Application of Machine Learning to Analyze Immune Cellular Compartments in a Pre-Transplant Cohort

Franz Fenninger (1), Vivian Wu (1), Karen Sherwood (1), Paul Keown (1,2) on behalf of the Genome Canada Consortium

Affiliation 1: Department of Medicine, University of British Columbia, Vancouver, B.C., Canada

Affiliation 2: Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, B.C., Canada

Aim

Chronic kidney disease affects 1 in 10 Canadians with estimated costs of over $2 billion/year. Advanced stages of the disease lead to uremia, which is accompanied by profound disturbance of the immune response comprising both impaired immune defense and enhanced inflammation. This dysregulation of the immune system is reflected in changes in function as well as composition of the immune system. We captured these changes by quantifying lymphocyte- (CD4 T-, CD8 T, B- and NK cells) and monocyte subpopulations as well as the expression of several activation markers during uremia.

Methods

As part of the Genome Canada Transplant Consortium program, we have developed an advanced flow cytometric immunophenotyping assay to detect high resolution cellular subpopulations based on specific cellular surface markers. Samples of 35 uremic patients and 12 healthy controls were immunophenotyped using 5 different panels and subsequently analyzed using a custom R/Bioconductor pipeline. Due to the complexity of these multidimensional data we used machine learning algorithms like uMAP to reduce complexity and and FlowSOM to cluster the immune cells into different subpopulations. Key immune populations that contribute to the state of chronic activation of the immune system in uremic patients were compared to healthy controls as well as correlated with other factors known to impact immune cells, such as time on / type of dialysis and primary diagnosis.

Results

In a preliminary analysis of the CD8 T cell panel we discovered an increased frequency of TEMRA CD27+ cells, a terminally differentiated effector memory T cell subset, in uremic patients (Fig. 1). This analysis also revealed other key players in the cellular compartments that show an immunophenotype of uremia and provides insight into how modifications in these cellular subsets drive disease progression.

Conclusions

With more understanding of the dysregulation observed in these disease states, we hope to recognize the onset of the disease earlier and develop more specific and targeted therapies.

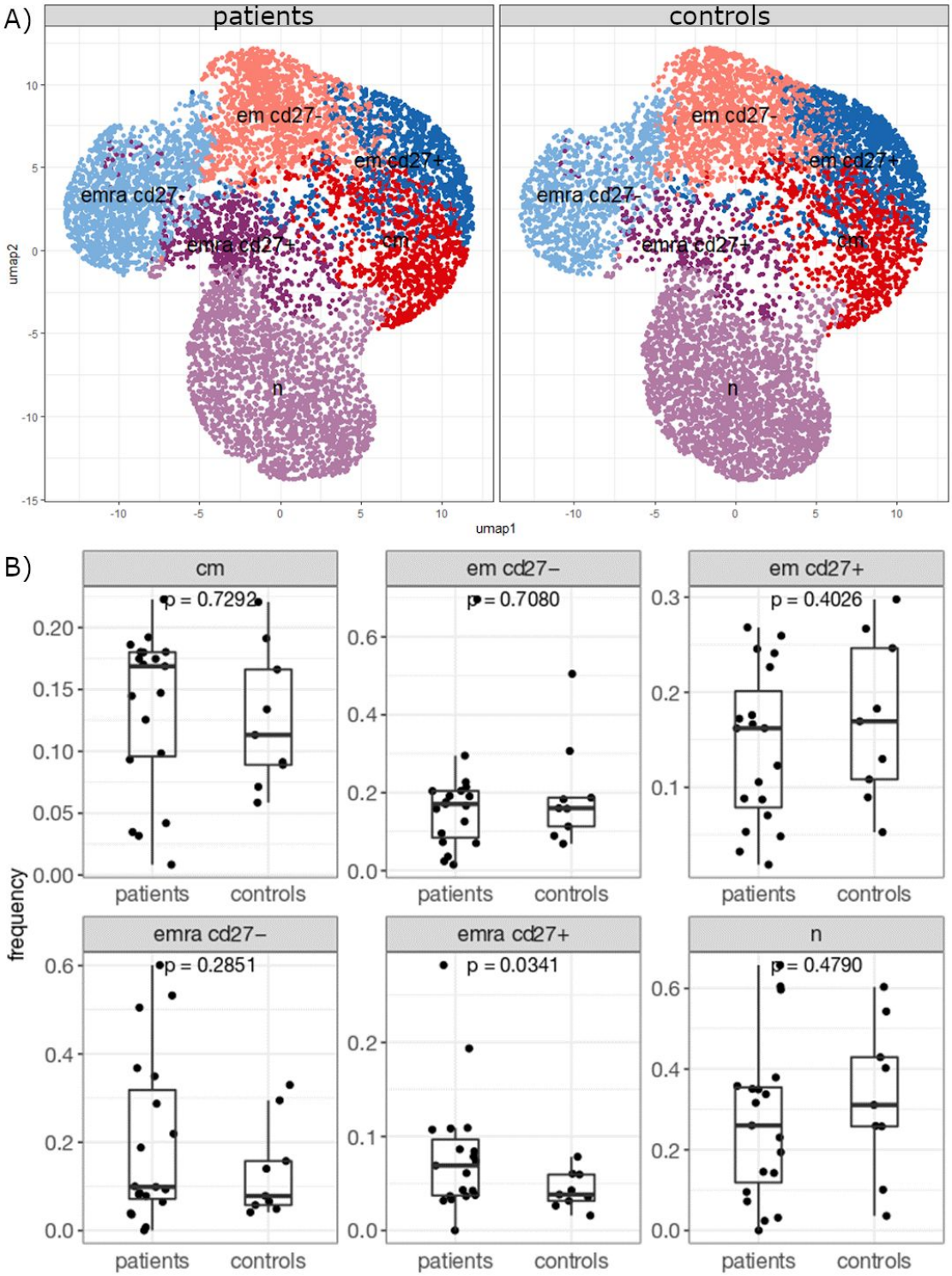


Fig 1) A) uMAP diagram comparing CD8 T cell subpopulations clustered using FlowSOM in uremic patients and healthy controls. B) Frequencies of the FlowSOM clusters (cm.. central memory, em.. effector memory, n.. naive)

FEASIBILITY OF RAPID DECEASED DONOR HLA TYPING AND EPITOPE LEVEL MATCHING USING ONT SEQUENCING

Karen Sherwood (1), Jennifer Beckrud (2), Paul Keown (1,2), on behalf of the Genome Canada Transplant Consortium

Affiliation 1: Department of Medicine, University of British Columbia, Vancouver, B.C., Canada

Affiliation 2: Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, B.C., Canada

Aim

To develop a high-resolution HLA typing deceased donor workup protocol from sample to epitope level matching in ~6hrs.

Methods

DNA from 50 patients (25 donors and recipients) representing a 6 month period of donor workups were selected for this study. All samples had been previously typed using current on-call LinkSeq RT-PCR (Linkage BioScience) and Holotype v3 NGS (Omixon) typing.

Samples were amplified using the Omixon Omnitype 11 loci single tube assay (full gene for HLA-A, -B, -C, -DQA, -DPA and partial genes for DRB1, DRB3/4/5, DQB1 and DPB1). Amplicons were then prepared using the 1D ligation kit (SQK-LSK109), and run on a standard R9.4 flow cell. Each flow cell was run for between 1-6 hours total sequencing time. Raw FAST5 files were live basecalled using the MinKNOW GUI using both the high accuracy mode. HLA allele assignment was performed blind by our collaborators at the data development team at Omixon and the Vancouver team using GenDX NGSEngine 2.18 with IMGT 3.40.0. Two field typings were then input to HLA Matchmaker v3 for each donor/recipient pair.

20 patients were also sequenced using the R10.3 flow cells with the same library and analysis pipeline as described.

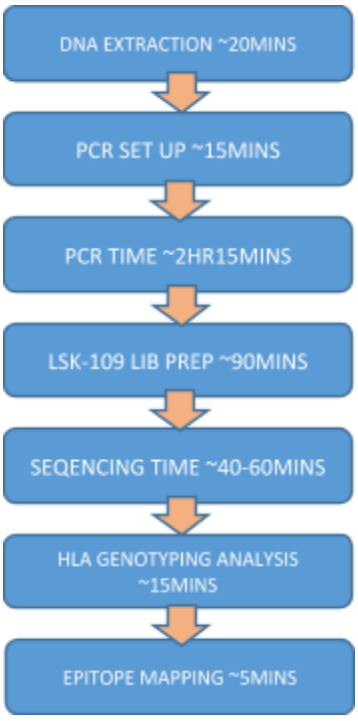
Results

HLA allele assignment performed blind by **Omixon collaborators.**

3rd or 4th field typing achieved for 100% of alleles
Concordance was only assessed to 2 field typing
No manual editing or interpretation
Omixon Pipeline: 97% accuracy for class 1 and 95% for class II

HLA allele assignment performed blind using **GenDX NGSengine** software.
4 field typing achieved for 100% of alleles.
Concordance was only assessed to 2 field typing
No manual editing or interpretation
GenDX Pipeline: 98% accuracy for class 1 and 97% for class 2

NOTE: SUFFICIENT DATA AVAILABLE AFTER ~45-60mins sequencing time TOTAL TIME 5hr 20min



Conclusions

The ability to perform HR typing and epitope matching in the context of a deceased donor workup would have major clinical benefits, in particular for highly sensitized recipients.

ROBUST RELIABLE NON-SEQUENCING INTERMEDIATE/HIGH RESOLUTION TYPING USING LINKAGE BIOSYSYTEMS LINKSEQ GENOTYPING ASSAY

Karen Sherwood (1), Carmen Phoon (2), Vince Benedicto (2), Paul Keown (1,2), on behalf of the Genome Canada Transplant Consortium.

Affiliation 1: Department of Medicine, University of British Columbia, Vancouver, B.C., Canada

Affiliation 2: Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, B.C., Canada

Aim

Given that sequencing based HLA genotyping assays for high resolution typing is not yet feasible in all labs for deceased donor workup, we have evaluated a non-sequencing based technique (LinkSeq RT-PCR) as an alternative assay and determined the feasibility of this approach in determining allele level HLA typing, sufficient for epitope mapping.

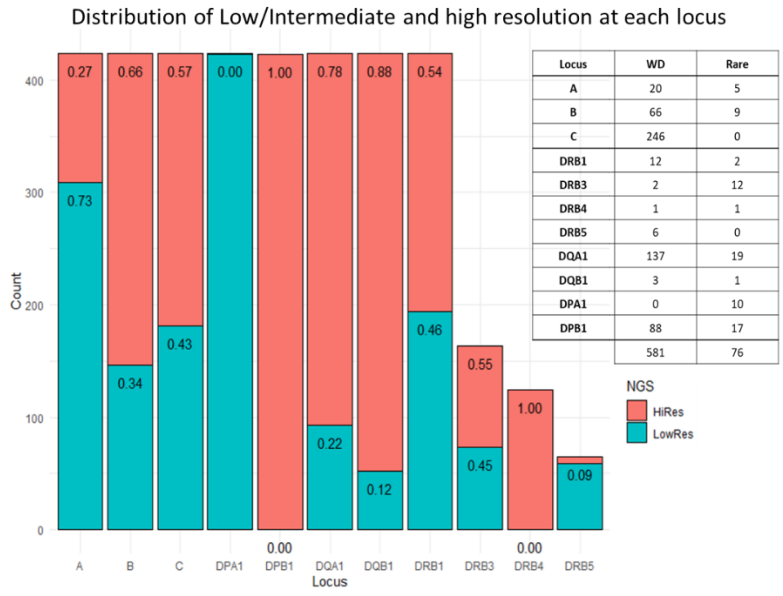
Methods

211 BC deceased donors from 2018-2019 (104 cases in 2018, 107 cases in 2019) were HLA genotyped using the LinkSeq SABR 384 Qpcr-SSP (Linkage Biosciences) assay kits at all 11 HLA loci (HLA-A, -B, -C, -DRB1/3/4/5, -DQA/B, -DPA/B). All samples were prepared according to the manufacturer's protocol and run on a Roche 480 Light Cycler Real-Time PCR instrument.

All deceased donors were retrospectively NGS sequenced at all 11-loci using the Omixon Holotype assay, following the manufacturer's protocol and analysed using the Omixon Twin v3.4 software for confirmation.

Results

The current performance of the LinkSeq qPCR-SSP assay is capable of resolving a number of alleles to high resolution (2 field HLA typing). However, many alleles are still not able to be fully resolved, resulting in low/intermediate resolution typing only.



Locus	WD	Rare
A	20	5
B	66	9
C	246	0
DRB1	12	2
DRB3	2	12
DRB4	1	1
DRB5	6	0
DQA1	137	19
DQB1	3	1
DPA1	0	10
DPB1	88	17
	581	76

Of the 4642 alleles typed, 98.8% alleles are common, only 1.1% alleles are well-documented and 0.1% alleles were rare (and not listed in the CWD2.0).

Most of the well-documented alleles are found in Class I (C) and Class II (DQA). Most of the rare alleles are found in Class II (DRB3, DQAQ and DPA/B).

Taking into account only Class II, high resolution HLA typing was able to be determined 80% of the time with no further investigation.

Conclusions

Whilst non-sequencing assays are not yet capable of resolving all alleles to high resolution, for class II loci, the LinkSeq kit coverage is sufficient to resolve 80% of the alleles observed. Given the importance of Class II loci in the development of antibody mediated rejection and the focus of epitope matching in the major Class II genes, it is promising that a rapid, easily standardisable assay can provide appropriate data for the BC deceased donor population to facilitate epitope matching for all our kidney patients.

RESOLVING ANTI-HLA SPECIFIC COMPLEXITIES USING PHENOTYPE BEADS: A DUAL ASSAY CONSIDERATION

Jason Wong (2), Karen Sherwood (1), Lenka Allan (2), Paul Keown (1,2) on behalf of the Genome Canada Transplant Consortium.

Affiliation 1: Department of Medicine, University of British Columbia, Vancouver, B.C., Canada

Affiliation 2: Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, B.C., Canada

Aim

To evaluate the use of Immucor LIFECODES Identification (IMID) kits to assess non-HLA specific reactivity patterns as observed on the One Lambda LABScreen single antigen bead (OLSAB) assay.

Methods

16 serums with Class I patterns and 20 serums with Class II patterns were run on OLSAB assay followed by IMID kits.

The OLSAB assay was set up following the Rapid Optimized SAB (ROB) protocol, which is a modified version of the manufacturer's protocol. An additional two final wash steps was added to decreased background fluorescence.

The IMID kits were set up following a modified manufacturer's protocol, using a centrifugation and flick method as opposed to vacuum manifold method.

Results

The two assays utilize different manufacturing processes of coating purified recombinant protein onto beads (OLSAB) versus using protein derived from EBV transformed cell lines with native phenotypic expression (IMID). We hypothesised that the difference in recombinant vs native protein may result in differences in observed non-HLA specific reactivities.

Our lab routinely tests patient serum using OLSAB kits to determine HLA Class I and Class II antibody specificities. Many of these well characterized reactivity patterns seen on the OLSAB assay appear to be non-HLA specific and cannot be correlated with positive flow crossmatches or single antigen testing by another vendor. In addition, epitope and cross-reactive analysis reveals no discernable explanation for the observed non-specific patterns.

The IMID assay does not show elevated raw MFI values nor particular groupings of CREG groups. Disproving the presence of many non-specific HLA specificities may lead to significant changes to reported PRA percentages.

DQA1*05:01/DQB1*02:01 reactivity is observed in the OLSAB assay but often yields a negative crossmatch. The IMID assay was able to confirm or eliminate alternative calls due to having additional DQA1*05 antigens in the beadset.

Conclusions

We believe using the IMID kits would be a beneficial tool to help deconvolute complex, non-specific reactivities seen on OLSAB in order to better reveal biological reactivity.

ASHI Abstract Submission 2020

Title

DEMONSTRATING THE FEASIBILITY OF EPLET-MATCHING IN A CANADIAN ORGAN ALLOCATION SIMULATION MODEL

Authors

Tran, Jenny N. (1), Oliver P. Günther (2), Karen R. Sherwood (3), and Paul A. Keown (1,3), on behalf of the Genome Canada Transplant Consortium

Affiliation 1: Department of Medicine, University of British Columbia, Vancouver, B.C., Canada

Affiliation 2: Günther Analytics, Vancouver, B.C., Canada

Affiliation 3: Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, B.C., Canada

Aim

Achieving histocompatibility between donors and recipients is desirable to improve transplant outcomes, but is challenging in large centres due to the extreme polymorphism of the human leukocyte antigen (HLA) genes. Matching by eplets, cardinal antibody targets on the HLA protein, is an innovative strategy to reduce risk of graft rejection but its effect and feasibility in a Canadian population remains unknown. Here, we modeled organ allocation using computer simulations to study the effects of eplet-matching in Canada.

Methods

Eplet profiles across the 11 HLA genes were determined in 1262 kidney patients/donors from British Columbia using next-generation sequencing and HLAMatchmaker. Simulations were performed for a base-model using identical-ABO and waitlist rank for matching, and an eplet-matching model additionally using eplet-mismatch scores. Simulations were run in R, based on provincial annual waitlist sizes (88 – 2032 patients) and donor rates (16 – 762 donors).

Results

All models with eplet-matching reduced mismatch scores. Compared with the national base-model, deliberate eplet-matching decreased the median mismatch from 27.35 to 9.3 across all genes; for class II genes from 16.8 to 1; for DRB1/3/4/5 from 6 to 0; and for DQB1 from 6 to 0. Waitlist and donor size was an important factor in determining compatibility, where ≥~250 patients allowed for the majority of matches to have a DQB1 score 0 (Fig1). Except for all genes combined and class II for small waitlists, the probability of a mismatch of 10 or lower was >95% across all waitlist sizes and gene loci.

Conclusions

Eplet-matching dramatically improves donor-recipient compatibility and is feasible in a waitlist of 250 patients. Our results offers an opportunity for a unique program of eplet-based matching to reduce organ rejection across Canada.

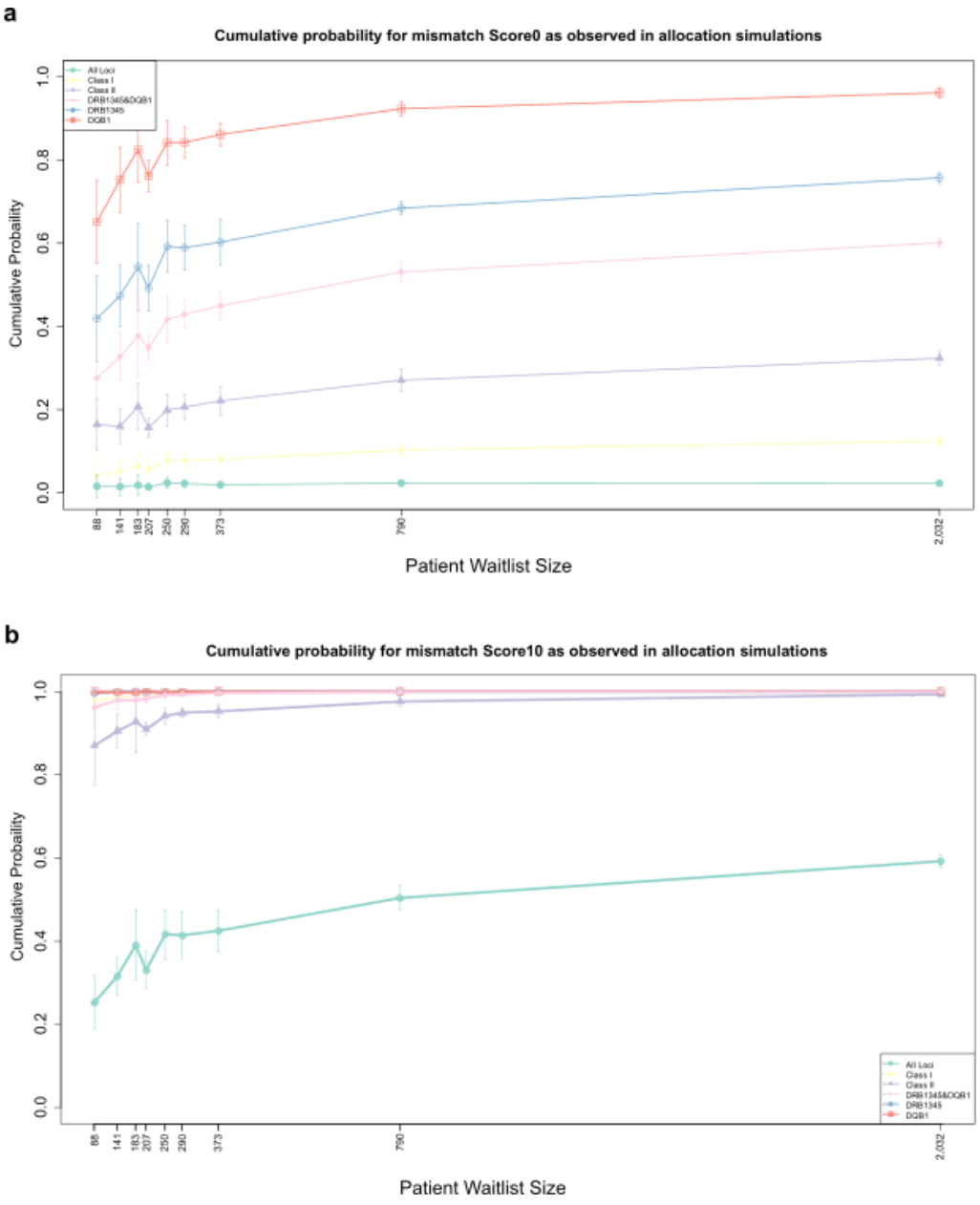


Figure 1. Results of an averaged set of 10 deliberative eplet-matching simulations at various gene loci with error bars. X axis shows the number of patients on provincial or national patient waiting lists and Y axis shows the averaged cumulative probability of achieving a total mismatch score of 0 (a) or 10 or lower (b) across all matched pairs at the end of the simulations.

Title

LYMPHOCYTE RECEPTOR SEQUENCING TO MONITOR ALLOIMMUNE RESPONSE: A SYSTEMATIC REVIEW

Authors

Wong, Paaksum (1), Karen R. Sherwood (2), and Paul A. Keown (1,2), on behalf of the Genome Canada Transplant Consortium

Affiliation 1: Department of Medicine, University of British Columbia, Vancouver, B.C., Canada

Affiliation 2: Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, B.C., Canada

Aim

The clinical importance of T-cell alloreactivity in the post-transplant period is clear. However, early predictors of alloreactivity are still not widely available, and TCR tracking may be fundamental in predicting and preventing irreversible graft damage. Recent research demonstrates the potential value of lymphocyte receptor sequencing as an indicator for alloreactivity. This systematic review examines current research in lymphocyte receptor sequencing in immune monitoring and assesses clinically feasibility for applications in transplantation.

Methods

Search and screening was conducted in accordance with PRISMA guidelines. We searched PubMed for English-language studies published between 2010 and 2020 that examine the relationships between changes in T cell and B cell repertoires, donor reactivity, and clinical outcomes. Manual filtering of search results was performed based on relevancy and predefined inclusion criteria, and data were extracted based on study and methodology characteristics.

Results

We found an extensive body of literature on lymphocyte receptor sequencing in transplantation. Initial screening yielded 764 articles of which 82 met the inclusion criteria. 21 were kidney transplantation studies (26%), 31 were other or general transplantation studies (38%), and 30 pertained to other diseases (36%). Sequencing the TCR β CDR3 region for TCR studies and IGHV region for BCR studies dominated repertoire quantification studies due to its high degrees of variability and prominent roles in antigen recognition. Clonal expansion and diversity indices are well-accepted benchmarks for receptor repertoire studies, with many demonstrating correlation between clonal expansion and adverse health outcomes. More studies focused on TCR methods compared to BCR, but no difference is seen in their effectiveness of predicting post-transplant course.

Conclusion

Methodological approaches of receptor sequencing are well-established and this novel technology is positioned to be adopted as a new and important clinical diagnostic assay for allorecognition and prediction of rejection.