in Canada, the Manitoba database is reviewed – there is no national wait-list registry in Canada. To validate the Manitoba findings, a comparison is made with the United Network for Organ Sharing (UNOS) registry, the national organ sharing registry in America.

a. Canadian Organ Replacement Registry (CORR)

CORR is a national information system that records, analyzes and reports the level of activity and outcomes of vital organ transplantation and renal dialysis activities. Data are collected from a variety of sources including hospital dialysis centres, transplant hospitals, as well as provincial/regional organ procurement organizations. **Importantly, the data collected by CORR are submitted voluntarily, opening the potential for incomplete data collection.** CORR is funded by the federal and provincial ministries of health through the Canadian Institute for Health Information (CIHI), which manages CORR. CORR also receives support from the Kidney Foundation of Canada for printing national data reports.

CORR **does not** receive patient level data regarding patients wait-listed for transplantation. Therefore, CORR is not able to compare the characteristics of patients who develop end organ failure with those of patients activated to the transplant waiting list. For example, the proportion of incident dialysis patients who are activated to the deceased donor kidney transplant wait-list cannot be determined with certainty. Similarly, the limited aggregate data available for the wait-listed population **preclude** analyses of the progression of patients through the wait-list process to transplantation. **To address this gap a specific analysis of the Manitoba wait-list is provided below**

Detailed statistical information regarding transplant recipients is available from CORR. However, because the reporting is voluntary there are significant limitations to the available data. Of importance, PRA (a measure of previous immunization to foreign HLA molecules) is not recorded for approximately 20% of kidney transplant recipients between 1998 and 2002.

The Incidence of ESRD in Canada

The incidence of ESRD in Canada continues to increase (Figure 1).

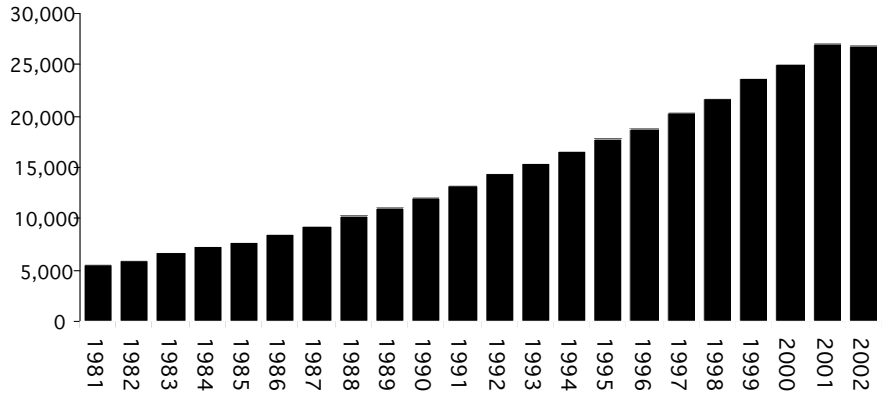


Figure 1. Prevalent ESRD patients in Canada (based on data reported in Facility Profile at year-end, not corrected for under-reporting).

The majority of ESRD growth is among older patients (≥ 65 years of age) (Figure 2).

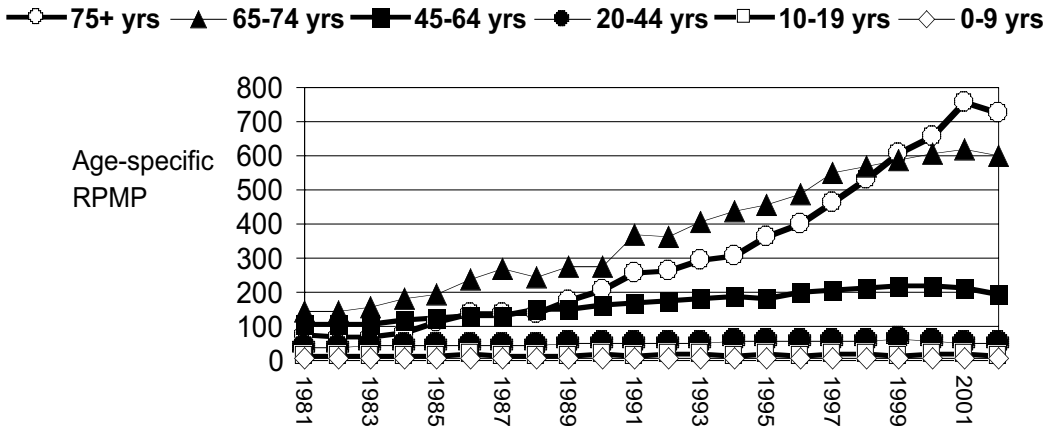


Figure 2. Incident ESRD patients by age group.

Although the CST has recently developed formal criteria for kidney transplant candidacy, the proportion of incident ESRD patients who may be considered transplant candidates cannot be determined with certainty because the data regarding the burden of comorbid disease in incident patients have not been validated. Projects to validate the comorbid disease conditions among incident ESRD patients in a cross-sectional manner are underway at CORR.

Among incident patients, pre-emptive transplantation is used infrequently. In 2002, only 125/4959 (2.5%) of incident ESRD patients received pre-emptive transplants. The frequency of pre-emptive transplantation is age related. In 2002, 31% of incident patients in the age range 0 to 19 years received pre-emptive transplants compared with 8%, 2.9% and 0.1% among incident patients aged 20 to 44 years, 45 to 64 years and ≥ 65 years.

Treatment of the Prevalent ESRD Population

The proportion of ESRD population treated with transplantation has decreased over time.

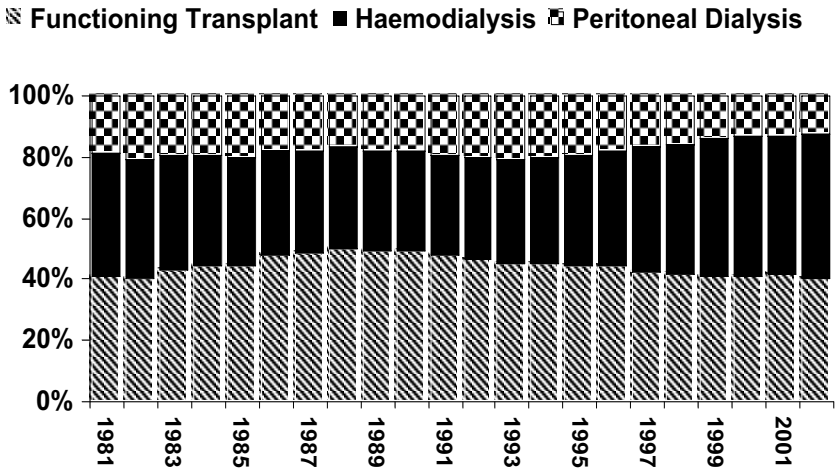


Figure 3. Distribution of prevalent patients by year end modality.

This is largely driven by the aging of the ESRD population. The proportion of ESRD patients treated with transplantation is clearly related to patient age (Figure 4).

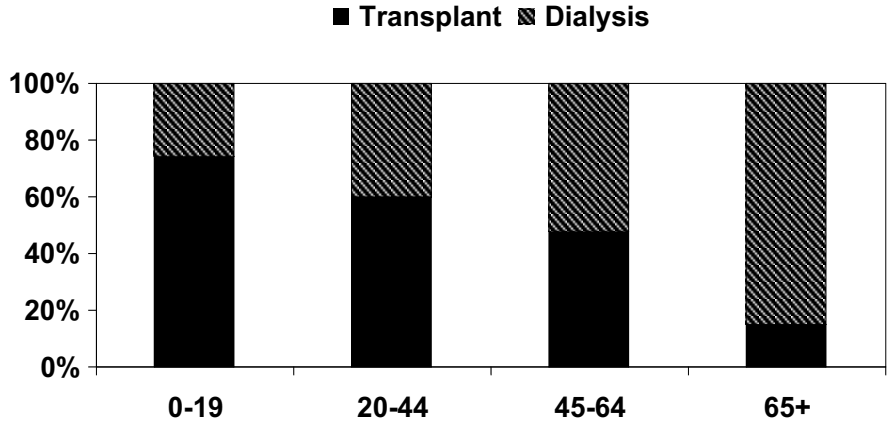
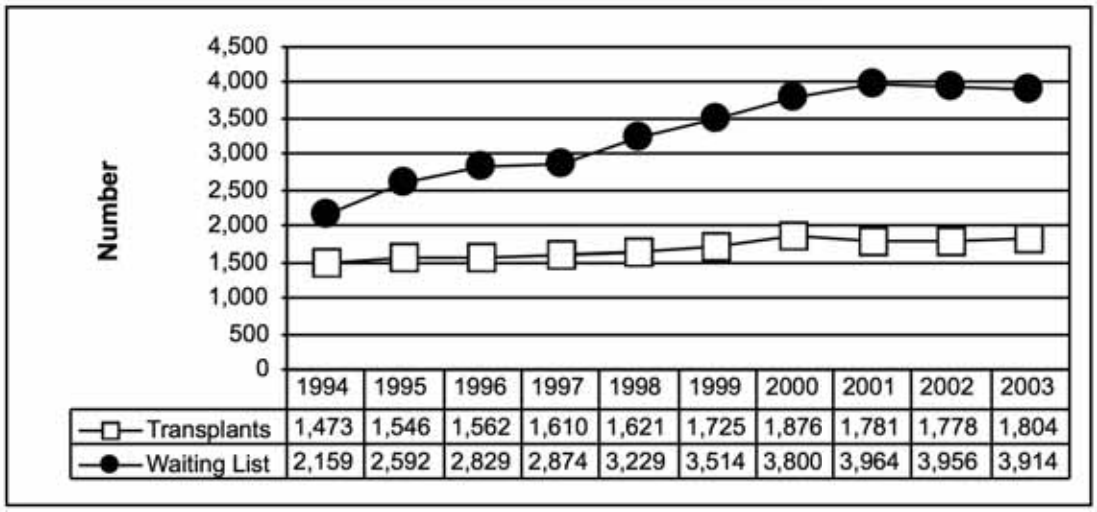


Figure 4. Treatment modality among prevalent ESRD patients by age in 2002.

Although patients of advanced age continue to derive a survival advantage from transplantation, < 50% of prevalent ESRD patients aged 45-64 years are treated with transplantation.

Transplant Wait-list

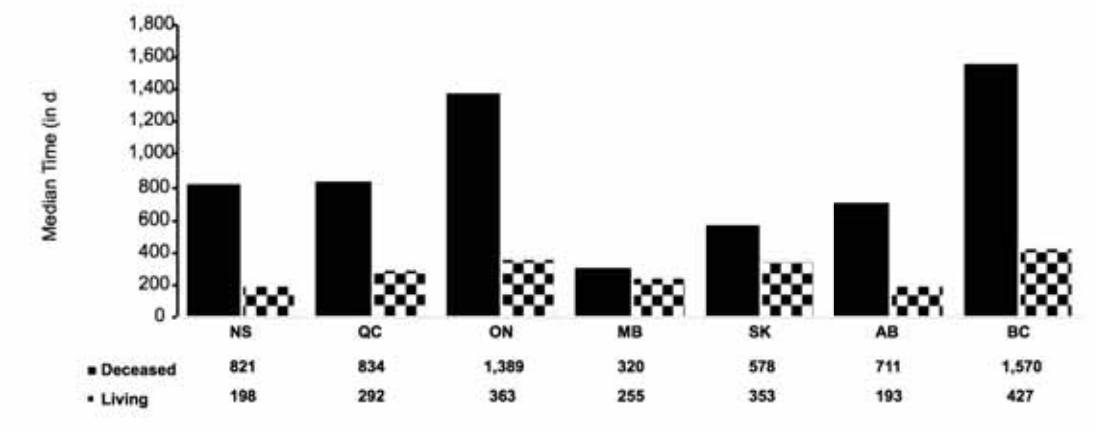
Despite the increasing incidence of ESRD, the number of patients awaiting organ transplantation has not increased significantly since 2000 (Figure 5).



	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003
Transplants	1,473	1,546	1,562	1,610	1,621	1,725	1,876	1,781	1,778	1,804
Waiting List	2,159	2,592	2,829	2,874	3,229	3,514	3,800	3,964	3,956	3,914

Figure 5. Number of transplants and patients on waiting list, of which 73% are waiting for kidney transplantation.

The reason why the waiting list numbers have not changed is unclear. One possibility is that referral practices for transplantation may be negatively impacted by the reality of long wait times. There is tremendous variability in both the number of patients wait-listed in each province and the median waiting times for transplantation (Figure 6). The reasons for this regional variability are uncertain and may include incomplete capture of wait-list candidates reported to CORR, regional differences in referral for transplantation and regional differences in the rate of transplantation. Unfortunately, these issues cannot be resolved from the existing data regarding the wait-list population.



	NS	QC	ON	MB	SK	AB	BC
▪ Deceased	821	834	1,389	320	578	711	1,570
▪ Living	198	292	363	255	353	193	427

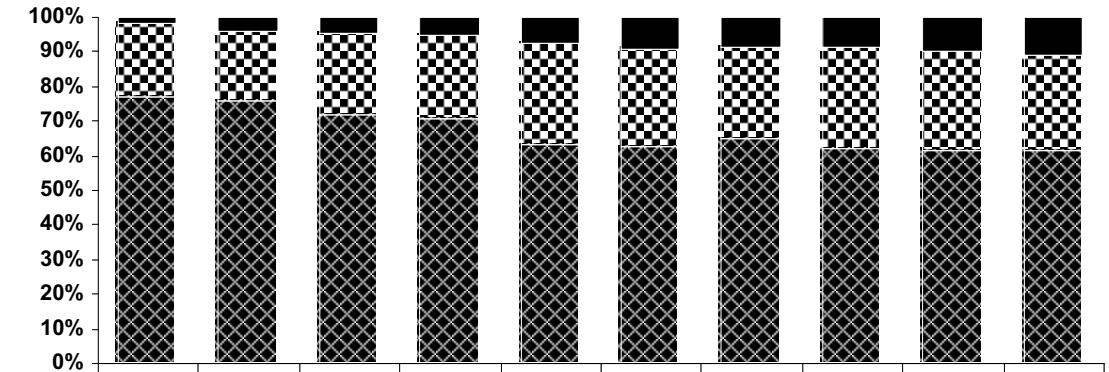
Figure 6. Median wait-times (days) for kidney transplantation by province in 2001.

The limited data regarding the wait-list population also precludes determination of sensitization (PRA), HLA typing and the prevalence of previous sensitizing events among the wait-listed population in Canada. Currently HLA typing and PRA titres are only available for transplant recipients. **To address this gap, an analysis of the Manitoba wait-list is provided below.**

Transplant Activity in Canada

The total number of transplants performed has remained constant over the last number of years. Despite decreases in deceased donor transplantation, the number of kidney transplants performed has been maintained with the expansion of living donor transplantation. Of note, the largest increases in living donor transplantation have been in regions with the lowest rates of deceased organ donation. Moreover, for the last few years the number of living kidney donors exceeded the number of deceased donors; however, at present, even they have hit a plateau. Nevertheless, living donor transplantation continues to be one of the most promising strategies to meet the growing need for transplantation.

Innovative approaches to permit living donor transplantation among sensitized patients and patients with blood group incompatible donors are not currently utilized, but may be important strategies to increase living donor transplantation in the future (e.g., a national paired living kidney donor exchange registry).



	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003
LURD	18	36	45	52	73	91	97	89	100	113
LRD	194	193	219	233	291	289	291	309	291	290
DD	700	711	678	684	629	633	716	654	627	650

Figure 7. Distribution of renal transplants by donor type.
(LURD = living unrelated donor, LRD = living related donor, DD = deceased donor)
Note: # DD = # DD transplants/2 (i.e., in 2003 the number of actual deceased donors is approximately 650/2 or 325 as a DD usually provides 2 kidneys)

Sensitization (% PRA): Impact on wait-time and transplant outcomes

The prevalence of sensitized patients awaiting transplantation in Canada **cannot** be determined from CORR data. Information from the United States and Manitoba (see below) indicate that approximately 23-30% of the wait-list population are sensitized with PRA > 20%. The fact that **very few** sensitized patients receive transplants (**these patients represent < 5% of all transplant recipients**) indicates that sensitization poses a **considerable barrier** to transplantation in Canada.

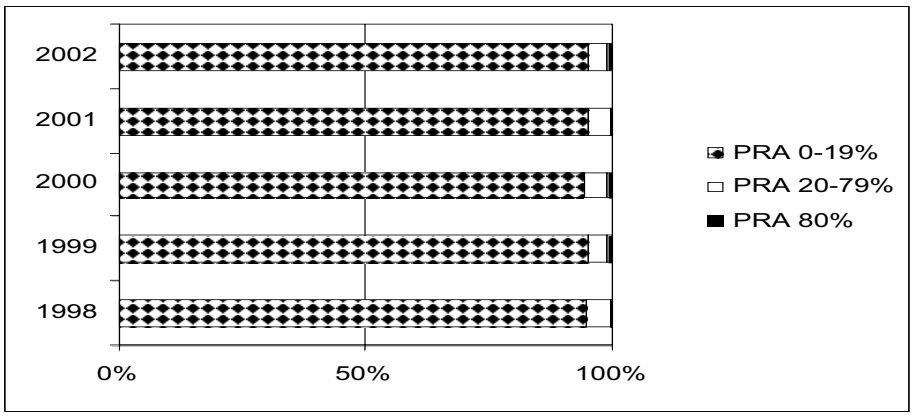


Figure 8. PRA in kidney transplant recipients: 95% had PRA <20%.

Increased PRA is clearly associated with decreased graft survival (Figure 9). Although, excellent short-term outcomes are achieved at one year after transplantation, 7.2% of all deceased donor transplants and 3.9% of all living donor transplants fail during the first post-transplant year (when death is censored as a cause of graft loss). Acute rejection accounts for 20% of graft failure during the first post-transplant year and accounts for 40% of such failures among transplant recipients with PRA > 20%. **In summary, national solutions are required to improve early graft outcomes, especially in the sensitized patient group.**

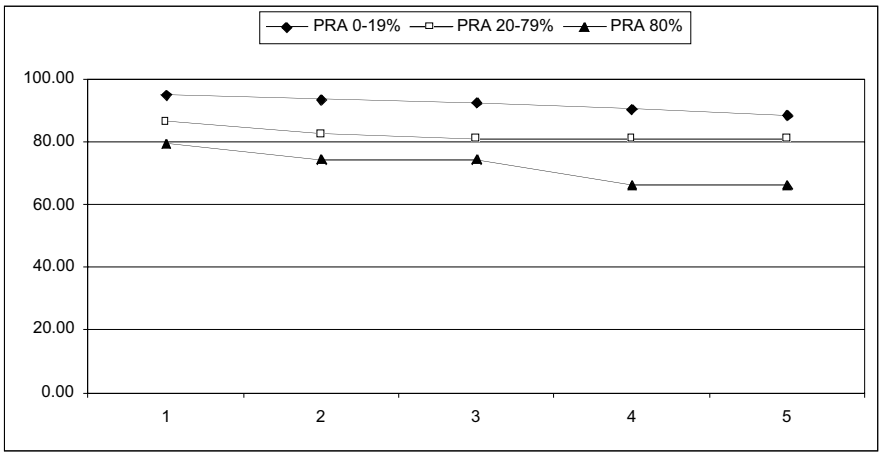


Figure 9. Deceased donor graft survival by PRA. X axis = years post transplantation. Y axis = proportion remaining event free.

Transplant Failure

Transplant failure has become the fifth leading cause of dialysis initiation in Canada. In 1998, 6% of all dialysis starts were failed transplant recipients returning to dialysis. The survival of transplant failure patients treated with dialysis is known to be poor and few of these patients receive repeat transplants. It is uncertain how many failed transplant recipients are reconsidered for transplantation. Many of these patients may be excluded from transplantation because they are highly sensitized and those patients that received repeat transplants have long wait-times (Figure 10).

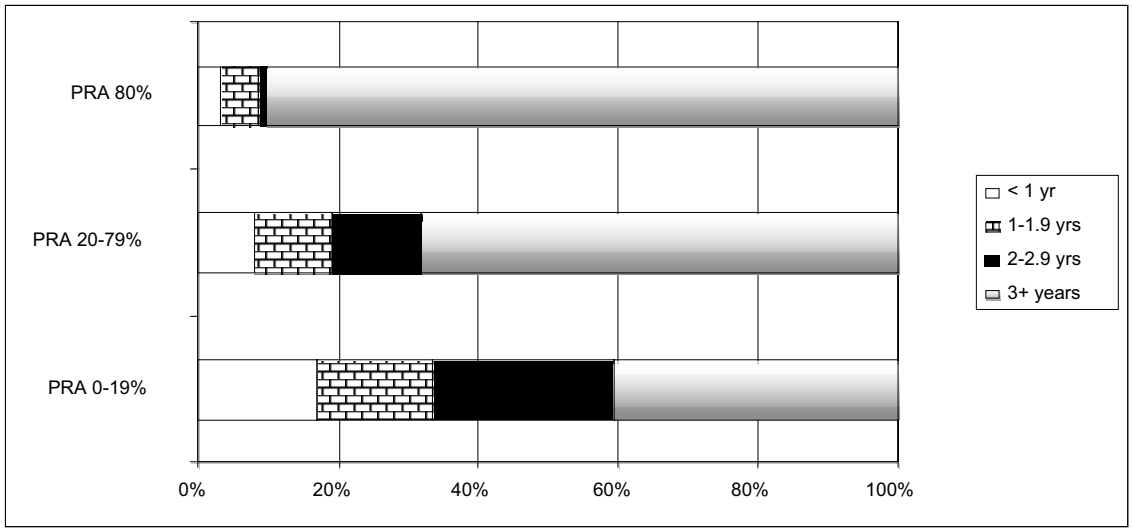


Figure 10. Wait-time for repeat transplantation by PRA.

Limitations of the CORR Database

The retrospective data collection and focus on post-transplant outcomes in CORR limit conclusions regarding opportunities to improve access to transplantation. To ensure equity and to implement strategies to increase transplantation, detailed data regarding the characteristics of the incident ESRD population and wait-list population are required including demographics, comorbid disease characteristics, sensitization status and HLA type.

Despite these limitations, the existing CORR data provide unique insights into the state of transplantation in Canada today and suggest considerable opportunities to increase both access to transplantation and post-transplant outcomes with widespread adoption of state-of-the-art immune surveillance testing.

b. Manitoba Wait-list

As of December 2004 there were 168 patients waiting for their first or second kidney transplant on the Manitoba Deceased Donor Wait-list (Table 1).

% Current PRA	0 - 19%	20 -79%	80 -100%
% on Wait-list	71%	18%	11%
Number of Patients	120	30	18
Mean Wait-time (years)	2.7 ± 1.9	5.0 ± 4.1	7.8 ± 5.6

Table 1. Time on Manitoba Deceased Donor wait-list vs. % PRA for patients awaiting first or second kidney transplant.

Of these ESRD patients, 29% have evidence of sensitization to foreign HLA molecules (i.e., PRA ≥ 20%), which correlates with prolonged time on the wait-list. Indeed, some patients with a current PRA > 80% have waited up to 18 to 20 years. This is true even if the analysis is restricted to those awaiting a first transplant (Table 2).

% Current PRA	0 - 19%	20 -79%	80 -100%
% on List for 1st Graft (n=130)	81%	12%	7%
Mean Wait-time (years)	2.6 ± 1.7	3.6 ± 3.4	7.8 ± 6.0

Table 2. Time on Deceased Donor Wait-list vs. % PRA for patients awaiting a first transplant.

Interestingly, while patients with a PRA ≥ 20% waiting for their first renal transplant in Manitoba over the period from 1992-2004 represent 19% of the wait-list, they received only 2.5% of the deceased donor kidneys. This is entirely consistent with the rest of Canada where < 5% of transplants in the country are conducted in patients with a PRA ≥ 20%. Indeed, if one considers patients with a PRA ≥ 80% this group in Manitoba makes up 7% of the waiting-list for a first transplant but on average receives only 0.5% of the deceased donor kidneys allocated to patients receiving their first transplant (Table 3). **Clearly, PRA ≥ 20% represents not only a barrier in terms of prolonged time on the wait-list but is strongly associated with lack of access to deceased donor kidneys.**

% Current PRA	0 - 19%	20 -79%	80 -100%
% of 1st Transplants (n = 237)	97.5%	2%	0.5%

Table 3. Allocation of first renal transplant vs. % PRA at time of transplant.

Of particular note, if one examines the composition of the Manitoba patient population with respect to gender, it is interesting to note that **78% of patients on the wait-list for a first transplant with a PRA ≥ 80% are women** (Table 4).

	% Current PRA (AHG-CDC)		
	0 to 19%	20 to 79%	80 to 100%
% on List Waiting for 1st Graft	81%	12%	7%
Male : Female (%) (56:43)	**63**:37	50:50	22:**78**

Table 4. Gender breakdown for those patients on the deceased donor wait-list prior to first transplant.

Clearly women are at a disadvantage at present if they have been sensitized. While this is not unexpected given that pregnancy is associated with sensitization (i.e., development of an HLA Ab against the father's HLA), our current system for organ allocation in Canada for this group is limited to the local donor pool (e.g., Manitoba), which prevents equity of access to deceased donor organs for such individuals. **A national solution is required to address the needs of the sensitized patient.**

c. United Network for Organ Sharing (UNOS) Registry

One might argue that the inequity of access for women might just be a Manitoba phenomenon. However, recent data provided from the UNOS Transplant Registry Research Department reveal that in America, women also make up the majority of sensitized patients waiting for their first renal transplants (Table 5). These data are more skewed compared to the Manitoba wait-list. Therefore, while the data is not available from the rest of Canada, it suggests a high likelihood that women indeed make up the majority of sensitized patients listed for first transplant on all provincial wait-lists in Canada.

% Current PRA	0 - 19%	20 -79%	80 -100%
% on List for 1st Graft	85%	10%	5%
Male: Female (%)	**64**:36	25:**75**	17:**83**
Number of Patients on Wait-list	40,766	4,603	2,510

Table 5. Gender breakdown of the UNOS wait-list (based on OPTN data as of Jan. 31, 2005).

In terms of organ allocation by the UNOS registry, it can be seen when compared to Canada **that the availability of a national registry improves the equity of access to first transplants for sensitized patients**. Indeed, 9% of first transplants in the UNOS registry (Table 6) occurred in patients with PRA ≥ 20% at the time of transplant, while this population makes up 15% of the UNOS wait-list (Table 5).

% Current PRA	0 - 19%	20 -79%	80 -100%
% of 1st Graft	91%	6%	3%
Number of 1st Graft (Dec 1999 to Nov 2004)	31,272	2,224	999

Table 6. Allocation of deceased donors to first renal transplant recipients (UNOS) vs. % PRA (based on OPTN data for first transplants for 5 year period between Dec 1999 and Nov 2004).

In terms of median wait-time on the UNOS registry for a kidney transplant, it is clear that even with the registry the sensitized patient has a prolonged wait-time to receive a transplant (Table 7). **Therefore, a registry can provide the foundation to improve access for the sensitized patient but the allocation strategy is critical.** Currently, UNOS is reassessing the allocation system for deceased donor kidneys and reviewing the technologies used to assess sensitization to try to improve some of the equity issues that still exist.

% Current PRA	0 - 19%	20 -79%	80 -100%
Median Wait-Time (Years)	3.1 (3.0 – 3.2)	5.3 (4.6 to 6.8)	6.4 (4.8 to **)

Table 7. Kaplan-Meier Median Waiting Time for Registrations on the Kidney List as of 1998.

Summary

It becomes evident from this review that while there is a shortage of donor organs in Canada, the sensitized patient bears a disproportionate amount of the burden. It is equally clear that a national registry (i.e., national donor pool to share amongst centres) goes a long way towards correcting this deficit. However, even with the registry, as will be seen in the following section, the quality and sophistication of the histocompatibility laboratory support is critical to resolving this issue for this disadvantaged group.

Part II:

Towards Clinical Consensus

3. Forum Process

In its 2004-2005 work plan, the CCDT Transplant Committee identified a pressing need to better understand current and best practices in the field of organ transplantation in Canada as part of a broader mandate to advise the Council of Deputy Ministers of Health on an effective organ donation and transplantation strategy. An area of particular concern and one where current practices are neither standardized nor optimized in Canada is the approach to the high risk (highly sensitized) patient. As a result, in collaboration with the Canadian Society of Transplantation, the CCDT agreed to convene a Forum of experts in an effort to develop consensus on key issues related to the assessment and management of immunologic risk in transplantation.

A Steering Committee, made up of a panel of leading Canadian experts and chaired by Dr. Peter Nickerson, was formed to organize the Forum. The Forum Steering Committee met in Toronto in the fall of 2004 to clarify objectives for the Forum and to develop a Forum Agenda. A delegate invitation list consisting of leading experts and practitioners in the fields of organ transplantation and HLA lab medicine was assembled along with a roster of potential speakers.

As the Consensus Forum was billed essentially as a working session, delegates were provided with a detailed reference list of the latest research papers and subject matter on the topic of immunologic risk. Several key research papers and reviews were circulated as required reading in advance of the Forum. The list of references and research papers are appended in the CCDT Consensus Forum Report. In addition, an environmental scan was performed prior to the Consensus Forum to determine the current assessment and management of immunologic risk pre-transplant across Canada.

The Forum consensus process involved a series of 3 intensive breakout sessions, each preceded by a round of expert presentations on the relevant breakout topics. Key questions for each breakout were prepared in advance and circulated just prior to each breakout. There were a total of 8 clinical breakout groups with approximately 8 participants per group with broad representation from across Canada. Overall, there were 6 kidney groups, 1 heart group, and 1 lung group. Each breakout group appointed a group facilitator, whose role was to keep the discussion focused and on track, and a recorder. The recorder was provided with a master record sheet for the purpose of capturing the group consensus positions on each question and topic. A Forum Recommendation Group (FRG) was formed for the purpose of consolidating the responses from the groups. At least one FRG member was assigned to each table and the members were charged with closely monitoring the proceedings and representing their group at the FRG meeting. After consolidating the groups' responses and consensus positions, the FRG reported back to the Forum delegates in plenary. The consensus process ran smoothly and was an effective means by which to capture the collective wisdom and best judgment of a large panel of well informed experts.

4. Environmental Scan and Expert Presentations

a. CCDT Survey of Canadian Laboratory and Clinical Practices

As part of the preparation for the CCDT meeting, *Assessment and Management of Immunological Risk in Transplantation*, the Steering Committee performed a survey of the heart, lung and kidney transplant programs across Canada. The purpose of the survey was primarily to understand the current practices, beliefs and opinions related to HLA Ab testing. In addition, the Committee wished to identify whether there was a difference between current practice and what the programs and HLA laboratory directors viewed as optimal.

The survey questionnaire was developed by a small Steering Committee and pre-tested with two volunteer respondents. The implementation of the survey was contracted out to Health Connexions and Dr. P. Campbell (University of Alberta) prepared the report for the CCDT. The survey questionnaire was sent by email and completed on the web. The survey was distributed to a list of 82 individuals who were either histocompatibility laboratory directors or medical and surgical directors of heart, kidney and lung adult and pediatric transplant programs across Canada. Program directors were asked to comment on risk assessment and lab directors were asked questions related to antibody screening and crossmatching.

Results of the Survey

Responses were received from a total of 34 recipients, a response rate of 41.5%. Eleven out of twelve lab directors completed the survey. Twenty-three program directors completed the survey, but only 2 heart and two lung program directors responded. This represented 7 heart programs, 4 lung programs and 13 kidney transplant programs in Canada (see Appendix 5 for a power point version of the report).

Screening for HLA Antibodies

Most programs screen routinely for Class I HLA antibodies, although 2 labs reported that they did not screen for Class I and 3 labs reported that they did not screen routinely for Class II antibodies. Nine of the 10 labs that reported the method used for Class I, use a solid phase assay alone or in combination with CDC methods (3 flow, 3 ELISA and 4 both). Seven labs use a solid phase assay for Class II screening (3 flow, 2 ELISA and 2 both). The method used for screening tends to reflect the methods available within the lab rather than the wishes of the program and doesn't vary from organ to organ. The frequency of screening sera from listed patients varied from time of listing only to monthly. The median frequency of screening was every 3 months. Most lab directors reported that sera were also screened after known sensitizing events. Of the 10 labs that are screening for antibodies, only 8 are defining specificities if antibodies are detected. Seven of these labs use solid phase methodology to determine specificities.

Source of Cells for Crossmatching

Most labs use peripheral blood for crossmatching for heart recipients and use spleen cells for crossmatching for lung transplants. All labs use peripheral blood for crossmatching kidney transplant recipients and 7 also use spleen. Eight labs also perform the final crossmatch with peak sera. Many of the lab directors indicated that they plan to add or revise their current methodologies.

T Cell Crossmatching

The methodology used for T cell crossmatching (TCXM) differed from organ to organ. The 7 heart programs perform TCXM by CDC based assays (4 AHG, 2 NIH-ext and 1 NIH). One lab also performs TCXM by flow cytometry (FC) if the PRA>15%. One lung program does TCXM by FC for all transplants, another does FC if the PRA>15% and the remaining 2 use CDC based methods (1 AHG and 1 NIH). TCXM for deceased donor kidney is mostly done by CDC methodology, often by AHG. Three labs are using FC for TCXM and 3 are using CDC-NIH-ext only. More TCXM is done by FC for living related kidney transplant, especially if the recipient is known to be sensitized.

B Cell Crossmatching

Only one heart transplant program performs B cell crossmatching (BCXM) for all heart transplants. Another program reported that all pediatric heart transplants have a BCXM. The BCXM is done by CDC-NIH in 2 centres and by FC in 1 centre. Three lab directors replied that a BCXM is done in situations of increased immunological risk. One lung transplant program does FC BCXM for all recipients. Three lung transplant programs do not perform routine BCXM, but 1 performs a FC BCXM if the PRA >15%. Six kidney transplant programs reported that a BCXM is performed routinely on deceased donor kidney transplant recipients. Two labs reported that a BCXM is only done if class II antibodies are detected on screening. The method of BCXM is CDC-NIH-ext in 5 labs and FC in 2 labs. Four labs indicated that they would do BCXM by FC for recipients of living donor kidneys but not deceased donor kidneys.

Transplantation Across a Positive T Cell Crossmatch

For both the heart and lung programs there is often difficulty performing prospective crossmatches due to cold ischemic times and therefore many of the crossmatches are performed retrospectively. The heart programs indicated that prospective TCXM was done if the recipients were of high immunological risk. One director indicated that a remote positive test would influence the decision to proceed. One program indicated that it would proceed if the TCXM was positive in an emergency situation. The lung programs indicated that they would not be influenced by a remote positive crossmatch and one program indicated that they would proceed with a positive T cell Flow XM. Four kidney program directors reported that they would perform a kidney transplant across a positive FC TCXM. Eight directors replied that a remote positive crossmatch would influence their decision to proceed.

Transplantation Across a Positive B cell Crossmatch

Both the lung and heart program directors indicated that a positive BCXM is not a barrier to transplant. Most kidney program directors that responded indicated that they would perform some kidney transplants across a positive BCXM. This was often in the situation where the FCXM was positive. Four directors indicated they would transplant across a CDC-NIH positive crossmatch.

Preference of Methods

The directors were asked what methods they would choose for crossmatching and antibody screening if resources were not an issue. All 23 program directors indicated that they would prefer solid phase assays to be used for HLA antibody screening. Eighteen program directors indicated that they would choose flow-based methods for HLA crossmatching. All the lab directors indicated they would prefer to use solid based assays (mostly flow-based methods) for antibody screening and flow-based methods for crossmatching.

Summary

The survey indicated that there is a disconnect at some centres between what the program directors and lab directors viewed as preferred methods of testing and what is currently being done. The range of testing performed in any one centre tended to reflect the methods available within that particular lab and did not seem to be influenced by the type of transplant. The program directors and lab directors generally support antibody screening and crossmatching by sensitive methods such as ELISA and flow. A number of labs indicated that they have plans to revise or add to their current methods.

b. Review of CSA Z900.2.3-03 Standards

The following was abstracted from the Z900.2.3-03 standards for **Perfusable Organs for Transplantation** published in February 2003 by the Canadian Standards Association.

> This is the first edition of CSA Standard Z900.2.03, *Perfusable Organs for Transplantation.* This Standard is part of a series of management system standards related to the safety of cells, tissues, and organs for transplantation and assisted reproduction. It was developed from work initiated by Health Canada's Expert Working Group on Safety of Organs and Tissues for Transplantation. This Standard was prepared by the Subcommittee on Perfusable Organs, under the jurisdiction of the Technical Committee on Safety of Cells, Tissues, and Organs for Transplantation and Assisted Reproduction and the Strategic Steering Committee on Health Care Technology, and has been formally approved by the Technical Committee. It has been submitted and approved by the Standards Council of Canada as a National Standard of Canada.
>
> **1.1** This Standard addresses issues related to the safety of human perfusable organs used for transplantation purposes. It includes aspects of safety for potential and actual donors and recipients, personnel, and others who may be exposed to, or affected by, the transplant of perfusable organs.
>
> **1.2** This Standard applies to establishments (or facilities) and individuals involved in the donor suitability assessment, retrieval, processing, preservation, packaging, labelling, storage, quarantine, evaluation, recordkeeping, adverse event reporting, distribution, importation or exportation, and recall of human organs intended for transplantation, including:
>
> a) organ donation organizations (ODOs);
>
> b) transplant programs and facilities (hospitals and special clinics); and
>
> **c) histocompatibility laboratories.**
>
> **14.3.1 Histocompatibility Laboratory**
>
> The standard operating procedures (SOP) manual, the operations of the laboratory, and participation in proficiency testing programs of the histocompatibility laboratory **shall conform to ASHI's *Standards for Histocompatibility Testing*.**

Observation

In comparison to the exhaustive requirements and detailed methods proposed regarding pre-transplant infectious disease testing for potential organ donors (i.e., HIV, HBV, HCV, etc.) under the CZ900.2.3-03 standards, there is little definition of requirements for histocompatibility testing other than to say that they should be compliant with ASHI standards. Compliance with ASHI standards only ensures that whatever test is performed is done in a manner consistent with "Good Laboratory Practices" (GLP). This in and of itself does not require any specific type of test. **Under the current proposed CSA standard, one could be compliant with the requirements but performing the least sensitive testing methodology for HLA Ab testing and crossmatching.**

c. Review of Recent Technology and Therapeutic Developments

As the Consensus Forum was billed essentially as a working session, delegates were provided with a detailed reference list of the latest research papers and reviews on the subject of immunologic risk assessment and management. Several key papers and reviews were circulated as required reading in advance of the Forum (Appendix 6). The list of references and research papers is appended in the CCDT Consensus Forum Report (Appendix 7). This section will provide the highlights of the expert presentations at the CCDT Consensus Forum.

[1] Immunologic Risk Assessment

Dr. Gebel from Emory University reviewed the latest developments in HLA antibody detection and the literature that demonstrate that even low levels of HLA antibody are associated with a high risk to the patient of early rejection or graft loss in kidney transplantation. His paper is published in the American Journal of Transplantation (Dec 2003) and is a comprehensive review of the literature as well as the official position of ASHI.

Serologic methodologies are the least sensitive tests for the detection of HLA antibody in a serum sample from a patient. While ELISA based methods are superior, it is clear that flow-based techniques are the most sensitive (Table 8).

Method	Positive	Negative
AHG-CDC	104	148
ELISA (QuickScreen™)	124	128
FLOW	(Gebel & Bray, Transplantation 2000, 69: 1370)	

Table 8: Relative sensitivities of methodologies to detect HLA Ab in patient's serum.

While a series of papers were reviewed, Gebel cited the Manitoba data as providing the following key insights (Table 9):

- Donor specific HLA Ab detected only by flow bead technology was associated with a high risk of rejection and graft loss in the early post-transplant period.
- Donor specific HLA Class II Ab is associated with the same degree of risk as donor specific HLA Class I Ab.

Specificity (Flow bead)

Donor Reactive HLA Antibody	Rejection First Month	Time to Rejection (Days)	Ab Mediated Graft Loss	Time to Graft Loss (Days)
Class I (15)	14 (93%)	6 (1-17)	4 (27%)	4 (1-14)
Class II (10)	8 (80%)	5 (2-7)	3 (30%)	5 (2-9)
Controls No Donor Reactive Ab				
HLA Ab (21)	3 (14%)*	13 (13-19)*	0 (0%)	* $p < 0.001$ vs. Class I or II
No HLA Ab (195)	60 (31%)*	12 (4-28)*	0 (0%)	

Note: Retrospective evaluation by flow bead technology found 15 patients with donor reactive Class I HLA Ab and 10 patients with donor reactive Class II HLA Ab. As control groups, there were 21 patients with HLA Ab detected by flow but none were donor specific and 195 patients without any HLA Ab detectable in the serum.

Table 9: Manitoba experience transplanting 243 Primary Transplants June 1992 to June 2003 across a negative AHG T cell and CDC B cell crossmatch.

The Manitoba Transplant Program formally switched to flow-based HLA Ab and crossmatch testing in Jan 2000 and has noted a marked improvement in early graft survival (Table 10).

Years	Method	% Survival (6 mo)
1993-1999	AHG-CDC T-cell, CDC B-cell AHG-PRA	89.1 $\pm$ 4.6
2000-2003	Flow T- and B-cell crossmatch	98.6 $\pm$ 1.7
	Flow bead PRA	$p < 0.003$

Note: Surgical causes of early graft loss excluded from analysis (i.e., vascular thrombosis)

Table 10. Early kidney transplant outcomes in the Manitoba Transplant Program after switching to flow-based HLA Ab analysis.

Dr. Zeevi from the University of Pittsburgh Medical Center presented literature that demonstrates that even low levels of HLA antibody (detected by ELISA or Flow) are associated with a high risk to the patient of early rejection or graft loss in heart and lung transplantation. Her paper is published in Transplant Immunology (2004) and is a comprehensive review of the literature as well as the official position of ASHI.

[2] Immunologic Risk Management

It was generally recognized that the immune sensitized patient had decreased access to both deceased and living donor transplants due to increased likelihood of a positive crossmatch with the potential donor. **In order to improve access for this patient population, three potential approaches were discussed by the invited experts**: [1] acceptable mismatches listed on a national registry, [2] immune modulation and transplantation across a positive crossmatch, and [3] establishing a national paired living kidney donor exchange registry.

Acceptable Mismatch/National Registry Strategy

In Dr. Gebel's second presentation, he shared the Emory experience of using high resolution flow-based technology to accurately identify "acceptable mismatch" HLA donor antigens in patients who are sensitized (PRA ≥ 20%). The goal in this approach is to clearly identify those HLA molecules against which a sensitized patient has antibodies (Ab) versus those HLA molecules for which they do not have antibodies. This latter group of HLA molecules represents acceptable mismatched HLA antigens that would be predicted to give a negative crossmatch with the recipient.

Using this approach and the access to the national donor pool (via UNOS) in the United States, the Emory Transplant Program improved access to deceased donor organs for their sensitized patient population (Table 11). Furthermore, given that 30% of the Emory wait-list is composed of patients with PRA ≥ 20% and that 25 to 40%/year of their transplants are now performed in patients with PRA ≥ 20%, they have corrected within their program the access problem that exists for the sensitized patient.

Patients Transplanted	PRA < 20%	PRA ≥ 20%
UNOS Registry Rates	86%	14%
Emory University Rates	60-75%	25-40%

Table 11. Increase in access to deceased donor organ at Emory using an "Acceptable Mismatch Strategy" based on flow-based HLA Ab analysis and the UNOS registry.

Dr. Davis from Duke University presented their experience using high resolution techniques to identify HLA Ab specificities in sensitized lung recipients followed by the listing on the UNOS registry of acceptable HLA mismatches. Because the Duke program does not perform a crossmatch prior to lung transplantation, they are using the donor HLA typing and the recipient HLA Ab specificity analysis to generate a "Virtual Crossmatch" as the basis for transplantation. A retrospective crossmatch is performed the next day. To date, the predicted and actual crossmatch results have been 100% concordant. Using this strategy, the Duke program has been

able to get more of their sensitized lung recipients transplanted and, as a result, has significantly shortened the wait-list time to lung transplant for sensitized patients.

Immunomodulation and Transplantation across a Positive Crossmatch

In terms of **kidney transplantation** in high risk patients, the following information was provided to the group: a recent NIH Forum on the subject (American Journal of Transplantation, July 2004), a review of the literature provided by Dr. A. Jevnikar (University of Western Ontario) and two presentations by experts (Dr. J. Gloor, Mayo Clinic and Dr. R. Montgomery, Johns Hopkins University).

Dr. Gloor presented the Mayo Clinic experience of transplanting living related donor-recipient pairs where there was HLA incompatibility in the recipient (i.e., an HLA Ab directed at the donor's HLA molecules). One of the principal findings of Dr. Gloor's studies is that those patients who had donor specific HLA Ab only detectable by flow techniques (i.e., flow +ve, AHG-CDC –ve) could be transplanted with excellent outcomes provided that the patient was medicated pre-transplant with high dose IVIG and a T cell depleting drug (Thymoglobulin). In the Mayo Clinic case series of 25 such transplants there has been 100% graft survival and only a 12% (n = 3) acute rejection rate (2 humoral treated successfully with short course IVIG/ Pheresis and 1 cellular treated successfully with steroids). The key to Gloor's success has been the ability to detect these low level antibodies pre-transplant using flow-based technology and intervening prior to transplant with modified immunosuppression.

Patient Risk	Pre-Transplant Management	Ab Rejection Rate	Graft Survival (1-2 yr)	References
CDC CXM +	NA	Contra-indicated	NA	
AHG CXM +				
LD	Low dose IVIG/PLEX ± anti CD20	40-50 %	80%	*Montgomery et al., Transplantation 70, 2000, Warren et al., AJT 4, 2004, Zachary et al. 76, 2003*
LD	High dose IVIG ± anti CD20	40-50 %	80-90%	*Jordan et al., Transplantation 76, 2003* *Glotz et al., AJT 2, 2002*
DD	High dose IVIG ± anti CD20	Not known	NA	
Flow CXM +, AHG CXM -	IVIG + Thymo	12%	100%	*Dr. Gloor Presentation (CCDT Consensus Forum)*

Note: In transplants that proceeded, crossmatches (CXM) were AHG positive *pre-desensitization* rather than at the time of transplant. Generally transplants proceed only if the crossmatch became CDC-AHG or NIH negative, but the management of Flow XM positive, AHG-XM negatives varies between centres.

Table 12. Review of outcomes to date transplanting kidneys across positive crossmatches with new therapeutic approaches.

Both Gloor and Montgomery presented their respective experiences in transplanting living related donor-recipient pairs where there were higher levels of donor specific HLA antibody present in the recipient (i.e., CDC or AHG-CDC crossmatch +ve). Under these circumstances the immunologic barrier was harder to cross. Specifically, there was a requirement pre-transplant to use more aggressive immunosuppression in the recipient (e.g., IVIG/Plasmapheresis/Thymglobulin/Rituximab) and while excellent outcomes could be achieved, they both identified an absolute barrier (i.e., AHG-CDC crossmatch +ve with a titre > 1:256) beyond which they felt it was not practical to proceed with desensitization protocols at present. A general summary of their findings and those of others to date is found in Table 12.

In terms of **lung transplantation** using immunomodulation prior to transplant in the sensitized patient, Dr. Davis presented the Duke series of 12 lung transplants managed peri-op with IVIG and/or plasmapheresis. The outcomes have been excellent with a low incidence and a decreased severity of acute rejection.

In terms of **heart transplantation** in high risk patients, Dr. West presented a summary of the UCLA and Toronto experiences with the major points being that [1] pre-formed donor specific HLA Ab are a risk factor for early humoral rejection and/or graft damage; and [2] due to clinical urgency, heart transplants have been performed in both adults and children despite a positive crossmatch, with variable outcomes using immunomodulation (e.g., IVIG and/or pheresis) in the peri-operative period.

Paired Living Donor Exchange Program

Dr. Montgomery related the Johns Hopkins experience with paired living donor exchange as a strategy to see highly sensitized patients transplanted. The concept is that while patient X may be sensitized to their specific in-family donor, they would not be to another living donor for patient Y. Likewise, patient Y who has a positive crossmatch to their own living donor would not react to the donor for patient X. By swapping donors, both patients (X and Y) are able to be transplanted now with a negative crossmatch (i.e., low risk). While they have initiated this program at Hopkins, Dr. Montgomery reported that it has been projected that a national paired living donor exchange in America would allow for 3,000 more kidney transplants in the first year and 750/year subsequently amongst sensitized patients.

Finally, Dr. E. Cole from Toronto presented the logistical hurdles that had to be crossed by the University of Toronto program in setting up their local paired living kidney donor exchange program that will address not only HLA incompatibilities due to HLA Ab against the donor but also ABO incompatibilities. It is clear that, in order for paired exchange programs to generate significant numbers of transplantable pairs, large incompatible donor and recipient pools are required. Thus, there is an exponential advantage of a national scheme as opposed to either local or provincial based programs.

5. Summary of CCDT Clinical Consensus Forum

The intent of the Forum was to generate recommendations for clinicians looking after kidney, heart and lung transplant recipients. It was clear prior to the breakout sessions that the majority of the data to date resided in the field of kidney transplantation. Moreover, it was equally appreciated that lung and heart transplantation is a more complex situation: there is no alternative (i.e., dialysis) for these patients and the time limitations between organ retrieval and implantation often require decisions to be made prior to the availability of crossmatch results. Therefore, it is not surprising that the consensus recommendations for kidney, lung and heart frequently differ between organs. Rather than try to capture all of the differences in this section, a more comprehensive set of guidelines and key considerations are recorded in Appendix 8. This section provides an overview of the general recommendations common to kidney, heart and lung transplantation; when the recommendations are referring to a specific organ they are clearly identified as such.

a. Recommendations for Pre-transplant Risk Assessment

General Considerations

There was consensus that HLA Class I or Class II directed IgG antibodies were clinically relevant conferring both short- and long-term risk to the patient. The consensus was that IgM HLA antibodies are not clinically relevant due to the lack of convincing data. Finally, while non-HLA antibodies may be potentially of clinical relevance there is no reliable methodology to test for them routinely at present.

Specific Recommendations

1. Screening for HLA Class I and Class II IgG should occur while patients are on the wait-list. The following caveats were agreed to:
   - Flow-based techniques were considered optimal.
   - If an IgG HLA antibody is detected then specificity of the antibody should be evaluated, optimally by flow-based techniques.
   - The preferred frequency of testing (screening and specificity) is not clear but likely between 3-6 months for kidney and heart patients.
2. A donor specific T cell and B cell crossmatch should be performed pre-kidney transplant. The following caveats were agreed to:
   - In general an AHG-CDC T cell and CDC B cell crossmatch may suffice, but in a sensitized patient a T cell and B cell flow crossmatch should be performed.
   - The crossmatch should be prospective, but in isolated circumstances in which the patient shows no HLA antibody by a flow-based technique a prospective crossmatch may be forgone and performed retrospectively.
3. In heart and lung transplantation the minimal practice should be to perform a retrospective flow-based crossmatch within 24 hours post-transplant.
4. Peripheral blood may be used for the screening crossmatch, but spleen or lymph node may be required in some circumstances.

b. Recommendations for Pre-transplant Risk Management

General Considerations

There was general consensus that high precision HLA Ab testing may increase the wait time for some patients as it will now identify them as sensitized, whereas before they were considered not to be sensitized by less sensitive methods. However, it was agreed that this would lead to better outcomes overall for patients and was an acceptable compromise. Indeed, it was strongly felt that high precision HLA Ab testing would be key to improving access for the highly sensitized heart, lung and kidney patient. In terms of transplanting a highly sensitized patient across a positive crossmatch, it is clear that some Canadian centres are currently transplanting these high risk patients and that this is a developing field where the optimal protocol is yet to be determined. This was especially true for heart and lung transplant recipients where there were no other options.

Specific Recommendations

For deceased donor transplants:

1. A positive current CDC or AHG-CDC T cell crossmatch is a contraindication to kidney transplant (there was no consensus as to whether a positive T cell flow crossmatch is a contraindication to transplant).
2. A remote positive T cell crossmatch by any method is not a contraindication to transplant but rather is a risk factor for early acute rejection and/or graft loss.
3. A positive B cell crossmatch should be approached in the same way as a positive T cell crossmatch.

For living donor kidney transplants:

1. While consensus was not reached, there is a willingness by some groups to proceed with a living donor transplant across a positive crossmatch when detected only by flow-based techniques.
2. While consensus was not reached, there is a willingness by some groups to proceed with a living donor transplant when the initial AHG-CDC crossmatch was positive if successful reduction in donor specific HLA Ab levels were achieved with a desensitization protocol.

c. Recommendations for Post-transplant Monitoring

1. For stable low risk patients there is insufficient data to justify routine monitoring. This was felt to be an area for research.
2. For patients with graft dysfunction post-transplant, pathologic assessment (with C4d staining) and serologic assessment for donor specific HLA Ab via a solid phase assay (preferably by flow-based techniques) should be available within 24 hours.
3. For high risk patients (known to have donor specific HLA Ab), pathologic assessment (with C4d staining) and serologic assessment for donor specific HLA Ab via a solid phase assay (preferably by flow-based techniques) should be available within 24 hours.

d. Recommended Future Directions

1. It was universally endorsed that Canada should establish a national high-risk patient registry for sensitized kidney, heart and lung patients (e.g., PRA > 80%).
    - It was recognized that critical to such an initiative, HLA laboratory technologies must be upgraded to high resolution technologies and standardized across all participating centres.
    - Given the number of issues that need to be sorted out, a CCDT forum dedicated to working out the logistics is required.
    - Consensus that an "acceptable mismatch" strategy should be pursued as the optimal strategy for sensitized kidney, heart and lung patients.
    - A management organization, with solid organ transplant professionals, will need to be charged to establish such a registry with funding provided by both federal and provincial governments.
2. The possible development of a national paired living kidney donor exchange registry should be considered.
    - Implementation across all participating centres of high precision HLA Ab testing is a critical foundational tool that would facilitate such an initiative.
3. A central registry for tracking outcomes prospectively should be created to guide future decision-making.

These recommendations represent an important contribution to the advancement of transplant knowledge and practice in Canada; they lay the foundation for progress and change, which will ultimately lead to improved patient and health care outcomes, by providing guidance to practitioners and policy makers alike. As a final note, while consensus was achieved on many fronts, there were areas and topics where consensus was not reached. However, this too should be viewed as an important outcome, one that points the way to continued research and dialogue.

Part III:

Business Case for Stakeholders

6. Logistics Group Deliverables

During the CCDT Consensus Forum, the task for the clinicians and allied health workers at the heart, lung, and kidney tables was to listen to the evidence presented by the guest speakers and integrate that with the literature provided, as well as with their own experience. They then worked through a list of consensus questions on the technical and clinical issues related to immunologic risk assessment and management in heart, lung, and kidney transplantation.

The logistics group listened to the plenary presentations and worked in parallel with the organ specific tables, but its goal was quite different. The logistics group was charged with developing a business case for the implementation of enhanced laboratory testing for organ transplantation and for the development of a communications strategy to maximize the probability that the clinical and technical recommendations and outcomes from the meeting would be implemented. These tasks were considered to be at least as important as the clinical recommendations generated from the meeting.

Specific Deliverables

- To conduct an environmental scan of the current status of kidney disease and renal transplantation in Canada using data provided from CORR, Manitoba wait-list and the UNOS registry.
- To review the current CSA Z900.2.3-03 standards for "Perfusable Organs for Transplantation" as they pertain to histocompatibility laboratory practices in support of solid organ transplantation.
- To review the current laboratory clinical practices in Canada presented in the CCDT Consensus Forum.
- To review the recent technologic and therapeutic developments presented at the CCDT Consensus Forum.
- To develop an analysis of the opportunities favoring high resolution testing and threats attributable to the status quo, based on the data provided (Ref. Section 7 following).
- To review clinical recommendations developed in the CCDT Consensus Forum.
- To develop an economic analysis of the cost/benefit to the health care provider of high resolution HLA antibody testing for kidney patients.
- To develop a business case to be used to communicate Forum recommendations to health care providers.

7. Analysis of Opportunities and Threats

Opportunities Favoring High Resolution Testing

- With the availability of high resolution flow-based testing, as well as new therapeutic agents (i.e., IVIG, Thymoglobulin), a transplant program could establish a high risk living donor program for sensitized patients who previously would remain years on the wait-list for a deceased donor kidney – *adds new donors to the pool.*
- Early graft loss commonly occurs due to undetected donor specific HLA antibodies. This suggests that newer, more sensitive, diagnostic technologies (i.e., flow-based) can be used to predict and prevent this occurrence. Proof of concept comes from the Manitoba Transplant Program whose early graft survival has gone from 89.1% to 98.6% since the implementation of high resolution flow-based testing in 2000 – *improves utilization of a limited resource.*
- Equal access to medical treatment: The majority (78%) of highly sensitized patients (PRA > 80%) in Manitoba and 83% of those on the UNOS registry (USA) awaiting a first renal transplant are **women,** as sensitization commonly occurs through pregnancy. This inequity of access to kidney transplants for women likely exists on all wait-lists in Canada.
- A Canadian registry for transplantation requires a reliable data set and high resolution flow-based testing would provide a baseline data set. Data collected by CORR is submitted voluntarily, opening the potential for incomplete data collection.
- A national paired living kidney donor exchange requires assurance that the donor's relative will receive a viable transplant; improved testing will provide assurance.
- Limited ability to analyze current Canadian transplant outcomes without a national registry and common data set. The limited aggregate data available for the wait-listed population preclude analyses of the progression of patients through the wait-list process to transplantation.
- Dollars exist within the current regional health care budgets and transfers will reduce pressure on dialysis budgets. The annual expenditure to the health care provider for the end stage renal disease (ESRD) patient on hemodialysis is $104,277/year, whereas the annual expenditure (beyond the first year) for transplantation is $32,196/year.
- Emory University (Atlanta, USA) has implemented high resolution flow-based testing to identify "acceptable" mismatched tissue antigens (HLA) in their highly sensitized population. Using this approach coupled with the UNOS National Registry, 25 to 40% of the kidney transplants at Emory are now performed in sensitized patients (sensitized patients make up 30% of their wait-list) – *the equity issue can be corrected.*
- A review of the CSA Z900.2.3-03 standards revealed that there is no minimum test method specified for histocompatibility laboratories supporting solid organ transplant programs. An environmental scan of Canadian histocompatibility laboratories and transplant programs revealed that the type and practice of testing provided in support of solid organ transplantation vary widely across Canada

- Seven of 10 Canadian HLA labs use flow-based methods, but only a minority is using it routinely as the standard of care. Additional funding is needed for flow-based technology to become routine.

Threats Attributable to the Status Quo

- Deceased organ donation per million populations (DPMP) in 2003 was 13.5 as compared to the 2005 target of 25 DPMP put forward in 1999 by the National Coordinating Committee for Organ and Tissue Donation and Transplantation. Living kidney donors have exceeded the number of deceased donor transplants.
- The incidence and prevalence of ESRD in Canada continues to increase, while the proportion of ESRD patients treated with transplantation has decreased over time. Less than 50% of prevalent ESRD patients aged 45-64 are treated with transplantation.
- PRA $\geq$ 20% represents not only a barrier in terms of prolonged time on the wait-list but is strongly associated with lack of access to deceased donor kidneys. Very few sensitized patients receive transplants (these patients represent < 5% of all transplant recipients), an indicator that sensitization poses a major barrier to transplant in Canada.
- Amongst ESRD patients, 30% of those on the wait-list have prior exposure to donor tissue antigens (HLA) from pregnancy, transfusions or prior transplants, resulting in preformed HLA antibodies (i.e., they are "sensitized"), which can lead to early rejection and graft loss.
- Sensitized ESRD patients, while comprising 30% of the wait-list, receive < 5% of the kidney transplants in Canada. They have prolonged wait-times compared to unsensitized ESRD patients due to the fact that kidneys are not shared between centres and local (i.e., centre) donor pools are too small to find an acceptable kidney donor for this disadvantaged group.
- Despite excellent short-term outcomes, 7.2% of all deceased donor transplants and 3.9% of all living donor transplants still fail during the first post-transplant year, requiring the patient to resume dialysis. Further improvement in early graft survival would result in significant cost savings to the health care provider.
- The annual expenditure to the health care provider for the end stage renal disease (ESRD) patient on hemodialysis is $104,277/year, whereas the annual expenditure (beyond the first year) for transplantation is $32,196/year.

8. Economic Evaluation of High Resolution HLA Ab Testing

General Overview

Implementation of high resolution HLA Ab testing in **all histocompatibility laboratories in Canada** has the potential not only to improve transplant outcomes at the local level but to lay the foundation for the development of national registries whose mandate is to improve the equity of access to organ transplantation for the highly sensitized patient. Therefore, an economic evaluation of the cost/benefit to the health care provider was undertaken to assess the financial impact of implementing universal flow-based testing. For the purposes of this discussion, cost was broken down into initial establishment costs and operational costs for the histocompatibility laboratory. Once these costs were outlined, an analysis of the cost/benefit was undertaken from the health care provider's perspective. The cost/benefit analysis (see below) is based on the lab operating costs and excludes the lab establishment costs as these vary from site to site.

Histocompatibility Laboratory Establishment Costs

The capital investment required in each laboratory will vary depending on what the current capabilities already are in each histocompatibility laboratory. If starting from new, the equipment contained in Table 13 would need to be purchased.

Equipment	**Amount**
Flow cytometer (4 colour)	$145,000
Robbins Microfuge 60	$ 3,000
Beckmann Centifuge Allegra 6	$ 7,500
Flow Analysis Station	$ 3,000
Pipettes x4	$ 1,600
Vortex Genie G-560	$ 250
Total	**$160,350**

Table 13: Capital Equipment Required to Operate Flow-based Testing

Beyond capital equipment there will be a validation period that will require samples to be tested and normal and abnormal ranges established. This will likely require approximately **$25,000** in expendables and a **technologist to spend 3 to 6 months** on the project.

Histocompatibility Laboratory Operational Costs

In order to generate a comparative cost analysis between serologic based technologies versus flow-based technologies, we utilized data provided by the CBS Immunogenetics Laboratory in Winnipeg, which supports the Manitoba Renal Transplant Program (Table 14).

Assumptions made (based on experience) to generate the test cost model are as follows:

- Labour time required to perform AHG-CDC PRA equivalent to work time required to perform Flow PRA.
- Labour time required to perform AHG specificity analysis equivalent to work time required to perform flow-based specificity analysis.
- Labour time required to perform AHG crossmatch equivalent to work time required to perform flow crossmatch.

While it will be important to maintain AHG-CDC crossmatch techniques in the lab to define AHG –ve, Flow +ve cases, solid phase HLA Ab testing will replace AHG-CDC based testing; therefore, the impact on staffing requirements should be limited (i.e., there will be some additional workload to accommodate the increased volume of HLA Ab specificity analysis performed when flow-based testing is utilized).

Serologic Methods	**Cost**
AHG CDC PRA + Specificity Analysis (Class I only)	$176.85/sera
AHG CDC T-cell + CDC B-cell crossmatch	$ 72.81/sera
Flow-based Methods	
Flow PRA (Class I and Class II)	$153.10/sera
Flow Specificity (Class I or Class II)	$351.69/sera/HLA Class
Flow T-cell and B-cell crossmatch	$ 76.55/sera

Note: Test costs include expendables, labour (including on-call stipends), administrative overhead, service contracts on equipment as well as equipment replacement (depreciation over an 8 year period).

Table 14. Global Cost/Test

The average wait-time prior to first transplant in Manitoba is 2.5 years and the number of sera tested over this time period would be 9/patient. In Manitoba, on average, 20% of sera tests positive by AHG PRA for an HLA Ab compared to 41% of the sera screened by flow-based techniques. Finally, for the purposes of the final crossmatch, on average, 4 sera are tested per patient. Taking all of this together, the **average** pre-transplant histocompatibility test **cost/patient prior to their first transplant in Manitoba** is:

AHG screen/specificity/crossmatch	$1,822.89
Flow-based screen/specificity/crossmatch	$2,981.84

Cost/Benefit Analysis of Flow-based versus AHG-CDC HLA Antibody Assessment

Dr. K. McLaughlin (University of Calgary) developed a decision analytic Markov model to evaluate the costs and effects of two different clinical strategies for immunological risk stratification of primary deceased donor renal transplant recipients. The two clinical strategies were [1] "serological screening only" where patients immunological risk was stratified using the result of antihuman globulin enhanced complement-dependent cytotoxicity crossmatch (AHG/CDCXM) and panel reactive antibody (AHG-PRA) titre only and [2] "flow-based screening only" where patient's immunological risk was stratified using the results of flow-based crossmatch and flow bead specificity analysis.

The Markov model was used to track clinical events and costs over time. The time horizon for this analysis was 25 years and in the model this was divided into monthly cycles. Patients could exist in three mutually exclusive health states: alive with a functioning primary deceased donor renal transplant; alive on hemodialysis after failure of the primary deceased donor renal transplant; and alive on peritoneal dialysis after failure of the primary deceased donor renal transplant. Patients could die from any of these health states. Patients could move between health states or die during each cycle as is shown in the figure. The probability of moving between health states and dying was based upon published data. The average life expectancy was calculated by summing the time spent in each health state. Quality of life was incorporated into the model by assigning a utility to each health state. Utilities are a measure of patient preference for a given health state and range from 0 to 1, representing death and perfect health respectively. Quality-adjusted life expectancy was calculated by multiplying the utility of health states by the time spent in that health state.

Outcome measures were total cost of patient care over 25 years; life expectancy, measured in life years; quality adjusted life expectancy, measured in quality adjusted life years (QALY); and transplant life expectancy, measured in transplant life years. These measures of cost and effect were discounted at a rate of 5%, the recommended rate in Canada.

The analysis used a simulated patient cohort of 1,000 patients below age 70 receiving a primary deceased donor renal transplant. The simulated patients were based upon the cohort of 23,275 primary deceased donor renal transplant recipients reported by Wolfe et al., using data from the U.S. Renal Data System. The distribution of age, gender, race and cause of ESRD for the simulated patient cohort were identical to those reported by Wolfe et al. Patients could have several possible outcomes following their transplant surgery. Patients could experience early renal allograft loss (i.e., within the first three months) that may be due to a variety of causes, such as surgical complications, refractory rejection, graft thrombosis, etc. Patients with a functioning renal allograft could have steroid-responsive rejection and/or steroid-resistant rejection, which may or may not respond to treatment. Those refractory to treatment experience graft loss and return to dialysis. Patients could lose their graft beyond three months, once again due to a variety of causes, such as acute rejection, chronic rejection, calcineurin inhibitor toxicity, recurrent disease, etc. Patients could lose their graft at any time due to death with a functioning graft.

Base-Case Analysis

In the primary analysis with serologic screening, flow-based screening was associated with greater life expectancy, quality-adjusted life expectancy and transplant longevity. The discounted gains for these variables for each patient in the flow-based screening strategy were 0.1 patient life years (approximately 1.2 months), 0.1 QALY (approximately 1.2 months) and 0.2 transplant life years (approximately 2.4 months) respectively. Flow-based screening was associated with a discounted cost saving of CDN $3,608 per patient transplanted.

Sensitivity Analysis

Using one-way sensitivity analyses there were no variables that altered the direction of the benefit associated with the screening strategies. The AHG/CDC false negative rate was the only influential variable. At a threshold level of 3% for the AHG/CDC false negative rate, there was no gain in life expectancy, quality-adjusted life expectancy or transplant longevity for flow-based screening compared to serology screening and at this level there was an additional cost of CDN $157 per patient for the flow-based strategy. It is generally reported that the false negative rate for serologic screening is in the range of 10 to15%.

It must be stated that this is only an abstract of the preliminary analysis and a final cost study is in progress that will be submitted for peer review publication. At that time a copy of the full analysis will be available as an appendix to this report. It should be emphasized, however, that the preliminary analysis is very conservative in its assumptions favoring no benefit to a flow-based screening strategy. Therefore, the actual benefit of a flow-based screening strategy is most likely greater than that represented in this abbreviated report.

Manitoba Solution to Fund High Resolution HLA Ab Assessment

In January 2000, Manitoba adopted flow-based crossmatching as the standard of care in solid organ transplantation. The laboratory equipment was purchased with funds ($150,000) provided by the Manitoba branch of the Kidney Foundation of Canada and the Winnipeg Regional Health Authority (WRHA), Manitoba Renal Transplant Program. Initial operating funds ($50,000/year) were transferred to the Immunogenetics Laboratory from the WRHA global budget. In 2002, an analysis revealed that short-term kidney graft survival had improved significantly from 89.1% ± 4.2% during 1993 to1999 to 98.3% ± 1.5% from 2000 to 2002. Upon reviewing the data, the WRHA and Manitoba Health approved continued funding of flow-based crossmatching and provided additional funding for high resolution flow bead HLA antibody specificity analysis.

To fund the additional tests, a further $80,000/year of **baseline funding was transferred to the laboratory program from both the WRHA Manitoba Renal Program and WRHA Medicine Program baseline budgets** (i.e., these were the clinical programs that were going to benefit from the higher resolution HLA Ab assessment provided by the laboratory program). The Manitoba solution highlights the *benefit of regionalization* of health care in that programs can transfer baseline funding among each other to realize a net cost savings to the regional health authority. Finally, in 2005, Manitoba Health and the WRHA have agreed to fund a high risk living related kidney donor transplant program providing an additional $100,000/year in total to the Canadian Blood Services (CBS), Immunogenetics Laboratory (for flow-based post-transplant monitoring) and to the WRHA Manitoba Renal Transplant Program (for drug therapies). This was approved after a pilot study to prove safety and feasibility.

Scope of Change

As emphasized in the general overview (see above), **the consensus of the CCDT Forum Steering Committee and Forum Recommendation Group was that systemic change should occur in all histocompatibility laboratories in Canada supporting a solid organ transplant program.** The principal rationale is that turn around time on histocompatibility testing for deceased donor transplants should be within 6 to 8 hours of sample collection in order to ensure optimal patient care (i.e., short cold ischemic times).

Furthermore, as most of the cost increase is derived from ongoing operational costs, even centralization will not significantly change the volume of work required, it would only save on some infrastructure. Moreover, given that these labs support bone marrow transplant programs, it is not feasible to close any one lab in order to regionalize function and effect cost savings. Finally, the future of solid organ transplantation will be related to drug minimization strategies, which in turn will require point of care post-transplant laboratory monitoring; a regionalized histocompatibility lab would prevent development of this strategy.

9. Recommendations for Health Care Providers

Based on the environmental scan, clinical consensus recommendations and the economic evaluation, the following recommendations are put forward:

- High resolution flow-based technologies are endorsed as the optimal standard of care in all histocompatibility laboratories supporting solid organ transplantation in Canada.
- Funding for high resolution flow-based technologies is to be provided by the provinces via the regional health authorities or hospitals. Beyond the up-front establishment costs, consideration should be given to a linkage of budgets so that savings can be used to fund additional lab testing (i.e., cost savings from dialysis linked to cost increase in the lab testing budget).

Furthermore, the CCDT Consensus Forum has charged the CCDT with the following tasks, which are on the CCDT work plan for 2005–2007:

- CCDT is to explore the logistics and cost associated with the establishment of a national high-risk patient registry to optimize and improve equity of access to deceased donor organs for highly sensitized patients.
- CCDT is to explore the possibility of a national paired living kidney donor exchange registry for patients who have living donors that cannot donate to their relative because of a positive HLA crossmatch or ABO incompatibility.
- CCDT is to communicate the Clinical Forum Recommendations to the CSA Transplantation Committee for review and possible implementation as amendment to the CSA Z900.2.3-03 standards for "Perfusable Organs for Transplantation."

Appendices

Appendix 1: CCDT Conference Steering Committee and FRG Members

Dr. David Hollomby	CCDT Liaison and Chair CCDT Transplantation Committee
Dr. Peter Nickerson (Chair)	Director, Immunogenetics Laboratory, Canadian Blood Services, Winnipeg
	Board Member, Canadian Society of Transplantation
	President-Elect, American Society of Histocompatibility and Immunogenetics
Dr. Anthony Jevnikar (Co-Chair)	Director, Renal Transplant Program, London, Ontario Past-President Canadian Society of Transplantation
Dr. Patricia Campbell	Director, HLA Laboratory, Edmonton, Alberta
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Dr. Tom Waddell	Director, Lung Transplant Program, Toronto, Ontario
Ms. Corinne Weernink	President of CAT, Transplant Coordinator, London, Ontario
Dr. Lori West	Director, Pediatric Heart Transplant Program, Toronto, Ontario
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CCDT Conference Support Group

John Gelder	Gelder, Gingras & Assoc.	Facilitator
Nancy Greene	GCSI	Administration
Kim Liss	CCDT	Project Manager

Appendix 2: CCDT Logistics Group Members

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	Chair, Canadian Society of Transplantation (CST) working group for national database development
	Assistant Professor of Medicine, University of British Columbia
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Frank Markel, Ph.D.	President and CEO, Ontario Trillium Gift of Life
Keith McConnell, MBA	Administrative Director, Winnipeg Regional Health Authority (WRHA) Laboratory Medicine Program
Kevin McLaughlin, MD, FRCPC	Assistant Professor of Medicine, University of Calgary
Darren Praznik, LLB	Executive Director, Governmental Affairs, Canadian Blood Services (CBS)

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Appendix 4: Conference Agenda

Friday, January 28, 2005

1600 - 1800 Registration

1800 Forum Opening

- **Welcome** - Dr. David Hollomby
- **Process of the Forum** - John Gelder
- **Overview** - Dr. Peter Nickerson
- **Survey Results** - Dr. Patricia Campbell
- **Methods of HLA Testing** - Dr. Howard Gebel

1930 Reception

2000 Dinner

Saturday, January 29, 2005

0730 - 0800 Breakfast

0800 Part I - Transplant Immunologic Risk Assessment

- **Evidence for Impact of Methods on Outcomes and Experience with Technologies: Kidney** - Dr. Howard Gebel
- **Evidence for Impact of Methods on Outcomes: Heart and Lung** - Dr. Adriana Zeevi
- **Questions and Answers**

0930 Break

0950 Part I - Breakout Sessions

1200 Lunch
Forum Recommendations Group (FRG) - Lunch

1300 Part II - Access to Organs for Patients at Immunologic Risk

- **Experience Transplanting at risk patients**
    - Kidney: Low levels of sensitization - Dr. James Gloor
    - Kidney: High levels of sensitization - Dr. Robert Montgomery
    - Lung - Dr. Duane Davis
    - Heart - Dr. Lori West

1500 Break

1520 Part II - Breakout Sessions

1730 Closing - Free Evening

1730 Forum Recommendations Group (FRG) and FRG Group Dinner

Sunday, January 30, 2005

0800 - 0830 Breakfast

0830 - 1030 Part III - Future Directions

- **Preliminary Report of FRG**
- **Toronto Experience Establishing Paired Donor Exchange**
  - Dr. Edward Cole
- **Part III - Breakout Session**

1030 - 1045 Break

1045 - 1145 Part IV - Implementation Challenges

- **Economic Impact of High Resolution Testing**
  - Dr. Kevin McLaughlin
- **Preliminary Report of the Logistics Group**
  - Dr. Peter Nickerson

1145 - 1200 Forum Wrap-up

1300 - 1500 FRG meeting

Appendix 5: Survey of Canadian Laboratories and Transplant Programs

Objectives

- An environmental scan of key stakeholders to:
  - Understand the current practices, beliefs and opinions related to HLA testing
  - Support the identification and development of best practices for pre-transplant immunological risk assessment

Scope

- Covered pre-transplant immunological risk assessment for:
  - Kidney
  - Heart
  - Lung
- Paediatric and Adult recipients

Methodology

- Survey developed by small steering committee and pre-tested with two volunteer respondents
- Sent by email and completed on web
- Distributed to a list of 82 key stakeholders across Canada
  - Lab directors
  - Medical/Surgical Transplant Program Directors
- Common questions for both lab and program directors
  - General info
  - Future plans
- Program Director specific questions
  - Risk assessment
- Lab Director specific questions
  - antibody screening and crossmatching
- Initial response rate was slow, primarily due to:
  - Very busy offices
  - Travel
- Follow up phone calls and a second letter sent to encourage higher response rate

Response Rate

- Responses were received from a total of 34 recipients
- Return rate = 41.5%
- 11 lab directors
- 22 program directors
- Responses were received from all regions of the country

Reports

- 2 Word Reports were developed:
- Responses x Lab Director
- Responses x Program Director x Type of Transplant Program
- In addition Excel spreadsheet containing raw data files for both Lab and Program Directors

General Observations

- Some participants filled in the whole survey (i.e., lab directors also completed program director sections and vice versa)
- Geographic representation was excellent
- Reasonable mix of lab and program directors
- However, only two respondents each from both the heart and the lung programs
- Most responses confirmed a variety of approaches being used for assessment and screening
- Many programs are planning to add or revise techniques within the next 6-18 months

Lab Directors

- 11 labs {Toronto, Montreal (2), Quebec City, Calgary, Saskatoon, Winnipeg, Edmonton, Halifax, Hamilton, London}

Program Directors

- Kidney n=18
- Heart n=2
- Lung n=

Sera Screening for anti-HLA antibodies

Screening for Class I antibodies

- 6/7 labs screen heart recipients
- 4/4 labs screen lung recipients
- 10/11 labs screen kidney recipient

Methods for Screening for Class I antibodies

CDC-NIH ext	=1
CDC-AHG + ELISA	=2
ELISA	=1
CDC AHG/NIH + FC	=2
CDC-AHG + ELISA + FC	=2
ELISA + FC	=1
FC	=1

Screening for Class II antibodies

- 5/7 labs screen heart recipients
- 3/4 labs screen lung recipients
- 8/11 labs screen kidney recipient

Methods of screening for class II antibodies

CDC-NIHext + ELISA	=1
ELISA	=1
ELISA + FC	=2
FC	=3
No method listed	=1

Frequency of screening-Heart

- 2 labs screen every 3 months
- 2 labs screen every 6 months
- The remaining 3 labs screen annually or at listing and at transplant
- Most commented that extra screening would be done after transfusion

Frequency of Screening-Lung

- One lab screens every 3 months
- One lab screens annually
- Two labs screen at time of listing
- All labs commented that extra screening would occur after sensitizing events

Frequency of Sera Screening-Kidney

- Six labs screen every 3 months
- Two labs screen every 6 months
- Two labs screen monthly when active
- One lab screens at listing
- Most labs indicated that extra screening would be done after sensitizing events

Lab Directors

- 8/10 labs indicated that they were defining specificities if antibodies are detected
- Methods for defining specificities

CDC-NIHext	=1
CDC-AHG +ELISA	=1
ELISA	=1
ELISA +FC	=1
CDC-NIH +FC	=1
CDC-AHG +FC	=2
FC	=1

T cell crossmatch (TCXM)

Heart

- Methods used for TCXM

CDC-NIH	=1
CDC-NIHext	=2
CDC-AHG	=4

One centre commented that sensitized individuals also had a flow crossmatch

Program Directors-Heart

- On the basis of which T-cell XM would you perform a transplant?

CDC method =1

ELISA =1

Lung

- Methods used for TCXM for lung transplants

CDC-NIH ext	=1
AHG-CDC	=2
FC	=1

One lab commented that sensitized individuals have a FC crossmatch

Program Directors-Lung

- On the basis of which T cell XM would you perform a transplant?

FC	=1
No response	=1

Method of TCXM-Kidney

	Deceased donor	Living Donor
CDC-NIHext	3	1
CDC-NIHext +AHG	2	
CDC-AHG	3	3
CDC-AHG + FC	3	5
CDC-NIH + FC		1
FC		1

- One lab indicated that the FCXM were usually done retrospectively and not routinely
- One lab commented that FCXM for living donor transplants are only done if high PRA or equivocal CDC-AHG

Program Directors-Kidney

- On the basis of which T cell XM result would you perform a kidney transplant? CDC ? Method =1

  CDC-NIH =1
  CDC-AHG =7
  CDC and FC =4
  FC =3
  No response =2

B-cell crossmatch (BCXM)

Program Directors-Heart

- Only 1 lab does BCXM for all heart transplants
- 1 lab indicated that all pediatric heart transplants have a BCXM
- 3 labs replied that it is done in situations of increased immunological risk
- Method for B-cell crossmatch (BCXM)

  CDC-NIH ext =2
  FC =1

Lab Directors-Lung

- 3/4 labs do not perform routine BCXM
- 2 labs indicated that they do BCXM in some circumstances

  CDC-NIH ext =1
  FC =1

Lab Directors-Kidney

- 6/11 labs perform a BCXM on all deceased donor kidney transplants
- Two labs reported that BCXM are only done if class II antibodies are detected on screening
- Method of BCXM

  CDC-NIHext =5
  CDC-NIH =1
  CDC-AHG =1
  FC =2

Lab Directors-Kidney

- 4 labs indicated that they would do BCXM by FC in living donors but not on deceased donors

Program Directors-Kidney

- On the basis of what B-cell XM result do you perform a transplant?

  CDC-method =1
  CDC-NIH =1
  CDC-AHG =3
  FC =5
  FC and CDC =2
  No B-cell XM =2
  No response =4

Program Directors-Kidney

- Only do BCXM if high immunological risk
- All living donors have BCXM, low risk deceased donor recipient only have TCXM
- If ELISA PRA neg will proceed
- Used in risk stratification not allocation

Transplantation across a Positive BCXM

Positive BCXM

- Both the lung and heart program directors indicated that a positive BCXM is not a barrier to transplant

Positive BCXM-Kidney

- 13/16 directors that responded indicated that they would perform some kidney transplants with a positive BCXM
- 7 indicated that this would be if the FCXM was positive
- 4 indicated they would transplant across a CDC-NIH positive crossmatch

Transplantation across a Positive TCXM

Program Directors-Heart

- Prospective TCXM if high risk
- One director indicated that a remote positive would influence decision to proceed
- One program indicated that it would proceed if the TCXM was positive in an emergency situation

Program directors-Lung

- One program indicated that they would proceed with a positive TCXM by FC
- Neither program would be influenced by a remote positive crossmatch

Program Directors-Kidney

- 4/17 directors that responded indicated that they would perform a kidney transplant with a positive TCXM
- All the responders that answered yes indicated that this would be in the case of a positive FCXM
- 8/16 labs replied that a remote positive crossmatch would influence their decision to proceed.

Sera and Cell Selection for Crossmatching

Lab Directors-Heart

- 4/7 labs perform a final crossmatch. 3/4 use sera from the day of transplant
- 4/7 labs use a peak sera
- 6/7 labs use peripheral blood for crossmatching
- 1 lab uses only spleen and 3 use both
- These crossmatches are usually retrospective

Lab Directors-Lung

- 3/4 labs do a final crossmatch using sera from the day of transplant
- 2/4 labs crossmatch with peak sera
- 2/4 labs use spleen cells for crossmatching
- 1 lab uses peripheral blood and one uses both

Lab Directors-Kidney

- All labs use peripheral blood for crossmatching
- 7/11 labs also use spleen
- 9/11 labs do a final crossmatch
- 5/11 labs use the most recent sera

- 5/11 labs use the sera from the day of transplant
- 8/11 labs use peak sera

Post-transplant monitoring

Lab Directors-Lung

- Only one lab indicated that any post-transplant monitoring was done in lung transplants and this was usually after an intervention and not routinely

Lab Directors-Heart

- Only one lab indicated that routine post-transplant monitoring is performed at 1,2,4 weeks and annually by FC
- Two other labs indicated screening as required

Lab Directors-Kidney

- 4/11 labs indicated that post-transplant monitoring was performed
- Only 2 labs reported a screening schedule
- One lab commented that they didn't do routine monitoring but only if clinically indicated

Lab Directors-Kidney

- Methods for post-transplant monitoring

CDC-NIH	=1
CDC-AHG + ELISA	=1
ELISA	=1
CDC-AHG + FC	=2

Program Directors

- Does your program have written policies or procedures regarding assessment of patient risk and when transplants should or should not be performed?

Yes	= 7
No	=13
No response	= 2

- If resources were not limited what methods would you preferentially use for antibody screening?

FC alone	=15
FC and ELISA	= 2
ELISA	= 2
CDC and ELISA	= 1
CDC and FC	= 1
CDC, ELISA and FC	= 1

- If resources were not limited what methods would you preferentially use for crossmatching?

FC	=12
CDC-AHG and FC	=5
CDC and ELISA	=1
ELISA	=1
CDC-AHG	=1
CDC-NIH	=1
CDC?method	=1

Summary

- At present there is a variety of assays being used to screen for anti-HLA antibodies and to perform crossmatching
- The range of testing tended to reflect the availability of testing at a particular centre rather than the choice of the programs and did not vary largely between different organ groups
- All directors would like to perform antibody screening by solid phase assays either alone or in addition to CDC methods
- Majority would like FC crossmatches to be done
- Most indicated a plan to introduce antibody screening by solid phase (mostly flow) and to introduce or increase the availability of flow crossmatches (i.e., from LD to all deceased donors)

Appendix 6: Pre-meeting Reading

Published Papers

Gebel HM, Bray RA, Nickerson P (2003). Pre-Transplant Assessment of Donor-Reactive, HLA-Specific Antibodies in Renal Transplantation: Contraindication vs. Risk. American Journal of Transplantation 2003; 3:1488-1500.

Reinsmoen NL, Nelson K, Zeevi A (2004). Anti-HLA antibody analysis and crossmatching in heart and lung transplantation. Transplant Immunology 2004; 13:63-71.

Takemoto SK, Zeevi A, Feng S, Colvin RB, Jordan S, Kobashigawa J, Kupiec-Weglinski J, Matas A, Montgomery R, Nickerson P, Platt JL, Rabb H, Thistlethwaite R, Tyan D, Delmonico FL (2004). National Conference to Assess Antibody-Mediated Rejection in Solid Organ Transplant. American Journal of Transplantation 2004; 4:1003-1041.

Management of Antibodies in Renal Transplantation - Overview

A. Definitions

Antibody mediated rejection (AMR)

- is acute (24 hours)
- involves various antibodies (HLA, ABO, anti endothelial) but primary concern is HLA
- can be predicted by risk pre-transplant events (sensitizing event such as transfusion, pregnancy and previous transplants), and by various methods of pre-transplant antibody detection, including B-cell XM (*Gebel et al., AJT 2003*)
- largest risk of HLA antibody formation may be with blood transfusions given at the time of transplant nephrectomy (10% risk of sensitization with previous transplant alone rises to 80%), or pregnancy (5% rises to 50%).
- antibody levels can modulate over time.

(Glotz et al., Trans Int 17, 2004)

In summary:

- pre-transplant risk assessment is essential for diagnosis of clinical AMR.
- avoidance of transfusions remains important
- monitoring for the development of antibodies after returning to dialysis is important.

B. Stratification of renal AMR risk with type of assay

Note: as CDC techniques are variable, a current or remote negative by CDC alone does not confer low risk for AMR ***(Takemoto et al, AJT 4, 2004)***

Presence of classical T cell, IgG XM is absolute contra-indication to renal transplant, and everything else may be relative with increasing risk of rejection and graft loss *(Montgomery et al, Transplantation, 78, 2004, Glotz et al., Trans Int 17, 2004).*

Table 1: Proposed kidney risk assessment for humoral rejection and early graft loss

	Contraindicated	High[1]	Intermediate[2]	Low[3]
Current positive CXM				
Direct CDC non-reducible	•			
Direct CDC modifiable		•		
AHG CDC		•	•	
Flow crossmatch				
Remote positive CXM				
Direct CDC			•	
AHG CDC			•	
Flow crossmatch			•	
Current and remote negative CXM				
Direct CDC			•[4]	
AHG CDC				•
Flow crossmatch				•

1 Minimally requires pretransplant intervention and post-treatment/transplant monitoring.
2 May require augmented immunosuppression and/or post-transplant monitoring.
3 Conventional therapy may be used.
4 See text.

Table from Takemoto et al, AJT 4, 2004

C. Pre-Transplant antibody reduction

C1. Plasmapheresis - no consensus on several factors:

- Volume (1-1.5 plasma volumes)
- Replacement type (usually albumin)
- Frequency (daily vs. q2 days)
- Number (titre dependent)
- Timing (delayed for 24-48 hours post IVIG)
- Endpoints (clinical vs. circulating DSA. *Not* well monitored using C4d in biopsies)

C2. IVIG

- Preparations (standard IVIG vs. CMV-HIG), but numerous manufacturers
- Sucrose based preparations nephrotoxicity in high concentrations not predictable
- "Most" IVIG contain 97% IgG, traces of IgA, IgM, aggregation can occur in high concentrations unless treated, may contain trace HLA, multiple mechanism of action against DSA

(Glotz et al, Trans Int 17, 2004)

Two main strategies for use:

a. Low dose 100 mg/kg, usually CMV-HIG, used with PLEX to remove unbound Ab

One advantage is that one can use any form of DSA monitoring (no interference with flow, ELISA, AHG) *(Montgomery et al, Transplantation 70, 2000, Warren et al AJT 4, 2004, Zachary et al 76, 2003)*

Plasmapharesis/low-dose CMVIg (100mg/kg) (34-36)

Protocol

QOD plasmapharesis (PP): one volume exchange replaced with albumin or FPP

CMVIG: 100 mg/kg following each PP

PreTx: Tacrolimus, MMF started with 1st PP/ICMVIg

Steroids and Daclizumab added at transplant

For ABO-incompatible recipient or high-risk CXM positive recipient – laparascopic splenectomy or anti-CD20 PP/CMVIg continued post-transplantation (3-6 QOD treatments)

Endpoint of therapy

For Anti-HLA antibody: Negative AHG CDC crossmatch

For ABO incompatibility: Isoagglutinin titer <= 1:16

Mechanism

Rapid reduction in anti-HLA or isoagglutinin Ab

Ab reduction allows immunomodulation at a lower Ig dose

Induces donor-specific unresponsiveness (HLA) or accommodation (ABOI)

Advantages

Predictable kinetics of plasmapharesis

No evidence on 'nonresponders', works for high titer DSA

Able to easily follow DSA levels during/after therapy

Disadvantages

Rebound occurs unless the transplant immediately follows pre-conditioning–not currently appropriate for patients waiting for a deceased donor transplant

Expensive and resource intensive

Probably more immunosuppressive

From Zachary et al, Transplantation 76, 2003

- 49 patients, antibody titres ranged for CDC XM, from 1-4096
- Mean PLEX numbers were 7±6
- 63% of patients lost donor HLA antibody, long lasting (13 months or greater), and not clearly related to original titre
- No elimination of anti viral (CMV, EBV) antibodies noted
- 59% had DSA at transplant with most being Flow XM= (20/29) with rejection rate of approx 40%

From Gloor et al AJT 3, 2003

- In LD transplants, 11/14 grafts functioning with PLEX, IVIg, anti CD20, splenectomy
- 2 grafts lost with accelerated vasculopathy
- PRA was 54%
- At time of transplant all had a neg T cell AHG XM
- At time of transplant 67% (8/12) had a positive T cell Flow crossmatch
- Baseline titre was < 1:4 in 71% and all had titre of less than 1:16
- AMR in 44% was reversible and occurred in those with high initial titres.

b. High dose (2 gm/kg), IVIg

May neutralize non HLA antibodies as well as HLA Ab, maximum dose to 140 gms

Can use CDC monitoring, in an inhibition assay, to monitor effect on all DSA. More difficult to use solid phase binding assay with secondary antibody as there is background (*Glotz et al., AJT 2, 2002, Jordan et al., Transplantation 66, 1998) Donor Specific Ab can be followed if it is of a reasonable titre with flow beads. Background attenuates with time after the IVIG (especially after 5-7 days) (Peter Nickerson, personal communication).*

High dose IVIG (1-2 g/kg) (32, 33)

Protocol

- In vitro PRA test to identify patients most likely to benefit from IVG therapy (45)
- Responders started on IVIG 2 g/kg on HD over 4 h
- Monthly x 4 doses
- Immunosuppression starts at time of transplant
- Transplantation with deceased donor kidney
- For live donors 1-4 doses – repeat crossmatch after each dose

Endpoint of therapy

- Negative enhanced CDC crossmatch

Mechanism

- Many putative immunomodulatory pathways identified
- Anti-idiotypic networks probably important (40)

Advantages

- Can be used to desensitize patients on the waiting list
- Less rebound in absence of donor antigen
- Less expensive than plasmapharesis
- Ease of administration

Disadvantages

- Nonresponders
- Need different techniques to follow DSA titers
- Less rapid Ab removal, unproven for high-titer DSA
- Toxicity and batch-to-batch variability

From Jordan et al Transplantation 76, 2003

- 40% had PRA > 50%
- 35 of 42 patients abrogated CDC-XM, most with single dose of 2 gm / kg
- Remaining 7 were Flow XM +
- 31 % developed rejection and total of 7% lost
- Graft survival of 89% at 2 years

Note: More recent paper quotes a graft loss of 25% in adherent pts at 30 months. 9/17 developed AR. *Jordan JASN 15:3256-62 2004*

D. Drug therapy

- CNI of choice?
- Anti-proliferatives - both MMF and Imuran used, cyclophosphamide historically **much** worse when used with PLEX in AMR
- Steroids

D1. Rituximab (anti CD20 mAb) *(Warren et al. AJT 4, 2004)*

- Does *not* target plasma cells, only B cells
- Not sufficient data to determine role in acute AMR

Anti-CD20 (35)

Mechanism

Rapid and durable ablation of the B-cell compartment

Advantages

Probably reduces precursor cells responsible for clonal expansion during AMR

May produce more effective antibody reduction when combined with plasmapharesis or IVIG

From Becker et al, AMJ 4, 2004

- High density of CD20+ cells found in steroid resistant rejection
- In case series, 24/27 patients with features of AMR responded to anti CD20, ALG, PLEX and steroids *without* ALG.

E. Post-operative management

E1. Clinical and pathological criteria for AMR

- AMR = 5-7% of patients and 12-37% of acute rejection biopsies
- Diagnosis of AMR = graft dysfunction + PMN/macrophage/thrombi ± fibrinoid necrosis ± tubular injury + C4d in PTC or Ig/C3 in arteries **+ anti-donor antibody at time of biopsy** *(Takemoto et al., AJT 4, 2004)*
- C4d positive in biopsy = circulating antibody with crossmatch positive post-transplant in 78-90% of patients) *(Bohmig & Regele Trans Int 16, 2003)*
- C4d mAb sources from Quidel (San Diego), or Biogenesis (Sandown, NH) can be used on frozen sections only, source for antibody for use on embedded sections?
- Prevalence in stable transplants is likely low, but not well studied yet
- C4d is not positive in kidney ischemia, cold storage, ATN *(Bohmig and Regele Trans Int 16, 2003)*
- C4d may remain positive for 8-22 days post loss of circulating Ab *(R. Colvin personal communication)*
- Poor outcome occurs in AMR without therapy

  1 year graft survival of 60% in C4d+ vs. C4d- patients

  Mean graft survival of 4 years vs. 8 years *(Feucht et al., Clin Exp Imm 86, 1991, Lederer et al., Kid Int 16, 2001)*

In the acute situation this is well accepted. In late biopsies this is less clear as there is less data.

- Outcome is much improved with therapy, including desensitization with IVIG, along with ALG, CNI, anti-proliferative pre-transplant is 95% at 2 years, in highly sensitized patients, (n=21) in deceased donor transplants *(Glotz et al., Trans Int 17, 2004)*. Impact of newer forms of therapy along with PLEX/IVIG not clear. Long-term data remains lacking.

Table 1: Positive crossmatch kidney transplant protocol

Pre transplant

- Plasmapharesis transplant days -4, -3, -1, 0
- IVIG 100 mg/kg/body weight after each plasmapharesis
- Crossmatch negative on day of transplant
- Splenectomy at time of transplant (if not already done)
- Rituximab 375 mg/m^2 transplant day -4
- MMF started transplant day -4

Post transplant
Plasmapharesis post operative day 1, 3
IVIG 100 mg/kg body weight after each plasmapharesis
Thymoglobulin indication
Maintenance immunosuppression with Tacrolimus, MMG, corticosteroids
Surveillance allograft biopsies post operative days 0, 3, 7, 4, 28, 90
IVIG = Intravenous immunoglobulin;
MMF=Mycophenolate Mofetil.

Table from Gloor et al, AJT 3, 2003

- The nature of antibodies in sensitized renal transplant recipients.

From Diaz et al., Clin Trans 18, 2004

- Pure IgM is rare; most are mixed (pattern 3) or pure IgG (pattern 4). IgM detection by addition of DTT. Pure class II Ab and non HLA are rare, most are mixed class I and class II HLA

Isotype pattern					
n	PRA (%)	Pattern 1 IgM[a] (%)	Pattern 2 IgG and IgM[b] (%)	Pattern 3 IgG and IgM[c] (%)	Pattern 4 IgM[d] (%)
14	>10 and <= 50	0 (0)	2 (14)	4 (29)	8 (57)
22	>50 and <= 80	0 (0)	2 (9)	5 (23)	15 (68)
23	>80	2 (9)	5 (22)	0 (0)	16 (69)
		2 (3)	9 (15)	9 (15)	39 (66)

Class of antibodies					
n	PRA (%)	HLA I[a] (%)	HLA I and HLA II[b] (%)	HLA II[c] (%)	Non-HLA[d] (%)
14	>10 and <= 50	3 (21)	10 (72)	0 (0)	1 (7)
22	>50 and <= 80	1 (4)	21 (96)	0 (0)	0 (0)
23	>80	5 (22)	16 (69)	0 (0)	2 (9)
		9 (15)	47 (80)	0 (0)	3 (5)

- The range of acute rejection is quite wide even with similar protocols.

From Jordan et al, AJT 3, 2003

Table 3: Summary of protocols involving immune globulin for sensitized patients

Center	John Hopkins (49)	University of Maryland (50)	Hopital Europeen Georges Pompidou (51)	Cedars-Sinai (52)
Desensitization protocol	PP/IVIG until crossmatch (-) MP 500 mg/day x 3 doses	MMF prior to PP, IVIG 500 mg/kg over 7 days, TAC, and CS started with first PP treatment, PP TIW x 2 weeks before transplant	MG 2 g/kg over 48h x 3 doses at 4-week intervals	MG 2 g/kg (140g max) x 1 dose for LD MG 2 g/kg (140g max) x 4 doses for CAD
No. of patients treated	4	15	15	48
No. of patients transplanted	4	11	13	44 (16 CAD, 28 LD)
Acute rejection (%)	100%	36.4%	7.7%	29%
Graft Loss (%)	0%	0%	15.4% (2)	6.8% (3)
Infection/malignancy (%)	N/A	9.1%	23.1%	0%
Follow-up period (months)	10 (4.3-17)	13.3 +- 2.4 (3-26)	12	24
Serum creatinine (mg/dL)	1.0 (0.8-1.2)	1.6 +- 0.2 (1.1-2.4)	N/A	1.4 + 0.5 (0.4-2.0)

LD= live donor; TAC=tacrolimus; CAD=cadaveric; CS=corticosteroids; PP=plasmapharesis; TIW=three times per week; IVIG= intravenous immune globulin; MP=methylprednisolone

- The cost is high.

From Jordan et al, AJT 3, 2003

Table 7: Patient cost comparison			
		ESRD patients without antibody lowering therapy	
	Patients transplanted with antibody lowering therapy	PRA > 30%	Blood type O
Wait time on UNOS list (years)	1	5.1	4.3
Per patient cost during wait time (dialysis, inpatient, outpatient, etc.)	$53 412	$269 731	$229 672
Transplant care ($17091/year for 36 months)	$51 273	$51 273	$51 273
Antibody lower protocol (avg)[1]	$35 540	-	-
Total cost per patient	$140 225	$321 004	$280 945
[1] John Hopkins costs; each institution should substitute center cost based on Table 4.			

Patient type	Ab treatment	Ab rejection rate	Outcome (2 yr)	references
High PRA, DD				
CDC XM+	NA	Contra-indicated	NA	
AHG XM+				
LD	Low dose IVIG/PLEX ± anti CD20	40-50 %	80%	*Montgomery et al, Transplantation 70, 2000, Warren et al AJT 4, 2004, Zachary et al 76, 2003*
LD	High dose IVIG ± anti CD20	40-50 %	80-90%	*Jordan et al Transplantation 76, 2003* *Glotz et al, AJT 2, 2002*
DD	High dose IVIG ± anti CD20	Not known	NA	
Flow XM +, AHG XM-	IVIG or IVIG/PLEX	Not known	NA	ATC abstracts only

Note: In transplants that proceeded, crossmatches were AHG positive *pre-desensitization* rather than at the time of transplant. Generally transplants proceed only if the crossmatch became CDC-AHG or NIH negative, but the management of Flow XM positive, AHG-XM negatives varies between centres.

Appendix 7: References

Reviews on Donor-Recipient Histocompatibility

- Braun WE. Laboratory and clinical management of the highly sensitized organ transplant recipient. Human Immunology 1989 26:245-260.
- Scornik JC. Detection of alloantibodies by flow cytometry: relevance to clinical transplantation. Cytometry 1995 22:259-263.
- Gebel HM, Bray RA, Nickerson P. Pre-transplant assessment of donor-reactive, HLA-specific antibodies in renal transplantation: Contraindication vs. risk. Am J Transplantation 2003 3:1488-1500.
- Reinsmoen NL, Nelson KN, Zeevi A. Anti-HLA antibody analysis and crossmatching in heart and lung transplantation. Transplant Immunology 2004 13:63-61.

CDC Crossmatch

- Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. N Engl J Med 1969 280:735-739.
- Belleil O, Mickey M, Terasaki P. Comparison of male and female kidney transplantation survival rates. Transplantation 1972 13:493-500.
- Iwaki Y, Terasaki PI. Primary non-function in human cadaver kidney transplantation: evidence for hidden hyperacute rejection. Clin Transplant 1987 1:125.

Enhanced CDC Crossmatch

- Amos DB, Bashir H, Bogle W, et al. A simple microtoxicity test. Transplantation 1970 7:220.
- Johnson AH, Rossen RD, Butler WT. Detection of alloantibodies using a sensitive antiglobulin microcytotoxicity test: identification of low levels of preformed antibodies in accelerated allograft rejection. Tissue Antigens 1972 2:215.
- Ting A, Hasegawa T, Ferrone 5, Reisfeld RA: Presensitization detected by sensitive crossmatch tests. Transplant Proc 1973 5:813.
- Cross DE, Whittier FC, Weaver P, Foxworth J. A comparison of the antiglobulin versus extended incubation time crossmatch results in 223 renal transplants. Transplant Proc 1977 9:1803-1806.
- Fuller TC, Cosimi AB, Russell PS: Use of an antiglobulin-ATG reagent for detection low levels of alloantibody-improvement of allograft survival in presensitized recipients. Transplant Proc 1978 10:463-466.
- Fuller T, Phelan D, Gebel H, Rodey C: The antigenic specificity of antibody reactive in the antiglobulin-augmented lymphocyte-toxicity test. Transplantation 1982 34:24-29.
- Kerman RH, Kimball PM, Van Buren CT, et al. AHG and DTE/AHG procedure identification of cross-match-appropriate donor-recipient pairings that result in improved graft survival. Transplantation 1991 51:316-320.

IgA, IgM and Autoantibodies

- Cross DE, Greiner R, Whittier FC. Importance of the autocontrol crossmatch in human renal transplantation. Transplantation 1976 21:307-311.
- Ting A, Morris PJ. Renal transplantation and B-cell crossmatches with autoantibodies and alloantibodies. Lancet 1977 2:1095-1097.
- Ettenger RB, Jordan SC, Fine RN. Cadaver renal transplant outcome in recipients with autolymphocytotoxic antibodies. Transplantation 1983 35:429-431.
- Pettaway CA, Freeman CC, Helderman HJ, Stastny P. Kidney transplant recipients with long incubation-positive, antiglobulin-negative T-cell cross-matches. Transplantation 1987 44:529-533.
- Chapman JR. Taylor CJ, Ting A, Morris PJ: Immunoglobulin class and specificity antibodies causing positive T cell crossmatches: Relationship to renal transplant outcome. Transplantation 1986 42:608-613.
- Iwaki Y, Lau M, Terasaki PI. Successful transplants across T warm-positive crossmatches due to IgM antibodies. Clin Transplant 1988: 81-84.
- Taylor CJ, Chapman JR. Ting A, Morris PJ: Characterization of lymphocytotoxic antibodies causing a positive crossmatch in renal transplantation. Relationship to primary and regraft outcome. Transplantation 1989 48:953-958.
- Barocci S, Valente U, Gusmano R, et al. Autoreactive lymphocytotoxic IgM antibodies in highly sensitized dialysis patients waiting for a kidney transplant: identification and clinical relevance. Clin Nephrol 1991 36:12-20.
- Vaidya S, Ruth J. Contributions and clinical significance of IgM and autoantibodies in highly sensitized renal allograft recipients. Transplantation 1989 47:956-958.
- Barger B, Shroyer TW, Hudson SL, et al. Successful renal allografts in recipients with cross-match-positive, dithioerythritol-treated negative sera. Transplantation 1989 47:240-244.
- McCalmon RT, Spees EK, Tardif GN, et al. Successful kidney transplantation in the presence of IgM alloantibodies. Clin Transplantation 1991 5:255-259.
- Roelen DL, van Bree J, Witvliet MD, et al. IgG antibodies against an HLA antigen are associated with activated cytotoxic T cells against this antigen, IgM are not. Transplantation 1994 57:1388-1392.
- McCalmon RT, Tardif GN, Sheehan MA, et al. IgM antibodies in renal transplantation. Clinical Transplantation 1997 11:558-564.
- Doran TJ, Susal C, Opelz G, Geczy AF. IgA class antibodies and flow cytometric cross-matching in renal transplantation. Transplantation 1999 67:309-314.
- Bryan CF, Martinez J, Muruve N, et al. IgM antibodies identified by a DTT-ameliorated positive cross-match do not influence renal graft outcome but the strength of the IgM lymphocytotoxicity is associated with DR phenotype. Clinical Transplantation 2001 15 Suppl 6:28-35.

B-Cell Crossmatch

- Pellegrino M, Belvedere M, Pellegrino AG, Ferrone S. B peripheral lymphocytes express more HLA antigens than T peripheral lymphocytes. Transplantation 1978 25:93-95.
- Ettinger RB, Terasaki PI, Opelz G, et al. Successful renal allografts across a positive cross-match for donor B-lymphocyte alloantigens. Lancet 1976 1:56-58.

- Ting A, Morris PJ. Renal transplantation and B-cell crossmatches with autoantibodies and alloantibodies. Lancet 1977 2:1095-1097.
- Ayoub G, Park MS, Terasaki PI, Opelz G. B cell antibodies and crossmatching. Transplantation 1980 29:227-229.
- Jeannet M, Benzonana G, Arni I. Donor specific B and T lymphocyte antibodies and kidney graft survival. Transplantation 1981 31:160-163.
- Ladza VA, Pollack R, Mozes MF, Jonasson O. Positive B cell crossmatches in highly sensitized patients – influence of antibody specificity on renal transplant outcome. Transplant Proc. 1987 19:782-784.
- Noreen HJ, van der Hagen E, Bach FH, et al. Renal allograft survival in CSA-treated patients with positive donor-specific B cell crossmatches. Transplant Proc 1989 21:691-692.
- Phelan DL, Rodey GE, Flye MW, et al. Positive B cell crossmatches: specificity of antibody and graft outcome. Transplant Proc 1989 21:687-688.
- Taylor CJ, Chapman JR. Ting A, Morris PJ: Characterization of lymphocytotoxic antibodies causing a positive crossmatch in renal transplantation. Relationship to primary and regraft outcome. Transplantation 1989 48:953-958.
- Karuppan SS, Lindholm A, Moller E. Characterization and significance of donor-reactive B cell antibodies in current sera of kidney transplant patients. Transplantation 1990 49: 510-515.
- Scornik JC, LeFor WM, Cicciarelli JC, et al. Hyperacute and acute kidney graft rejection due to antibodies against B cells. Transplantation 1992 54:61-64.
- ten Hoor GM, Coopmans M, Allebes WA. Specificity and Ig Class of preformed alloantibodies causing a positive crossmatch in renal transplantation. Transplantation 1993 56:298-304.
- Ladza VA. Identification of patients at risk for inferior renal allograft outcome by a strongly positive B cell flow cytometry crossmatch. Transplantation 1994 57:964-969.
- Ghasemian SR, Light JA, Currier CB, et al. The significance of the IgG anti-B-cell crossmatch on renal transplantation outcome. Clin Transplant 1997 11:485-487.
- Bittencourt MC, Rebibou J-M, Saint-Hillier Y, et al. Impaired renal graft survival after a positive B-cell flow-cytometry crossmatch. Nephrol Dial Transplant 1998 13:2059-2064.
- Lobashevsky AL, Senkbeil RW, Shoaf J, Mink C, Rowe C, Lobashevsky ES, Burke R, Hudson S, Deierhoi MH, Thomas JM. Specificity of preformed alloantibodies causing B cell positive flow crossmatch in renal transplantation. Clin Transplant. 2000 14:533-542.
- Mahoney RJ, Taranto S, Edwards E. B-cell crossmatching and kidney allograft outcome in 9031 United States transplant recipients. Human Immunology 2002 63:324-335.

Flow Crossmatch

- Garovoy MR, Rheinschmilt MA, Bigos M, et al. Flow cytometry analysis: A high technology crossmatch technique facilitating transplantation. Transplant Proc. 1983 15:1939-1944.
- Chapman JR, Deierhoi MH, Carter NP, et al. Analysis of flow cytometry and cytotoxicity crossmatches in renal transplantation. Transplant Proc 1985 17:2480-2481.
- Bray RA, Lebeck LK, Gebel HM. The flow cytometric crossmatch: dual color analysis of T cell and B cell reactivities. Transplantation 1989 48:834-840.
- Scornik JC, Brunson ME, Schaub B, et al. The crossmatch in renal transplantation. Transplantation 1994 57:621-625.

- Scornik JC, Clapp W, Patton PR, Van der Werf WJ, Hemming AW, Reed AI, Howard RJ. Outcome of kidney transplants in patients known to be flow cytometry crossmatch positive. Transplantation. 2001 71:1098-1102.
- Vaidya S, Cooper TY, Avandsalehi J, et al. Improved flow cytometric detection of HLA alloantibodies using pronase. Transplantation 2001 71:422-428.

PRA (Serology, ELISA, and Flow)

- Pei R, Wang G, Tarsitani C, Rojo S, Chen T, Takemura S, Liu A, Lee J. Simultaneous HLA Class I and Class II antibodies screening with flow cytometry. Hum Immunol 1998 5:313-322.
- Gebel HM, Bray RA. Sensitization and sensitivity: defining the unsensitized patient. Transplantation 2000 69:1370-1374.
- Zachary AA, Ratner LE, Graziani JA, et al. Characterization of HLA Class I specific antibodies by ELISA using solubilized antigen targets: II. Clinical relevance. Human Immunology 2001 62:236-246.
- Pei R, Lee J-H, Shih N-J, Chen M, Terasaki PI. Single human leukocyte antigen flow cytometry beads for accurate identification of human leukocyte antibody specificities. Transplantation 2003 75:43-49.

Serologic vs. Flow Crossmatch in Renal Transplantation

- Thistlethwaite JR, Buckingham MR, Stuart JK, et al. T cell immunofluorescence flow cross-match results in cadaver donor renal transplantation. Transplant Proc 1987 19:722-724.
- Iwaki Y, Cook DJ, Terasaki P, et al. Flow cytometry crossmatching in human cadaver kidney transplantation. Transplant Proc 1987 19:764-766.
- Cook DJ, Terasaki PI, Iwaki Y, et al. An approach to reducing early kidney transplant failure by flow cytometry crossmatching. Clin Transplant 1987 1:253-256.
- Lazda VA, Pollak R, Mozes MF, Jonasson O. The relationship between flow cytometer crossmatch results and subsequent rejection episodes in cadaver renal allograft recipients. Transplantation 1988 45:562-565.
- Talbot D, Givan AL, Shenton BK, et al. The relevance of a more sensitive crossmatch assay to renal transplantation. Transplantation 1989 47:552-555.
- Mahoney RJ, Ault KA, Given SR. et al: The flow cytometric crossmatch and early renal transplantation. Transplantation 1990 49:527-535.
- Berteli AJ, Daniel V, Mohring K, et al. Association of kidney graft failure with a positive flow cytometric crossmatch. Clin Transplantation 1992 6:31-34.
- Ogura K, Terasaki PI, Johnson C, et al. The significance of a positive flow cytometric crossmatch test in primary renal transplantation. Transplantation 1993, 56:294-298.
- Christiaans MH, Overhof R, ten Haaft, et al. No advantage of flow cytometry crossmatch over complement-dependent cytotoxicity in immunologically well-documented renal allograft recipients. Transplantation 1996, 15:1341-1347.
- Mahoney RJ, Norman DJ, Colombe BW, et al. Identification of high-and low-risk second kidney grafts. Transplantation 1996 61:1349-1355.
- LeFor WM, Ackermann JRW, Alveranga DY, et al. Flow cytometry crossmatching and primary cadaver kidney graft outcome: relevance of T and B cell targets, historic sera and autologous controls. Clinical Transplantation 1996 10:601-606.

- Utzig MJ, Blumke M, Wolff-Vorbeck G, et al. Flow cytometry cross-match: a method for predicting graft rejection. Transplantation 1997 63:551-554.
- Pelletier RP, Orosz CG, Adams PW, et al. Clinical and economic impact of flow cytometry crossmatching in primary cadaveric kidney and simultaneous pancreas-kidney transplant recipients. Transplantation 1997 63:1639-1645.
- Kimball P, Rhodes C, King A, et al. Flow cross-matching identifies patients at risk for postoperative elaboration of cytotoxic antibodies. Transplantation 1998 65:444-446.
- Bryan CF, Baier KA, Nelson PW, et al. Long-term graft survival is improved in cadaveric renal retransplantation by flow cytometric crossmatching. Transplantation 1998 66:1827-1832.
- Kerman RH, Susskind B, Buyse I, Pryzbylowski P, Ruth J, Warnell S, Gruber SA, Katz S, Van Buren CT, Kahan BD. Flow cytometry-detected IgG is not a contraindication to renal transplantation: IgM may be beneficial to outcome. Transplantation. 1999, 68:1855-1858.
- El Fettouh HA, Cook DJ, Bishay E, Flechner S, Goldfarb D, Modlin C, Dennis V, Novick AC. Association between a positive flow cytometry crossmatch and the development of chronic rejection in primary renal transplantation. Urology. 2000 56:369-372.
- O'Rourke RW, Osorio RW, Freise CE, Lou CD, Garovoy MR, Bacchetti P, Ascher NL, Melzer JS, Roberts JP, Stock PG. Flow cytometry crossmatching as a predictor of acute rejection in sensitized recipients of cadaveric renal transplants. Clin Transplant. 2000, 14:167-173.
- Karpinski M, Rush DR, Jeffery J, et al. Flow cytometric crossmatching in primary renal transplant recipients with a negative anti-human globulin enhanced cytotoxicity crossmatch. J Am Soc Nephrol 2001 12:2807-2814.
- Cho YW, Cecka JM. Cross-match tests – an analysis of UNOS data from 1991-2000. Chapter 22 In Clinical Transplants 2001 p237-246. Cecka and Terasaki, Eds.

Remote Positive Current Negative Cross-match in Renal Transplants

- Cardella CJ, Falk JA, Nicholson MJ, et al: Successful renal transplantation in patient with T-cell reactivity to donor. Lancet 1982 2:1240-1243.
- Sanfilippo F, Vaughn WK, Spees EK, Bollinger RR. Cadaveric renal transplantation ignoring peak reactive sera in patients with markedly decreasing pretransplant sensitization. Transplantation 1984 38:119-124.
- Matas AJ, Nehlsen-Cannarella S, Tellis VA, Kuemmel P, et al. Successful kidney transplantation with current-sera-negative/historical-sera-positive T-cell crossmatch. Transplantation 1984 37:111-112.
- Rosenthal JT, Rabin B, Taylor RJ, et al. Positive T cell crossmatch with stored recipient sera in cadaveric renal transplantation. Transplantation 1985 39:310-311.
- Falk JA, Cardella CJ, Halloran P, et al. Transplantation can be performed with positive (noncurrent) cross-match. Transplant Proc 1985 17:1530-1532.
- Goeken NE: Outcome of renal transplantation following a positive crossmatch with historical sera: The ASHI survey. Human Immunology 1985 14:77-85.
- Kerman RH, Flechner SM, Van Buren ET, et al: Successful transplantation of cyclosporine-treated allograft recipients with serologically positive historical but negative preoperative donor crossmatches. Transplantation 1985 40:615-619.

- Turka L, Goguen JE, Gagne JE, Milford EL. Presensitization and the renal allograft recipient. Transplantation 1989 47:234-240.
- Taylor CJ, Chapman JR, Ting A, Morris PJ. Characterization of lymphocytotoxic antibodies causing a positive crossmatch in renal transplantation. Relationship to primary and regraft outcome. Transplantation 1989 48:953-958.
- ten Hoor GM, Coopmans M, Allebes WA. Specificity and Ig Class of preformed alloantibodies causing a positive crossmatch in renal transplantation. Transplantation 1993 56:298-304.
- Bryan CF, Shield CF, Warady BA, et al. Influence of an historically positive cross-match on cadaveric renal transplantation. Transplant Proc. 1999 31:225-227.
- Avlonitis VS, Chidambaram V, Manas DM, et al. The relevance of donor T cell-directed immunoglobulin G in historic sera in the age of flow cytometry. Transplantation 2000 70:1260-1263.
- Karpinski M, Rush DR, Jeffery J, et al. Flow cytometric crossmatching in primary renal transplant recipients with a negative anti-human globulin enhanced cytotoxicity crossmatch. J Am Soc Nephrol 2001 12:2807-2814.
- Baron C, Pastural M, Lang P, et al. Long-term kidney graft survival across a positive historic but negative current sensitized cross-match. Transplantation 2002 73:232-236.

Retransplant Renal Transplants

- Kerman RH, Flechner SM, Van Buren ET, et al: Successful transplantation of cyclosporine-treated allograft recipients with serologically positive historical but negative preoperative donor crossmatches. Transplantation 1985 40:615-619.
- Taylor CJ, Chapman JR, Ting A, Morris PJ. Characterization of lymphocytotoxic antibodies causing a positive crossmatch in renal transplantation. Relationship to primary and regraft outcome. Transplantation 1989 48:953-958.
- Kerman RH, Van Buren CT, Lewis RM, et al. Improved graft survival for flow cytometry and antihuman globulin cross-match-negative retransplant recipients. Transplantation 1990 49:52-56.
- ten Hoor GM, Coopmans M, Allebes WA. Specificity and Ig Class of preformed alloantibodies causing a positive crossmatch in renal transplantation. Transplantation 1993 56:298-304.
- Mahoney RJ, Norman DJ, Colombe BW, et al. Identification of high-and low-risk second kidney grafts. Transplantation 1996 61:1349-1355.
- Bryan CF, Baier KA, Nelson PW, et al. Long-term graft survival is improved in cadaveric renal transplantation by flow cytometric crossmatching. Transplantation 1998 66:1827-1832.

Antibody as a marker of CTL memory in Kidney Transplantation

- Roelen DL, van Bree J, Schanz U, et al. Differential inhibition of primed alloreactive CTLs in vitro by clinically used concentrations of cyclosporine and FK506. Transplantation 1993 56:190-195.
- Roelen DL, van Bree J, Witvliet MD, et al. IgG antibodies against an HLA antigen are associated with activated cytotoxic T cells against this antigen, IgM are not. Transplantation 1994 57:1388.
- Van Kampen CA, Roelen DL, Versteeg-van der Voort Maarschalk MFJ. Activated HLA class I-reactive cytotoxic T lymphocytes associated with a positive historical crossmatch predict early graft failure. Transplantation 2002 74:1114-1119.

Antiidiotypic Antibodies

- Reed E, Hardy M, Benvenisty A, et al. Effect of antiidiotypic antibodies to HLA on graft survival in renal allograft recipients. N Eng J Med 1987 316:1450.
- Phelan DL, Rodey GE, Anderson CB. The development and specificity of antiidiotypic antibodies in renal transplant recipients receiving single-donor blood transfusions. Transplantation 1989 48:57.

HLA localization in Kidney Tissue

- Natali PG, De Martino C, Quaranta V, et al. Expression of Ia-like antigens in normal human non-lymphoid tissues. Transplantation 1981 31:75-78.
- Fuggle SV, Errasti P, Daar AS, Fabre JW, et al. Localization of major histocompatibility complex (HLA-A, B, C and DR) antigens in 46 kidneys. Transplantation 1983 35:385-390.
- Evans PR, Trickett LP, Smith JL, et al. Variable expression of HLA-DR antigens in the human kidney. Dis Markers 1984 2:251.

Position Papers re Foregoing Crossmatch in Kidney Transplantation

- Kerman RH, Susskind B, Ruth J, et al. Can an immunologically, nonreactive potential allograft recipient undergo transplantation without a donor-specific crossmatch? Transplantation 1998 66:1833-1834.
- Matas AJ, Sutherland DE. Kidney transplantation without a final crossmatch. Transplantation 1998 66:1835-1836.
- Taylor CJ, Smith SI, Morgan CH, et al. Selective omission of the donor cross-match before renal transplantation. Transplantation 2000 69:719-723.
- Martin S. A potential revolution in pretransplant histocompatibility testing: selective omission of the donor crossmatch. Transplantation 2000 69:709-710.

Non-HLA antibodies

- Martin S, Brenchley PE, Postlethwaite RJ, Johnson RWG, Dyer PA. Detection of anti-epithelial cell antibodies in association with pediatric renal transplant failure using a novel microcytotoxicity assay. Tissue Antigens 1991 37:152-155.

HLA antibodies in Heart Transplantation

- Smith JD, Danskine AJ, Laylor RM, Rose ML, Yacoub MH. The effect of panel reactive antibodies and the donor specific crossmatch on graft survival after heart and heart-lung transplantation. Transpl Immunol 1993 1:60-65.
- Lau CL, Palmer SM, Posther KE, Howell DN, Reinsmoen NL, Massey HT, Tapson VF, Jaggers JJ, D'Amico TA, Davis RD Jr. Influence of panel-reactive antibodies on posttransplant outcomes in lung transplant recipients. Ann Thorac Surg 2000 69:1520.
- Tambur AR, Bray RA, Takemoto SK, Mancini M, Costanzo MR, Kobashigawa JA, D'Amico CL, Kanter KR, Berg A, Vega JD, Smith AL, Roggero AL, Ortegel JW, Wilmoth-Hosey L, Cecka JM, Gebel HM. Flow cytometric detection of HLA-specific antibodies as a predictor of heart allograft rejection. Transplantation 2000 70:1055.
- Przybylowski P, Balogna M, Radovancevic B, Frazier HO, Susskind B, Van Buren C, Katz S, Kahan DB, Kerman R. The role of flow cyotmetry-detected IgG and IgM anti-donor antibodies in cardiac allograft recipients. Transplantation 1999 67:258.

- Hess ML, Hastillo A, Mohanakumar T, Cowley MJ, Vetrovoe G, Szentpetery S, Wolfgang TC, and Lower R: Accelerated atherosclerosis in cardiac transplantation: Role of cytotoxic B-cell antibodies and hyperlipidemia. Circulation 1983 68:94.
- Yowell RL, Hammond E H, Bristow MR, Watson FS, Renlund DG, O'Connell JB. Acute vascular rejection involving the major coronary arteries of a cardiac allograft. J Heart Trans 1988 7:191.
- Palmer DC, Tsai CC, Roodman ST, Codd JE, Miller LW, Sarafian JE, Williams GA. Heart graft arteriosclerosis. An ominous finding on endomyocardial biopsy. Transplantation 1985 39:385.
- Cherry R, Nielsen H, Reed E, Reemtsma K, Suciu-Foca NM, Marboe CC. Vascular (humoral) rejection in human cardiac allograft biopsies: relation to circulating anti-HLA antibodies, J Heart Lung Transplant 1992 11:24.
- Constanzo-Nordin MR, Heroux AL, Radvany R, Koch D, Robinson JA. Role of humoral immunity in acute cardiac allograft dysfunction. J Heart Lung Transplant 1993 12:S143-6.
- Rose EA, Smith CR, Petrossian GA, Barr ML, Reemtsma K. Humoral immune responses after cardiac transplantation: Correlation with fatal rejection and graft atherosclerosis. Surgery 1989 106:203.
- Suciu-Foca N, Reed E, Marboe C, Harris P, Xi YP, Yu-Kai S, Ho E, Rose E, Reemtsma K, King D. The role of anti-HLA antibodies in heart transplantation. Transplantation 1991 51:716.
- Reed EF, Hong B, Ho E, Harris PE, Weinberger J, Suciu-Foca N. Monitoring of soluble HLA alloantigens and anti-HLA antibodies identifies heart allograft recipients at risk of transplant-associated coronary artery disease. Transplantation 1996 61:566-72.
- Zavazava N, Bottcher H, Ruchholtz WM. Soluble MHC class I antigens (sHLA) and anti-HLA antibodies in heart and kidney allograft recipients. Tissue Antigens 1993 42:20.
- George JF, Kirklin JK, Shroyer TW, Naftel DC, Bourge RC, McGiffin DC, White-Williams C, Noreuil T. Utility of posttransplantation panel reactive antibody measurements for the prediction of rejection frequency and survival of hear transplant recipients. J Heart Lung Transplant 1995 14:856.
- Smith JD, Danskine AJ, Rose ML, Yacoub MH. Specificity of lymphocytotoxic antibodies formed after cardiac transplantation and correlation with rejection episodes. Transplantation 1992 53:1358.
- Barr ML, Cohen DJ, Benvenisty AI, Hardy M, Reemtsma K, Rose EA, Marboe CC, D'Agati V, Suciu-Foca N, Reed E. Effect of anti-HLA antibodies on the long-term survival of heart and kidney allografts. Transplant Proc 1993 25:262.
- Nelson K, Allen MD, Youngs D, Aziz S, Montero R, Fishbein D, Slachman F. Significance of B cell crossmatches in cardiac transplantation. Human Immunology 1992 34 (Supplement 1):71.
- Zucker MJ, Fuzesi L, Ribner HS, Pancoska P. Biventricular dysfunction after heart transplantation: Reversal with extracorporeal immunoadsorption. J Heart Lung Transplant 1995 14: 228.
- Nikaein A, Alivizatos PA, Monahan K, Stone MJ. The role of anti-class II HLA anitbodies in heart transplantation. Transplantation 1995 59:439.
- Leech SH, Mather PJ, Eisen HJ, Pina IL, Margulies KB, Bove AA, Jeevanandam V. Donor –specific HLA Antibodies after Transplantation are associated with deterioration in cardiac function. Clin Transplant 1996 10:639.
- Itescu, S, Tung TC, Burke EM, Weinberg AD, Mancini D, Michler RE, Suciu-Foca NM, Rose EA. An immunological algorithm to predict risk of high-grade rejection in cardiac transplant recipients. Lancet 1998 352:263.

- Itescu S, Tung TC, Burke EM, Weinberg AD, Moazami N, Artrip JH, Suciu-Foca NM, Rose EA, Oz MC, Michler RE. Preformed IgG antibodies against major histocompatibility complex class II antigens are major risk factors for high-grade cellular rejection in recipients of heart transplantation. Circulation 1998 98:786.
- McCarthy JF, Cook DJ, Massad MG, Sano Y, O'Malley KJ, Ratliff NR, Stewart RW, Smedira NG, Starling RC, Young JB, McCarthy PM. Vascular rejection post heart transplantation is associated with positive flow cytometric crossmatching. Eur J Cardio Thorac Surg 1998 14:197.
- Aziz S, Hassantash SA, Nelson K, Levy W, Kruse A, Reichenbach D, Himes V, Fishbein D, Allen MD. The Clinical significance of flow cytometry crossmatching in heart transplantation. J Heart Lung Transplant 1998 17:686.
- Tambur AR, Winkel E, Heroux A, Kao W, Pamboukian S, McLeod M, Parrillo JE, Costanzo MR. Flow panel reactive antibody monitoring following heart transplantation. Transplant Proc 2001 33:3295.
- Michaels PJ, Espejo ML, Kobashigawa J, Alejos JC, Burch C, Takemoto S, Reed EF, Fishbein CM. Humoral rejection in cardiac transplantation: risk factors, hemodynamic consequences and relationship to transplant coronary artery disease. J. Heart Lung Transplant 2003 22:58.

HLA Antibodies in Lung Transplantation

- Frost AE, Jammal CT, Cagle PT. Hyperacute rejection following lung transplantation. Chest 1996 110:559.
- Choi JK. Kearns J, Palevsky HI, Montone KT, Kaiser LR, Zmijewski CM, Tomaszewski JE. Hyperacute rejection of a pulmonary allograft: Immediate clinical and pathologic findings. Am J Respir Crit Care Med 1999 160:1015-1018.
- Bittner HB, Dunitz J, Hertz M, Bolmann MR III, Park SJ. Hyperacute rejection in single lung transplantation: Case report of successful management by means of plasmapheresis and antithymocyte globulin treatment. Transplantation 2001 71:649.
- Scornik JC, Zander DS, Baz MA, Donnelly WH, Staples ED. Susceptibility of lung transplants to performed donor-specific HLA antibodies as detected by flow cytometry. Transplantation 1999 68:1542.
- Sundaresan S, Mohanakumar T, Smith MA, Trulock EP, Lynch J, Phelan D, Cooper JD, Patterson GA. HLA-A locus mismatches and development of antibodies to HLA after lung transplantation correlate with the development of bronchiolitis obliterans syndrome. Transplantation 1998 65:648.
- Gammie JS, Pham SM, Colson YL, Kawai A, Keenan RJ, Weyant RJ, Griffith BP. Influence of panel-reactive antibody on survival and rejection after lung transplantation. J Heart Lung Transplant 1997 16: 408.
- Schulman LL, Ho EK, Reed EF, McGregor C, Smith CR, Rose EA, Suciu-Foca NM, Immunologic monitoring in lung allograft recipients. Transplantation 1996 6:252.
- Smith MA, Sundaresan S, Mohanakumar T, Trulock EP, Lynch JP, Phelan D, Cooper JD, Patterson GA. Effect of development of antibodies to HLA and cytomegalovirus mismatch on lung transplantation survival and development of bronchiolitis obliterans syndrome. J Thorac Cardivasc Surg 1998 116:812.
- Jaramillo A, Smith MA, Phelan D, Sundaresan S, Trulock EP, Lynch JP, Cooper JD, Patterson GA, Mohanakumar T. Development of ELISA-detected anti-HLA antibodies precedes the development of bronchiolitis obliterans syndrome and correlates with progressive decline in pulmonary function after lung transplantation. Transplantation 199 67:1155.

- Reznik S, Jaramillo A, Zhang L, Patterson GA, Cooper JD, Mohanakumar T. Anti-HLA antibody binding to HLA class I molecules induces proliferation of airway epithelial cells: a potential mechanisms for bronchiolitis obliterans syndrome. J Thorac Cardiovasc Surg 2000 119:39.
- Palmer SM, Davis RD, Hadjliadis D, Hertz MI, Howell DN, Ward FE, Savik K, Reinsmoen NL. Development of an antibody specific to major histocompatibility antigens detectable by flow cytometry after lung transplant is associated with bronchiolitis obliterans syndrome. Transplantation 2002 74:799.
- Girnita AL, McCurry KR, Iacono AT, Duquesnoy R, Awad M, Spichty KJ, Yousem SA, Burckart G, Dauber JH, Griffith BP, Zeevi A. HLA-specific antibodies are associated with high grade and persistent-recurrent lung allograft acute rejection. J Heart Lung Transplant (in press).
- Magro CM, Harman AP, Klinger D, Orosz C, Adams P, Waldman J, Knight D, Kelsey, Ross P Jr. Use of C4d as a diagnostic adjunct in lung allograft biopsies. Am J Trans 2003 3:1143.
- Magro CM, Ross P Jr, Kelsey M, Waldman WJ, Harman AP. Association of humoral immunity and bronchiolitis obliterans syndrome. Am J Trans 2003 3:1155.

Appendix 8: Clinical Practice Guidelines

Clinical Practice Guidelines

CCDT Forum Report

Assessment and Management of Immunologic Risk in Transplantation

(Montréal, January 28-30, 2005)

Table of Contents

Part III: Post-Transplant Monitoring

Part I: Assessment of Immunologic Risk Pre-Transplant

Section A: Defining Antibodies that Confer Immunologic Risk

I.A.1 Class I IgG Antibodies

Kidney, Heart and Lung

There is general consensus that Class I IgG antibodies have the potential to confer risk for early acute rejection and/graft loss, as well as to confer a risk for poor long-term outcome.

Key Considerations

- A risk if donor specific.
- If not donor specific, data is unclear whether they still confer a risk or not.
- Impact on risk of HLA antibody titre and its ability to fix complement or not still awaits clarification.

I.A.2 Class II IgG Antibodies

Kidney, Heart and Lung

There is general consensus that Class II IgG antibodies have the potential to confer risk for early acute rejection and/graft loss, as well as to confer a risk for poor long-term outcome.

Key Considerations

- A risk if donor specific.
- If not donor specific, data is unclear whether they still confer a risk or not.
- Impact on risk of HLA antibody titre and its ability to fix complement or not still awaits clarification.

I.A.3 IgM Antibodies

Kidney

There is no consensus as to whether IgM antibodies have the potential to confer risk for early acute rejection and/graft loss.

Key Considerations

- In general it is agreed that there is insufficient data to support or refute the premise.
- It is felt that labs should be capable of differentiating between an IgM vs. IgG HLA antibody.
- If IgM is detected then it is felt that this would not change clinical management.

Heart

The consensus is that IgM antibodies do not confer a risk for early acute rejection and/or graft loss.

Key Considerations

- To date no documented evidence.
- Requires further study before any change in approach.

Lung

It is not known whether IgM antibodies confer a risk for early acute rejection and/or graft loss, but at present it is not considered clinically relevant.

Key Considerations

- Lack of data precludes a more definitive statement.
- When IgM is detected it does not change approach to the patient.

I.A.4 Non-HLA Antibodies

Kidney, Heart and Lung

There is general consensus that non-HLA antibodies (e.g. anti-phospholipid, anti-endothelial antibodies) have the potential to confer a risk for early acute rejection and/graft loss, but that at this point it is not practical to test for them.

Key Considerations

- General experience of the group is that they could identify cases that would be consistent with non-HLA Ab (rare but relevant).
- No one routinely tests for, as no practical assays are available.
- May be especially important late post-transplant in relation to chronic pathology.
- A key area for future research.
- A serum repository for cases of humoral rejection in the absence of HLA antibody may be useful to support research in this area but not a high priority.

Section B: Immune Work-up Pre-transplant

I.B.1 Immune Investigations While on the Wait-List for Transplant

I.B.1.1 Routine Testing for Class I IgG

Kidney, Heart and Lung

It is recommended that

- Screening for Class I IgG be performed upon placement on the wait-list.
- Testing should be performed after sensitizing events (e.g., blood transfusions, LVAD, etc.).
- The frequency of testing for kidney and heart patients should be q 3-6 months while on the wait-list.
- A solid phase technique should be used for these evaluations, optimally by flow-based techniques.
- If a Class I IgG antibody is detected then antibody specificity needs to be determined, optimally by flow-based techniques.

Key Considerations

- For some individuals ELISA and Flow were seen as equivalent to solid phase techniques.
- Specificity analysis important for all organs if "virtual crossmatch" approach is to be eventually implemented as part of a national sharing strategy.

I.B.1.2 Routine Testing for Class II IgG

Kidney, Heart and Lung

It is recommended that

- Screening for Class II IgG be performed upon placement on the wait-list.
- Testing should be performed after sensitizing events (e.g., blood transfusions, LVAD, etc.).
- The frequency of testing for kidney and heart patients should be q 3-6 months while on the wait-list.
- A solid phase technique should be used for these evaluations, optimally by flow-based techniques.
- If a Class II IgG antibody is detected then antibody specificity needs to be determined, optimally by flow-based techniques.

Key Considerations

- For some individuals, ELISA and Flow were seen as equivalent to solid phase techniques.
- Specificity analysis important for all organs if "virtual crossmatch" approach is to be eventually implemented as part of a national sharing strategy.

I.B.1.3 Routine Testing for IgM

Kidney, Heart and Lung

It is not recommended that IgM be routinely looked for while on the wait-list.

Key Considerations

- Clinical relevance is unclear.
- In research studies, or in specific cases, IgM assessment may be useful.
- It is felt that labs should be capable of differentiating between an IgM vs. IgG HLA antibody

I.B.2 Minimal Histocompatibility Evaluation Prior to Transplant

I.B.2.1 Prospective Donor Specific T cell Crossmatch

Kidney

A prospective T cell crossmatch is recommended prior to transplant. In general an AHG-CDC crossmatch may suffice but in sensitized patients a flow crossmatch should be performed. Peripheral blood cells may be used as the donor antigen source for the crossmatch, but spleen or lymph node may be required in some circumstances.

Key Considerations

- Important to stratify risk in deceased donor transplantation. If no evidence of recipient sensitization (i.e., solid phase screen negative and no history of sensitization) then one may consider forgoing a prospective crossmatch and conduct it retrospectively.
- Living donor transplants should always have a prospective crossmatch as it is an elective procedure.

Heart

A prospective flow-based T cell crossmatch is recommended in sensitized patients when feasible (i.e., a local donor) using peripheral blood cells as the donor antigen source, but spleen or lymph node may be required in some circumstances. If no evidence of sensitization by flow-based techniques then a prospective crossmatch may be foregone and conducted retrospectively.

Key Considerations

- It is critical to have HLA typing of the donor available and high resolution HLA Ab specificity analysis on recipient's sera. This will allow for the possibility for a "virtual crossmatch" (see below).

Lung

A prospective T cell crossmatch is not required prior to transplant.

Key Considerations

- Limitations in cold ischemic time make it difficult to perform a prospective T cell crossmatch prior to implantation.
- A retrospective flow-based T cell crossmatch should be performed within 24 hours of transplant.

I.B.2.2 Prospective Donor Specific B-cell Crossmatch

Kidney

A prospective flow-based B-cell crossmatch is recommended prior to transplant in sensitized patients who have evidence of a Class II IgG antibody by routine flow-based sera screening. Peripheral blood cells may be used as the donor antigen source for the crossmatch, but spleen or lymph node may be required in some circumstances.

Key Considerations

- Important to stratify risk, in deceased donor transplantation if no evidence of recipient sensitization (i.e., solid phase screen negative and no history of sensitization) then one may consider forgoing a prospective crossmatch and conduct it retrospectively.
- Living donor transplants should always have a prospective crossmatch as it is an elective procedure.

Heart

A prospective flow-based B-cell crossmatch is recommended in sensitized patients when feasible (i.e., a local donor) using peripheral blood cells as the donor antigen source, but spleen or lymph node may be required in some circumstances. If no evidence of sensitization by flow-based techniques then a prospective crossmatch may be foregone and conducted retrospectively.

Key Considerations

- It is critical to have HLA typing of the donor available and high resolution HLA Ab specificity analysis on recipient's sera. This will allow for the possibility for a "virtual crossmatch" (see below).

Lung

A prospective B-cell crossmatch is not required prior to transplant.

Key Considerations

- Limitations in cold ischemic time make it difficult to perform a prospective B-cell crossmatch prior to implantation.
- A retrospective flow-based B-cell crossmatch should be performed within 24 hours of transplant.

I.B.2.3 Prospective "Virtual Crossmatch"

Kidney

Do not recommend use of a "virtual crossmatch" in a sensitized patient. Rather a prospective crossmatch should be performed by flow-based techniques.

Key Considerations

- Insufficient data at present to rely on virtual crossmatch approach.

Heart

Do not recommend use of a "virtual crossmatch" as a minimal standard in a sensitized patient. Rather a prospective crossmatch should be performed by flow-based techniques if feasible (i.e., local donor) or retrospectively and available within 24 hours.

Key Considerations

- Insufficient data at present to recommend use of a virtual crossmatch approach as the minimum practice standard.
- May not be able to perform prospective crossmatch due to time limitations.
- Key to have molecular HLA typing from the donor prior to transplant and flow-based specificity analysis of HLA Ab of the recipient available if virtual crossmatches are to be utilized in the future.

Lung

Do not recommend use of a "virtual crossmatch" as a minimal standard in a sensitized patient. Rather a retrospective crossmatch should be available within 24 hours.

Key Considerations

- Key to have molecular HLA typing from the donor prior to transplant and flow-based specificity analysis of HLA Ab of the recipient available if virtual crossmatches are to be utilized in the future.

Part II: Management of Immunologic Risk Pre-Transplant

Section A: Pre-Transplant Management of Deceased Donor Transplants

II.A.1 Positive CDC or AHG-CDC T cell crossmatch

Kidney

It is recommended that a positive CDC or AHG-CDC T cell crossmatch be considered a contraindication to transplantation.

Key Considerations

- To proceed would require a desensitization protocol, which at this point for deceased donor transplants would require sufficient infrastructure.

Heart and Lung

It is recommended that a positive CDC or AHG-CDC T cell crossmatch be considered as a risk factor for early acute rejection and/or graft loss but is not a contraindication to transplantation.

Key Considerations

- In general, heart and lung transplants would attempt to avoid crossing this barrier but competing considerations may require this barrier to be crossed.
- If proceed to transplant then immunosuppressive approach would change.

II.A.2 Negative AHG-CDC, Positive Flow-Based T cell Crossmatch

Kidney

It is recommended that a negative AHG-CDC, positive flow-based T cell crossmatch be considered as a risk factor for early acute rejection and/or graft loss but there is no consensus that it represents a contraindication to transplantation.

Key Considerations

- To proceed would require a desensitization protocol, which at this point for deceased donor transplants would require sufficient infrastructure and expertise.

Heart and Lung

It is recommended that a negative AHG-CDC, positive flow-based T cell crossmatch be considered as a risk factor for early acute rejection and/or graft loss.

Key Considerations

- In general, heart and lung transplants would attempt to avoid crossing this barrier but competing considerations may require this barrier to be crossed.

II.A.3 Remote Positive T cell crossmatch

Kidney, Heart and Lung

It is recommended that a positive remote T cell crossmatch be considered as a risk factor for early acute rejection and/or graft loss.

Key Considerations

- Approach to immunosuppression may need to be modified and early post-transplant monitoring increased.

II.A.4 Positive CDC B-cell crossmatch

Kidney

It is recommended that a positive CDC B-cell crossmatch be considered a contraindication to transplantation.

Key Considerations

- To proceed would require a desensitization protocol, which at this point for deceased donor transplants would require sufficient infrastructure.
- Critical to make sure that it is a true positive B-cell crossmatch (i.e., that there is in fact a donor specific antibody previously detected by solid phase techniques, give the false positive rate of B-cell crossmatches).

Heart and Lung

It is recommended that a positive CDC B-cell crossmatch be considered as a risk factor for early acute rejection and/or graft loss, but is not a contraindication to transplantation.

Key Considerations

- In general, heart and lung transplants would attempt to avoid crossing this barrier but competing considerations may require this barrier to be crossed.
- If proceed to transplant then immunosuppressive approach may need to be changed.

II.A.5 Negative CDC, Positive Flow-Based B-cell Crossmatch

Kidney

It is recommended that a negative CDC, positive flow-based B-cell crossmatch be considered as a risk factor for early acute rejection and/or graft loss but there is no consensus that it represents a contraindication to transplantation

Key Considerations

- To proceed would require a desensitization protocol, which at this point for deceased donor transplants would require sufficient infrastructure and expertise.
- Critical to make sure that it is a true positive B-cell crossmatch (i.e., that there is in fact a donor specific antibody previously detected by solid phase techniques, give the false positive rate of B-cell crossmatches).

Heart and Lung

It is recommended that a negative CDC, positive flow-based B-cell crossmatch be considered as a risk factor for early acute rejection and/or graft loss.

Key Considerations

- In general, heart and lung transplants would attempt to avoid crossing this barrier but competing considerations may require this barrier to be crossed.

II.A.6 Remote Positive B-cell crossmatch

Kidney, Heart and Lung

It is recommended that a positive remote B cell crossmatch be considered as a risk factor for early acute rejection and/or graft loss.

Key Considerations

- Approach to immunosuppression may need to be modified and early post-transplant monitoring increased.

Section B: Pre-Transplant Management of Living Donor Transplants

II.B.1 Positive Crossmatch due to Donor Specific Antibody (Class I / II)

Kidney

In contrast to the recommendations for deceased donor transplants, there was no consensus recommendations that could be made. It was clear that some centres are willing to proceed with a living donor transplant in the presence of a donor specific antibody. This was mainly the case if the donor specific antibody was detected by flow-based methods only. However, there were even some centres willing to proceed if the initial AHG-CDC positive crossmatch could be converted to negative with desensitization protocols.

Key Considerations

- To proceed requires pre-transplant desensitization even for flow positive donor specific antibodies.
- To proceed requires early post-transplant monitoring.
- Consensus that best option may be a national paired organ donor exchange program.

Part III: Post-Transplant Monitoring

III.A Routine Post-Transplant Monitoring in Stable Low Risk Patients

Kidney and Lung

It is not recommended that routine post-transplant HLA antibody monitoring occur.

Key Considerations

- This was considered an area for active research.

Heart

It is recommended that routine post-transplant flow-based assessment of HLA antibody and pathology monitoring occur.

Key Considerations

- General sense that sufficient data are present to warrant such an approach.

III.B Routine Post-Transplant Monitoring in High Risk Patients

Kidney, Heart and Lung

It is recommended that routine post-transplant solid phase based HLA antibody assessment and pathology monitoring be performed.

Key Considerations

- In heart and kidney it was felt that pathology assessment must include C4d staining.
- Pathology assessment must be available within 24hrs for kidney, heart and lung transplants.
- Most preferred flow-based assessment of HLA antibody.
- HLA antibody assessment needs to be quantitative to allow optimization of therapy.

III.C Post-Transplant Diagnostic Assessment with Graft Dysfunction

Kidney, Heart and Lung

It is recommended that post-transplant solid phase based HLA antibody assessment and pathology assessment be performed.

Key Considerations

- In heart and kidney it was felt that pathology assessment must include C4d staining.
- Pathology assessment must be available within 24hrs for kidney, heart and lung transplants.
- Most preferred flow-based assessment of HLA antibody.
- HLA antibody assessment needs to be quantitative to allow optimization of therapy.