

Extramural Meeting 2024

Patient Based Cases 2023

Yvonne Zoet

Eurotransplant Reference Laboratory

LEIDEN





A **5 years** old boy with **blood group B pos** was registered on the kidney waitlist

Recipient HLA type: A*02:05, A*11:01, B*50:01, B*54:01, C*01:02, C*06:02, DRB1*07:01, DRB1*12:02, DRB3*03:02, DRB4*01:03, DQB1*02:02, DQB1*03:01, DQA1*02:01, DQA1*06:01, DPB1*03:01, DPB1*04:01, DPA1*01:03, DPA1*02:02

Antibody information:

No immunizing events

2 sera were tested with a 3-month time interval before waitlist registration:

CDC: negative

LMX positive for Class II

Luminex SA shows Class II specificities



Patient case 2023-01 : Luminex SA results

Lifecodes	DR9	DR4	DR7	DR52	DR103	DR1	DR16	DR15	DR14	DR12	DR13	DR3	DR10
Serum 1	6263	5684	5527	4574	3031	2970	2838	2687	2486	2451	2336	2311	1806
Serum 2	1739	1719	1615	1424	604	674	582	593	578	*	*	*	*

Values in background adjusted MFI, *Values below MFI 500 are not depicted

DP3	1	2	5700,7
<u>DR9</u>	2	0	2639,65
<u>DR4</u>	4	1	2172,21
<u>DR7</u>	1	0	2015,34
<u>DR12</u>	1	1	1933,9
<u>DR52</u>	1	2	1530,94
<u>DR103</u>	1	0	1490,37

One lambda serum 1

DP3	1	2	4313,6
<u>DR12</u>	1	1	1664,7
<u>DR9</u>	2	0	1416,56
<u>DR4</u>	5	0	1365,68
DQ6	1	5	1284,56
DQ7	2	3	1253,8
DR1	1	1	1216,76

One lambda serum 2

Found in both sera/test kits Patient's HLA type

Patient case 2023-01: Definition of Unacceptable Antigens?



9/49 centers defined one or more unacceptable antigens:

Specificity	DR4	DR9	DR52
Frequency	8	8	4

Specificity	DR1	DR15	DR16	DR17	DR18	DR10	DR11	DR13	DR14	DR51	DR53
Frequency	3	3	3	3	3	3	3	3	3	3	3

Specificity	DR12	DP3	DR7	DR8
Frequency	2	2	1	1

Found in both sera/test kits **Patient's HLA type**

Most other centers remarked that in this pre-transplantation situation without known immunizing events the MFI values are overall too low for unacceptable antigen definition

Patient case 2023-01: Additional testing?



- Follow up: repeat antibody testing after 3-6 months
- Repeat the test on treated serum: presorb, serum cleaner, EDTA, Adsorb-Out, freezing and thawing
- Auto crossmatch: By CDC and or FCM
- Confirmation of non-specific reactivity: Crossmatch (flow, B-cells, T-cells) for specific allo-reactivity or B-cell screening
- Repeat HLA typing of patient; HLA-typing of mother
- Allocation Crossmatch on separated T- and B-cells
- SAB complement fixing antibodies

Explanations for the presence of (false positive) antibodies



- Medication/Therapy: IVIG, therapeutic antibodies, or other medication?
- Underlying (autoimmune) disease
- Recent infections/vaccinations
- Antibodies against denatured HLA/structures of artificially developed bead array antigens/recombinant HLA molecules
- It is not uncommon to see positive reactions in SAB tests in young children without immunizing events
- In the One Lambda test often an unspecific DP1 signal is seen



There is no explanation for the antibodies found.

Some of the antibody specificities were listed:

A24, DR1, DR15, DR16, DR17, DR18, DR4, DR11, DR12, DR13, DR14, DR7, DR9, DR10, DR52

No unacceptable antigens were listed

The patient was transplanted with a deceased donor kidney.

HLA-type of the donor: A2, A68, A28, B7, B8, Bw6, Cw7, DR15, DR2, DR7, DR51, DQ6, DQ1, DQ9, DQ3, DQA-01, DQA-02, DP-0401, DP-13, DPA-01, DPA-02



The male recipient, aged 45 years, blood group B pos, returned to the kidney waitlist after failure of the first graft

Recipient HLA type:

A*02:01, A*A24:02 B*07:02, B*50:01, C*06:02, C*07:02 DRB1*07:01, DRB4*01:03, DQB1*02:01, DQA1*02:01, DPB1*04:01

Immunizing events:

Cadaveric donor kidney in 2004, which failed in 2018 Available donor HLA type:
A3, A9, A23, B35, B21, B50, DR5, DR11, DR7, DQ3, DQ7, DQ2

Antibody information:

Luminex screen: negative for class I, positive for class II
CDC screening: 8% with and without DTT
Luminex SAB Class I results were negative
Luminex SAB II results from recent serum

Patient case 2023-02; Antibody profile



Spec	DQA1*04:01	DQA1*06:01	DQA1*05:01	DQA1*03	DQA1*01	DQA1*02:01
MFI	20667	20610	20076	19600	19356	19259

Spec	DQB1*03	DQB1*04	DQB1*06	DQB1*05	DQB1*02
MFI	20713	20616	20245	18337	13673

Spec	DRB1*09	DRB1*01:03	DRB1*11	DRB1*14	DRB1*13:01	DRB1*12:02	DRB1*04:02
MFI	8632	6760	3043	2876	2863	2265	2082

Note the following DQA-DQB combinations:

	MFI
DQA1*02:01 DQB1*02:02	14437
DQA1*02:01 DQB1*02:01	1438

Explanation of the antibody reactivity pattern



- By the donor HLA-type. Transplantation took place in 2004
No donor material was left to perform a high-resolution typing
- The most likely donor class II typing would be: DR11-DQB1*03:01-DQA1*05:05
DR7-DQB1*02:02-DQA1*02:01
- Antibodies against DQ7 (immunizing antigen), DQ8, DQ9, DQ5, DQ6 and DQ4 could all be explained by a shared eplet (46VY)
- DQA1*05:01 immunization could be explained by the donor DQA1*05:05. DQA1*05:01 and DQA1*05:05 have identical amino acids present on solvent accessible positions
- Antibodies against DQB1*02:02 could be explained by the proposed DQ2 typing of the donor.
- DR antibodies are more difficult to explain by the donor HLA type, only DR11 antibodies can be explained.



Until recently high resolution typing of cadaveric donors was not available

Translating the donor HLA-type into the most likely high-resolution HLA-type as well as imputation or linkage for the DQA chain, may help to understand the immunization status of a patient

Please be aware that in reality the HLA-type may be different

Try to obtain as much immunological information as possible

Patient case 2023-03: Recipient information



Male recipient aged 43 years, blood group A pos, transplanted with a deceased donor kidney in 2014

The organ failed in 2018

Recipient HLA type: A31, A33, B18, B72, Bw6, Cw2, Cw7, DR4, DR10, DR53, DQ5, DQ8, DQA-01, DQA-03, DP-03, DP-0401, DPA-01

HLA type of the failed donor kidney:

A3, B7, B35, Bw6, Cw4, Cw7, DR4, DR12, DR52, DR53, DQ7

Antibody information:

Recent Luminex screen: negative for class I, positive for class II

Recent autologous crossmatch: positive

No CDC antibodies; SAB antibodies: see following slide

Crossmatch on unseparated spleen cells without DTT: Positive; with DTT: Negative

HLA type donor offer: A3, B7, B37, Bw4, Bw6, Cw6, Cw7, DR4, DR10, DR53, DQ5, DQ1, DQ8, DQ3



Recipient HLA type:

A31, A33, B18, B72, Bw6, Cw2, Cw7, DR4, DR10, DR53, DQ1,DQ5, DQ3,DQ8, DQA-01, DQA-03, DP-03, DP-0401, DPA-01

First donor's HLA type:

A3, B7, B35, Bw6, Cw4, Cw7, DR4, DR12, DR52, DR53, DQ3, DQ7

Current Donor offer HLA type:

A3, B7, B37, Bw4, Bw6, Cw6, Cw7, DR4, DR10, DR53, DQ5,DQ1, DQ8, DQ3

Green: match

Blue: mismatch without proven antibodies (CDC or Luminex SA)

Orange: repeated mismatch

Unacceptable Antigens as defined by the recipient's laboratory: A2, A28, B8, DR3, DR6

Patient case 2023-03: Unacceptable antigens



	A2	A28	B8	DR3	DR6
Historic MFI (One Lambda)	4946	3987	3490	4532	2533
Current MFI (Immucor)	181	184	114	145	298

MFI: raw values

No unacceptable antigens are present in the donor's phenotype according to single antigen data: A31, A33, B18, B72, Bw6, Cw2, Cw7, DR4, DR10, DR53, DQ1,DQ5, DQ3,DQ8, DQA-01, DQA-03, DP-03, DP-0401, DPA-01

15 centers indicate that the repeated mismatches are considered unacceptable



Crossmatch:

- Negative with DTT
- Autologous crossmatch is positive
Indicates IgM antibodies (no contraindication)



Repeated mismatches

- No antibodies directed against the repeated mismatches
- Standard risk
(data Dave Roelen; presented at the annual ET meeting in 2023, available via <https://my.eurotransplant.org/organization/?target=annual-meeting>)

Request: New cases!



Preferably concerning (recipients awaiting) deceased donor kidney transplantations

For example:

- for recipients with an aberrant antibody profile
- with unexpected crossmatch outcomes
- for a recipient waiting longer than average
- with interventions done to facilitate transplantation
- and other.....

Please send a short information to the ETRL: etrl@eurotransplant.org

- All recipient, donor and center data will be anonymized -

Thanks to the labs who already made cases available!

Thank You

For participating in the patient-based cases EPT



The ETRL-team
etrl@eurotransplant.org