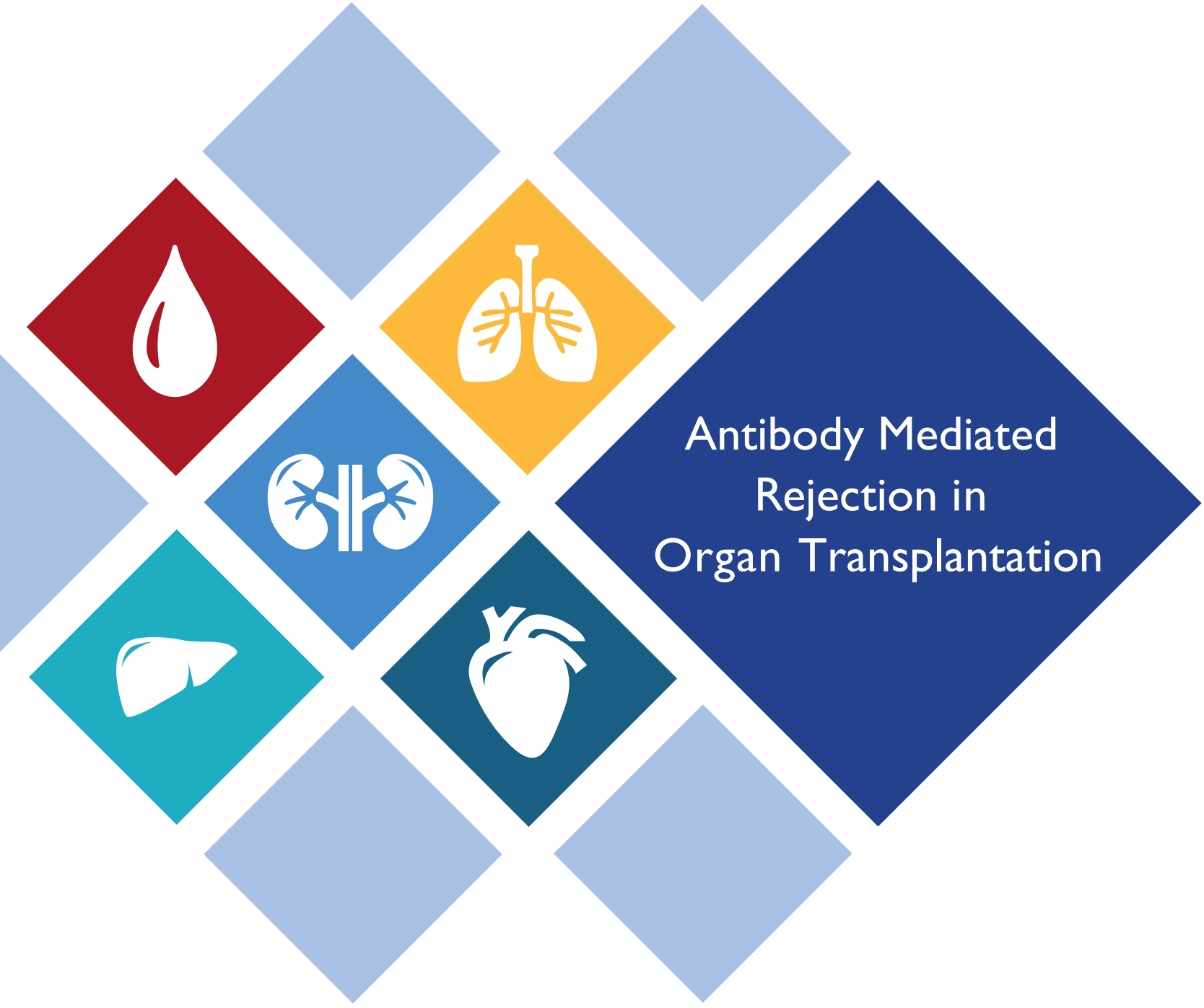

Antibody Mediated Rejection in Organ Transplantation

ONE LAMBDA

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One Lambda, Inc. has been the global leader in transplant diagnostics for over thirty years. Our comprehensive product portfolio includes a broad range of industry-leading antibody detection and monitoring solutions, HLA Typing products, laboratory instrumentation, and software. All our technologies are designed for flexibility and scalability to provide reliable results and fast turnaround.

Our history of innovation, reputation for quality, and industry-leading customer support are what enable us to fulfill our mission of improving the lives of transplant patients around the world.

Visit www.onelambda.com to discover how we can help you improve patient outcomes.

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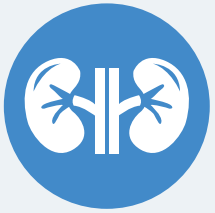

Introduction to HLA Antibodies

Kidney

Heart

Lung

Liver

Bone Marrow

Evolution of Antibody Detection

ONE LAMBDA
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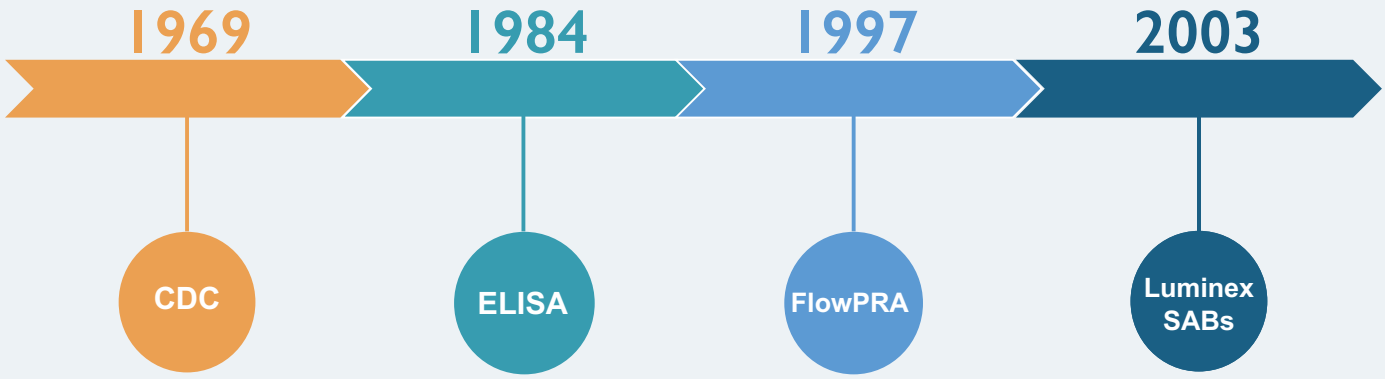
Decades of clinical evidence has shown that HLA-specific antibodies can be stimulated by a number of mechanisms. These include response to graft mismatched HLA antigens, paternal mismatched antigens through pregnancy, and via HLA antigens expressed on donor cells after a blood transfusion.

The discovery of hyperacute antibody-mediated rejection (ABMR) due to HLA-specific antibodies led to the introduction of the cytotoxic crossmatch in 1969, which drastically reduced the incidence of hyperacute rejection. Exactly 50 years later, ABMR remains a major challenge to successful transplantation. Today, a multitude of tools and strategies are available to improve risk stratification for the best patient outcome.

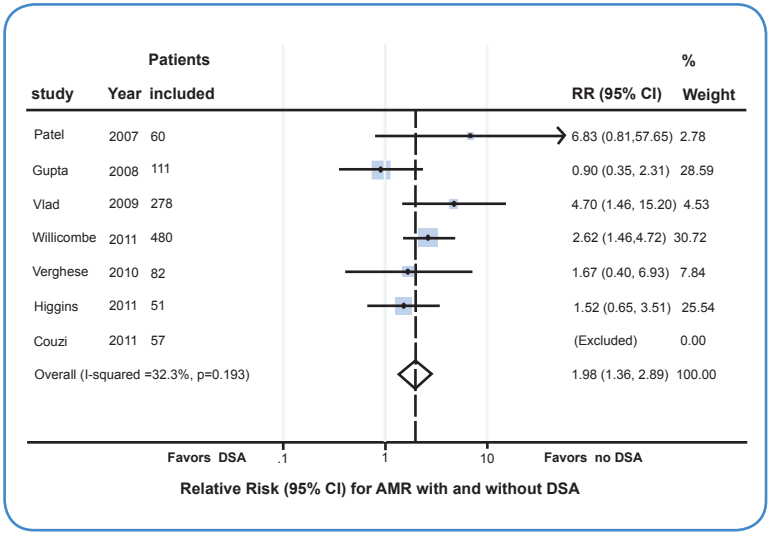


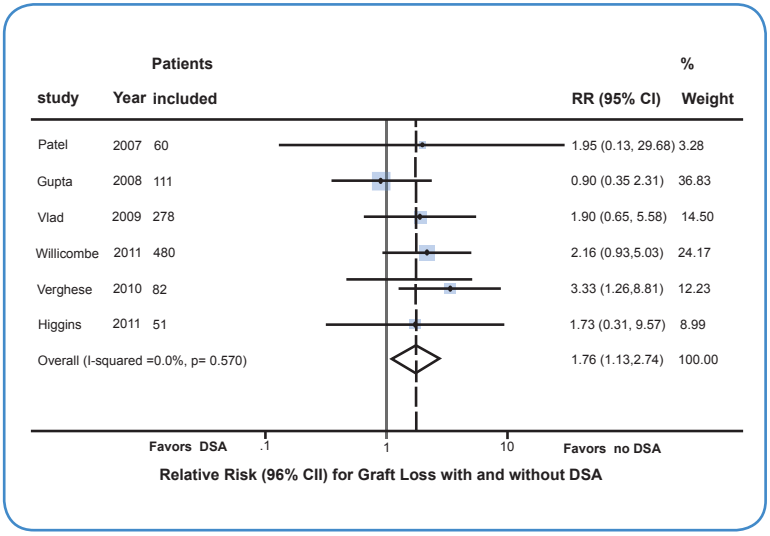
Donor Specific Antibody

What is a Donor Specific Antibody?

In addition to pregnancy and transfusion stimulated antibodies, patients can form HLA antibodies in response to transplant-mismatched antigens. These antibodies are commonly referred to as donor specific antibodies (DSAs).

In recent years, the literature has shown the importance of detecting DSA with enhanced sensitivity over and above traditional cellular crossmatch. In a pooled analysis of retrospective cohort studies, the presence of donor specific antibody (DSA) detected by solid phase assay, even with a negative flow cross-match, demonstrated statistical significance for increased risk for biopsy-proven ABMR and graft failure.



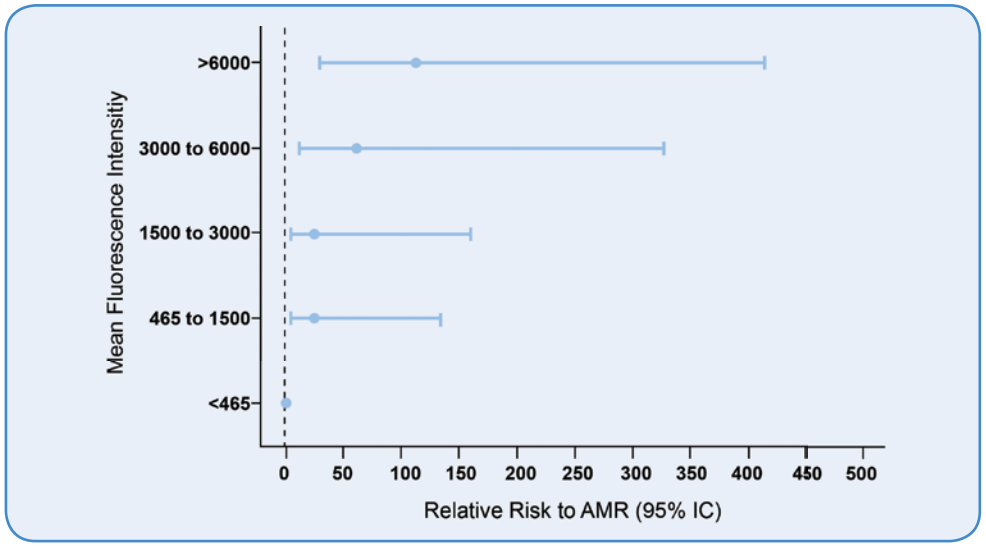


Mohan S, et al. JASN, 2012

Mean Fluorescence Intensity (MFI)

The readout from solid phase Luminex based assays is the mean fluorescence intensity (MFI) value. This value represents a semi-quantitative figure. Laboratories will define their own MFI cut-off values to determine where a clinically relevant HLA antibody is called. This value may be adjusted according to the HLA loci to which an antibody is directed and may also take into account additional factors related to patient immunological history, such as the presence of a repeat transplant mismatch or a known pregnancy-exposed antigen.

The example provided below shows the increased risk of ABMR when a threshold DSA MFI of 465 is exceeded and demonstrates how the risk of ABMR is increased with a concomitant rise in DSA specific MFI.



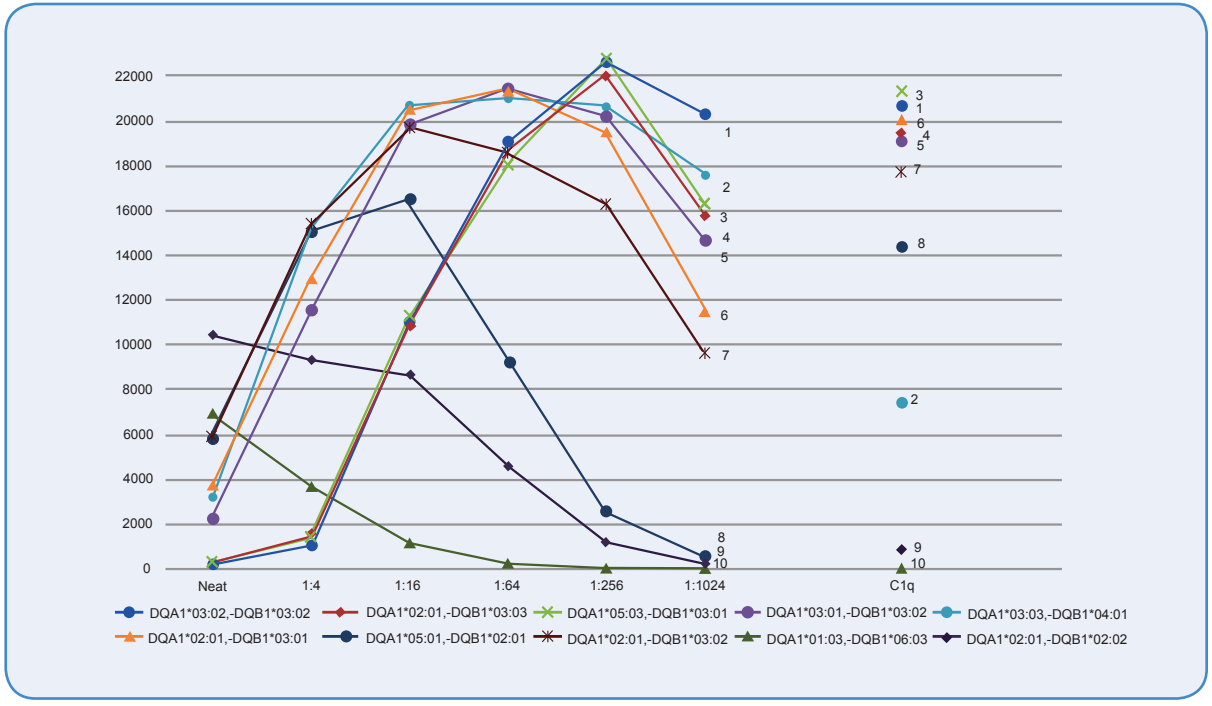
Adapted from: Lefaucheur C, et al. JASN, 2010

Donor Specific Antibody

MFI does not reflect antibody concentration

The study below shows how MFI does not always accurately reflect antibody concentration and that low MFI values may sometimes be associated with high titer antibodies. This phenomenon is often referred to as the "prozone effect," but this description is not entirely accurate as there may be multiple reasons why high titer antibodies give low MFI values. Putative mechanisms include complement factor inhibition, steric hindrance, antibody agglutination, and the presence of competing IgM.

Laboratories have developed different strategies to counter these effects, including serum dilution and pre-treatment with EDTA.

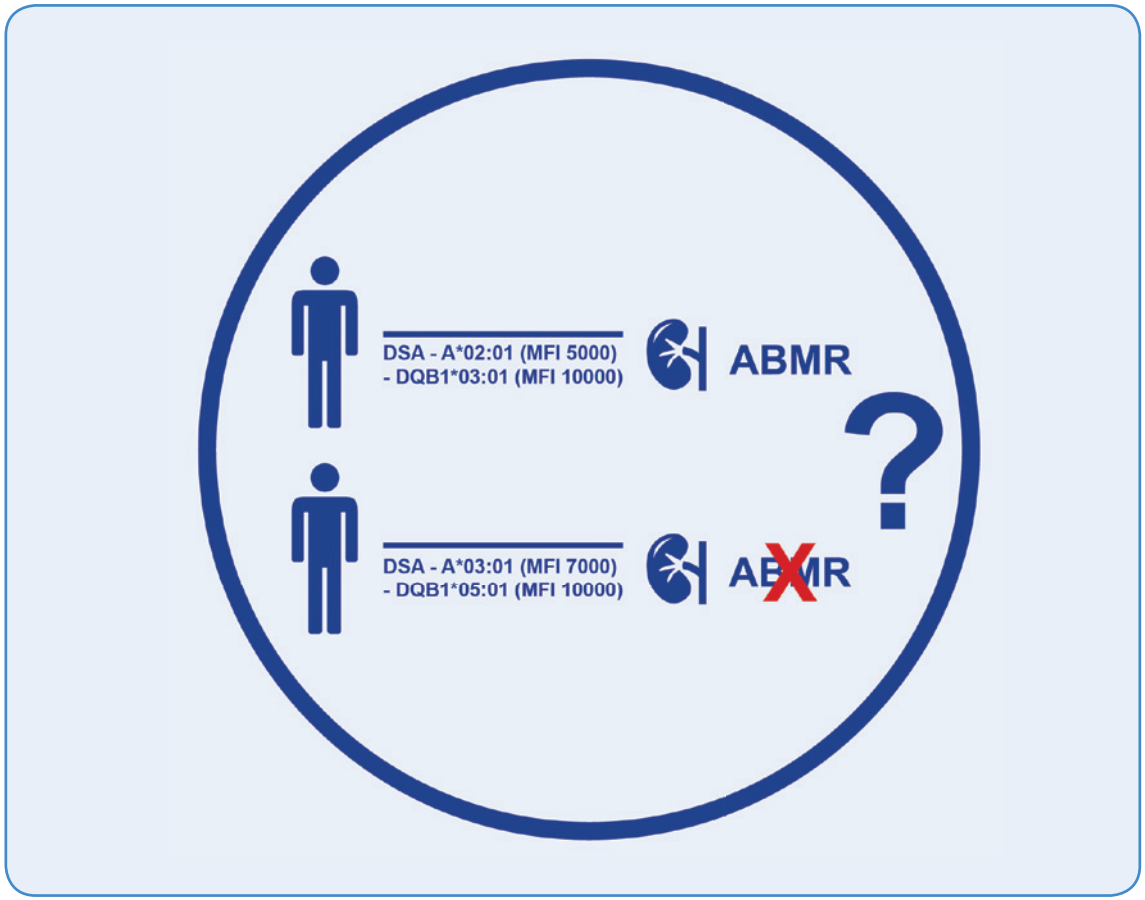


Antibodies present in serum in high titer may present as low MFI. Serum titration is required to reveal the real antibody concentration.

Tambur AR, et al. Am J Transplant, 2015

THE MFI Conundrum...

Many factors are likely to impact the capacity of DSA to cause organ rejection. Antibody affinity, avidity, and complement fixing ability are key considerations as are the specificity and tissue expression levels of the target donor antigen.

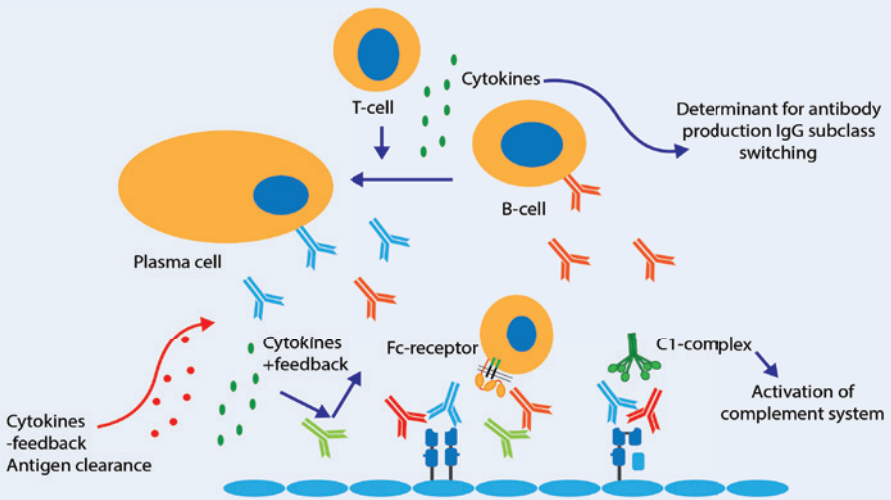


Donor Specific Antibody

Factors Influencing the Humoral Response

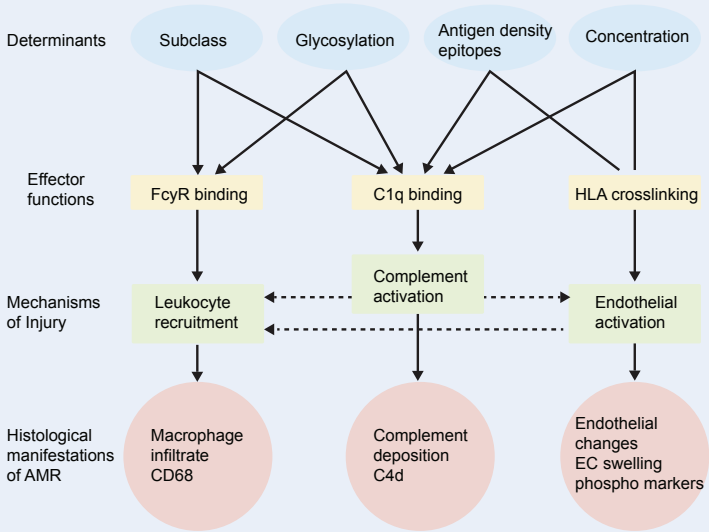
- Antibody Characteristics
  - Amount, number, HLA class
  - Binding strength to the target epitope
  - Ability to activate complement
  - Capacity to recruit cells via Fc-receptors
- Density of HLA-molecule expression on endothelial- cells
- Protective factors and absorptive capacity of endothelial cells

Adapted from: Schaub S, et al. Transpl Int, 2014



Antibody concentration determines activation of pathways that lead to ABMR lesions

Through the actions of structural, binding region, and kinetic properties, HLA specific antibodies can lead to a number of effector function pathways, which include Fc receptor binding and complement activation. Subsequent recruitment of immune effector cells ultimately leads to the histological manifestations of antibody mediated organ damage.

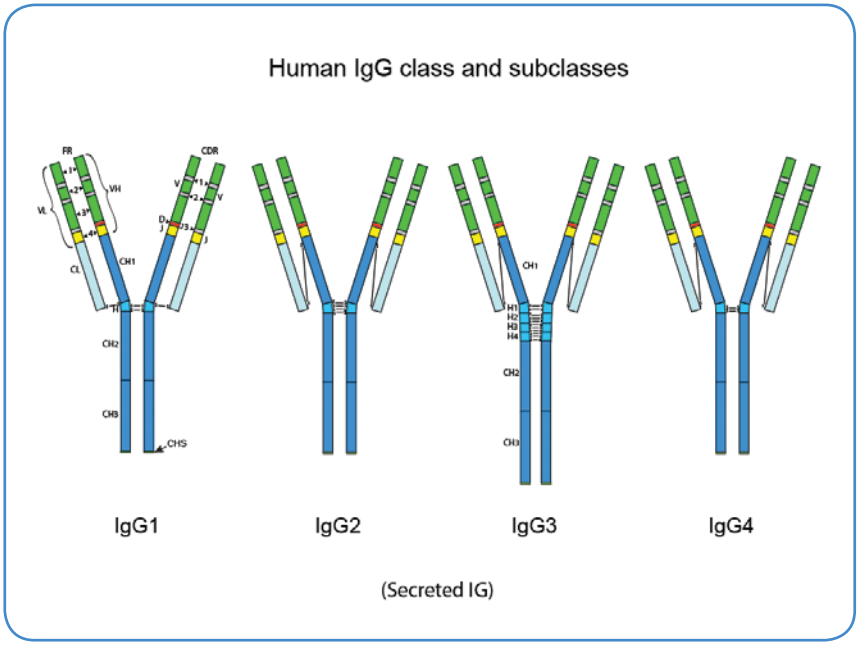


Thomas KA, et al. Trends Mol Med, 2015

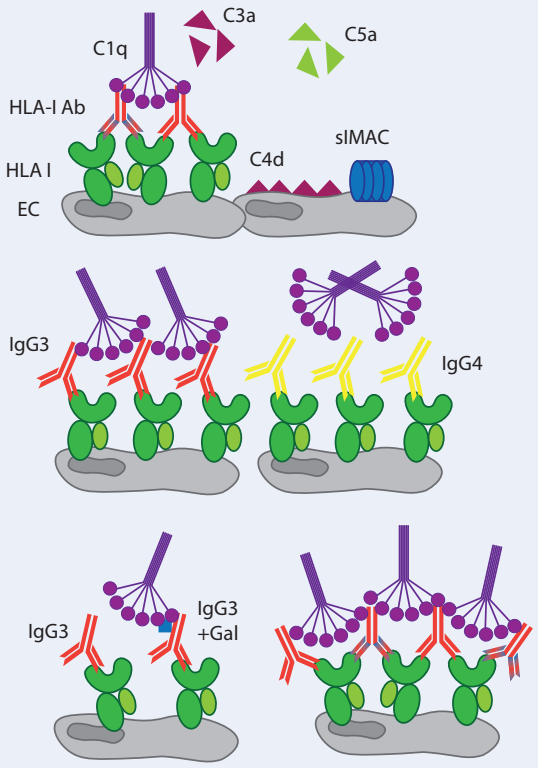
IgG Subclasses

What are IgG Subclasses?

IgG subclasses (IgG1-4) exhibit functional differences, such as the ability to fix complement and to bind to Fc receptors. IgG1 and IgG3 are the most effective at complement activation.



Schroeder HW, Jr and Cavacini L, J Allergy Clin Immunol, 2010



Complement Fixation

Although IgG3 has the greater binding efficiency to complement component C1q, IgG1 is the most effective at mediating complement dependent cell lysis.

Thomas KA, et al. Trends Mol Med, 2015

IgG Subclasses

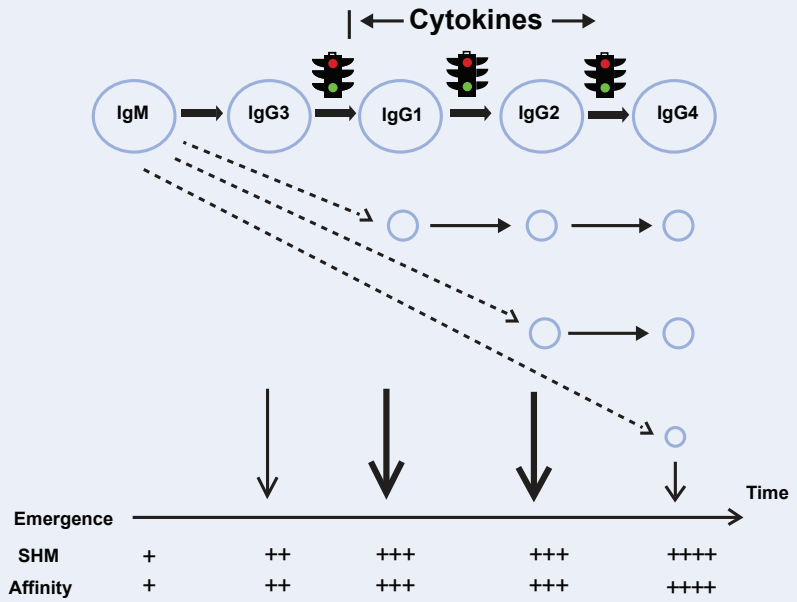
IgG Subclass Switching

The production of the various IgG subclasses occurs at different rates in the distinct germline gene order IgG3>IgG1>IgG2>IgG4. This system is strictly governed and controlled by the activity of T-lymphocytes as demonstrated in early mouse models where T-cell deficient mice exhibited greatly reduced rates of immunoglobulin class switching.

Adapted from: Nimmerjahn F and Ravetch JV, Science, 2005 and Bruhns P, et al. Blood, 2009 and Avery, et al. J Immunol, 2013

IgG Subclass Switching (continued)

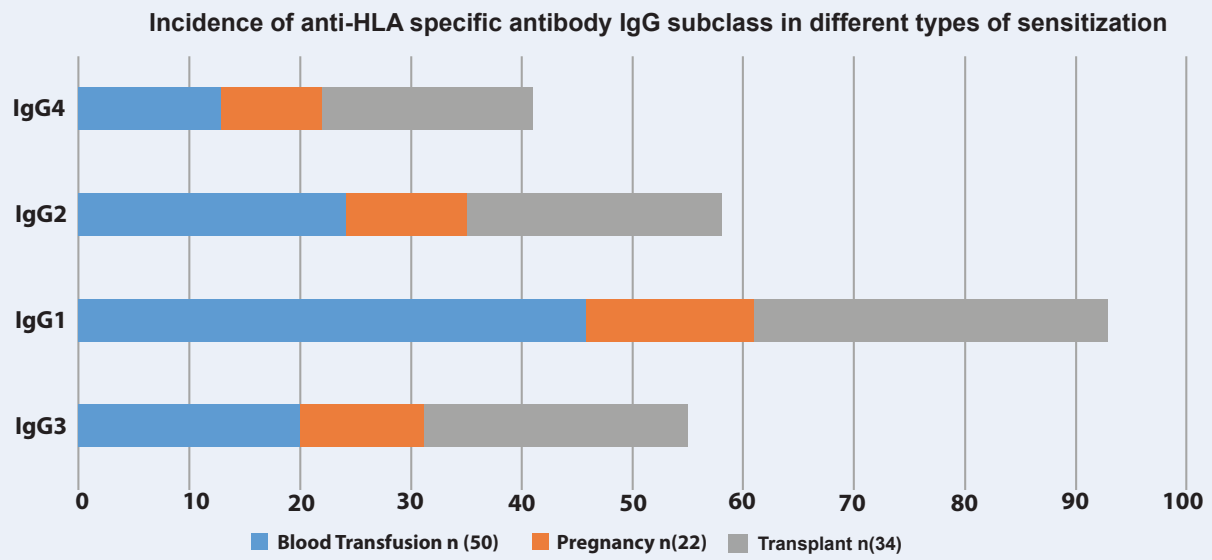
The early antibody response is typified by the presence of a powerful IgM response which usually subsides rapidly and is replaced by a more gradual and sustained IgG response, often resulting in greatly increased IgG titers.
Throughout this T-cell mediated cytokine driven process, the variable (antigen-binding) region of the antibody molecule remains unchanged but the isotype determining Fc region is altered. Thus the specificity remains the same, but the potential effector function of the antibody is altered.



Collins AM and Jackson KJ, Front Immunol, 2013

IgG Subclasses

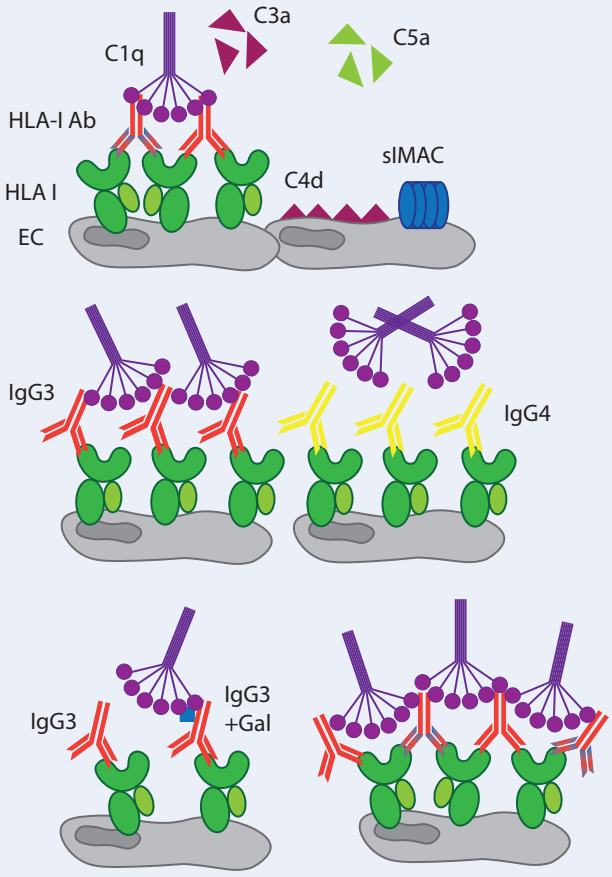
Pre-transplant sensitization



Different immunizing events generate different profiles of IgG subclasses. While transfusion alone presents a response with lower levels of IgG1; response to transplantation shows a tendency to a wider range of subclasses with higher levels of each subclass.

Adapted from: Lowe D, et al. Hum Immunol, 2013

Complement Activation



Conditions for complement binding

The level of complement activation might be dictated by density of HLA antigen on the surface of the cell. Proximity of antibody Fc regions is increased when multiple antibodies can bind the same molecule of HLA on the cell surface. Patients with high-titer polyclonal DSAs of multiple IgG subclasses may present exacerbated complement activation during times of inflammation.

Thomas KA, et al. Trends Mol Med, 2015

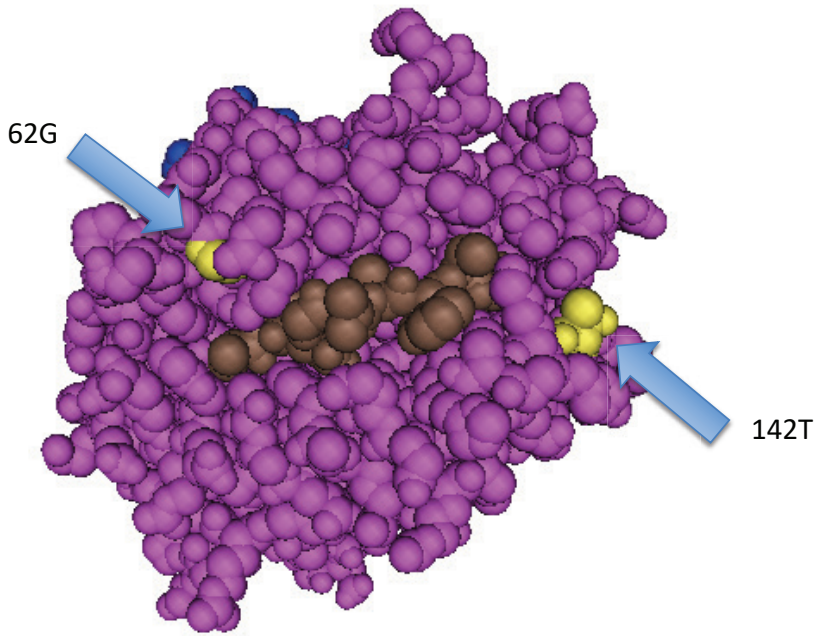
HLA Antibodies: Epitopes

What is an Epitope?

Traditionally, the degree of donor mismatching has been defined by virtue of counting the antigen mismatches at HLA-A,B, and DR loci. However, mismatched antigens have multiple epitopes that can induce formation of HLA-specific antibodies.

An epitope is the specific part of an antigen to which an antibody binds. Originally, epitopes formed the basis of serological cross-reactivity patterns, but today our knowledge of the amino acid sequence and molecular structures of HLA molecules have allowed us to further define the structural basis of HLA epitopes.

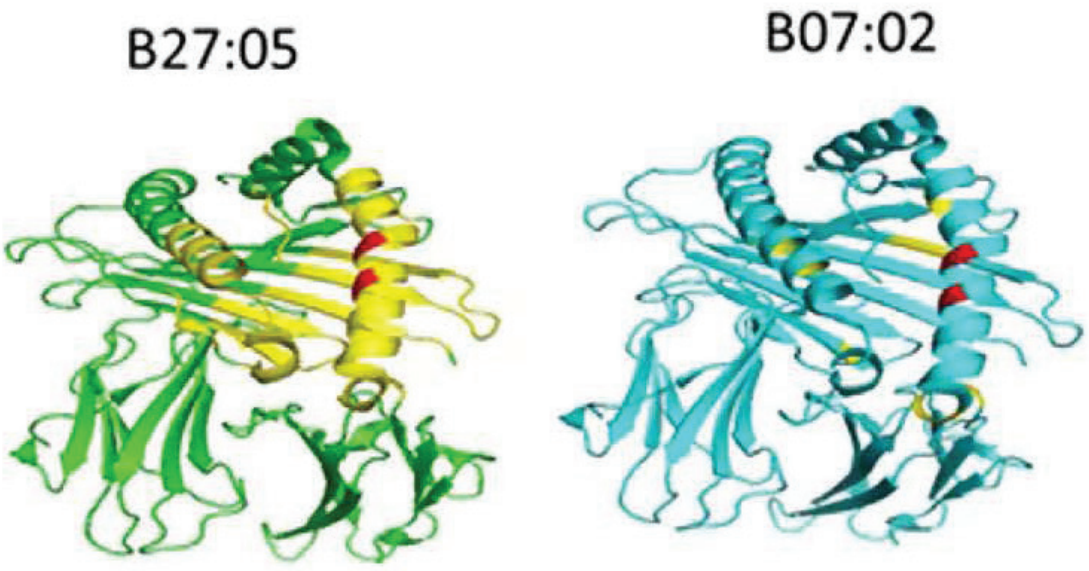
Three-dimensional structure of the HLA-A2 molecule (top-view). The highly polymorphic 62G and 142T epitopes are labeled and sit on either side of the bound peptide (brown).

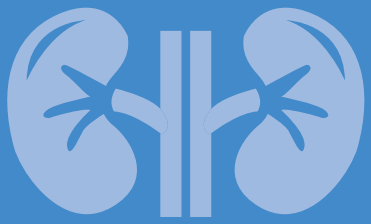


HLAMatchmaker

HLA Matchmaker is a computer algorithm that suggests histocompatibility at the epitope level. It uses the concept that antigenic proteins have functional epitopes consisting of amino acid residues that are about 3 Ångstroms apart from each other and at least one of them is non-self. Polymorphic residues within this radius are termed "eplets."

The molecular locations of class I eplet pairs that correspond to antibody-verified epitopes on the HLA-B27 and HLA-B7 molecules.

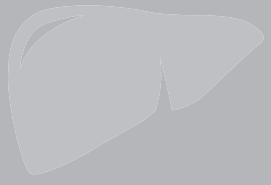




Kidney

Heart

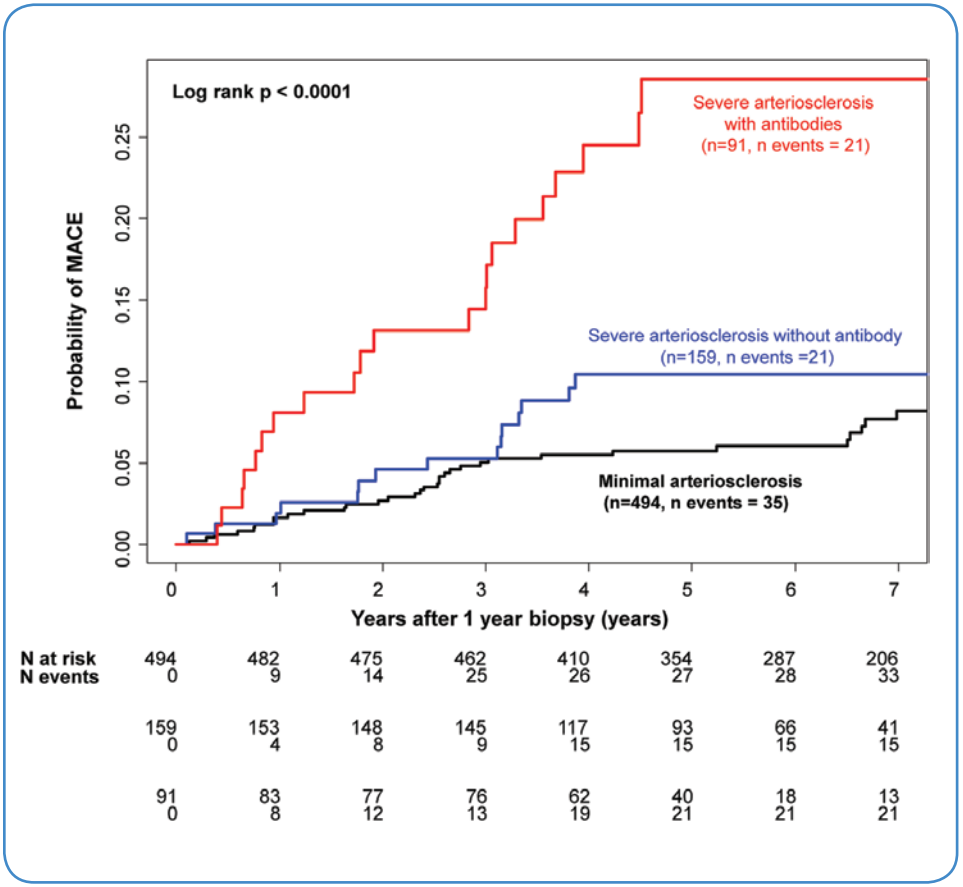
Lung



Liver

Bone Marrow

Impact of Donor Specific Antibodies in Accelerated Arteriosclerosis

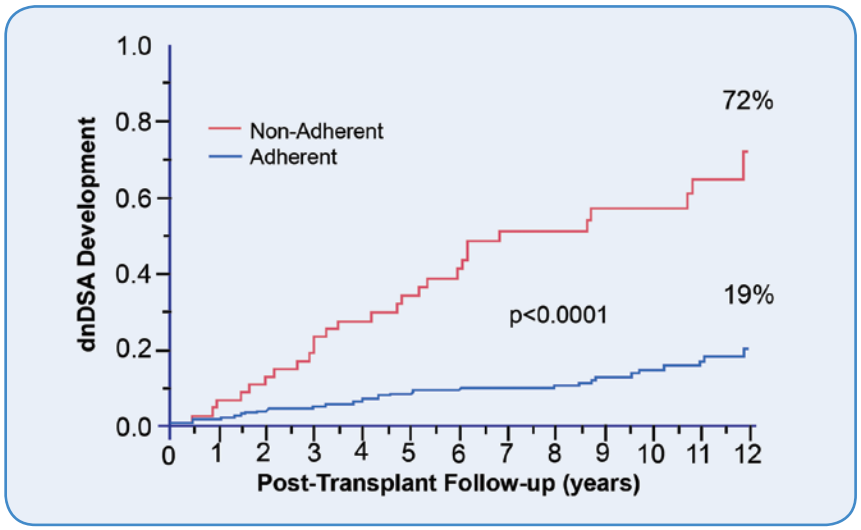


Loupy A, et al. Circ Res, 2015

Arteriosclerosis is a pathological condition characterized by brointimal thickening of the arteries, leading to the dysfunction of various organs. It is recognized as a primary cause of end-stage renal disease and kidney loss.

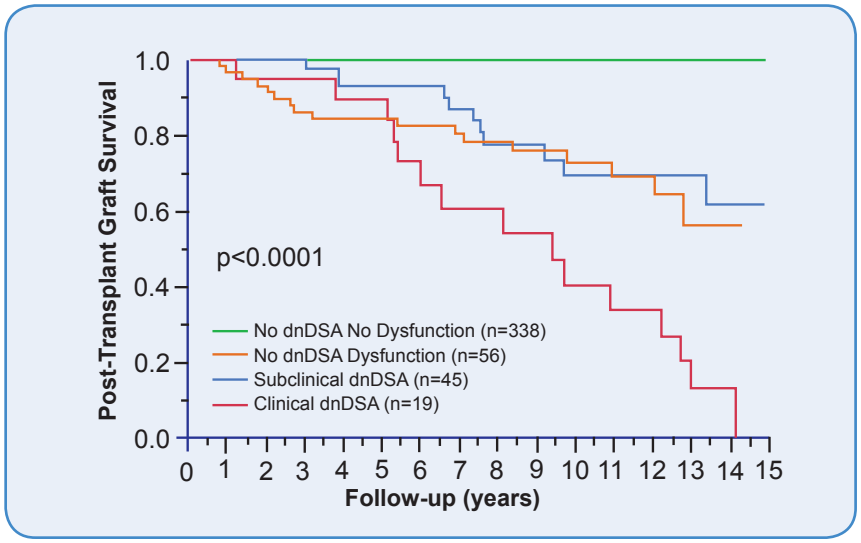
By evaluating 1065 kidney transplants, this study found that circulating anti-HLA antibodies are major determinants of severe arteriosclerosis, independent of traditional cardiovascular risk factors. The presence of anti-HLA antibodies is also related to an increased risk of major adverse cardiovascular events. This shows the importance of identifying patients with circulating anti-HLA antibodies to screen them for cardiovascular diseases and to aggressively treat traditional risk factors.

ABMR in Kidney Transplantation



DSA Post-Transplant

In a population of 508 low risk kidney transplant recipients (95% first transplants and PRA<25%), 64 formed DSA post-transplantation in a median time of 49 months. Of those DSA, 69% were directed against HLA class II. This study clearly highlights the importance of patients remaining adherent to immunosuppression to reduce the likelihood of DSA formation post-transplant.

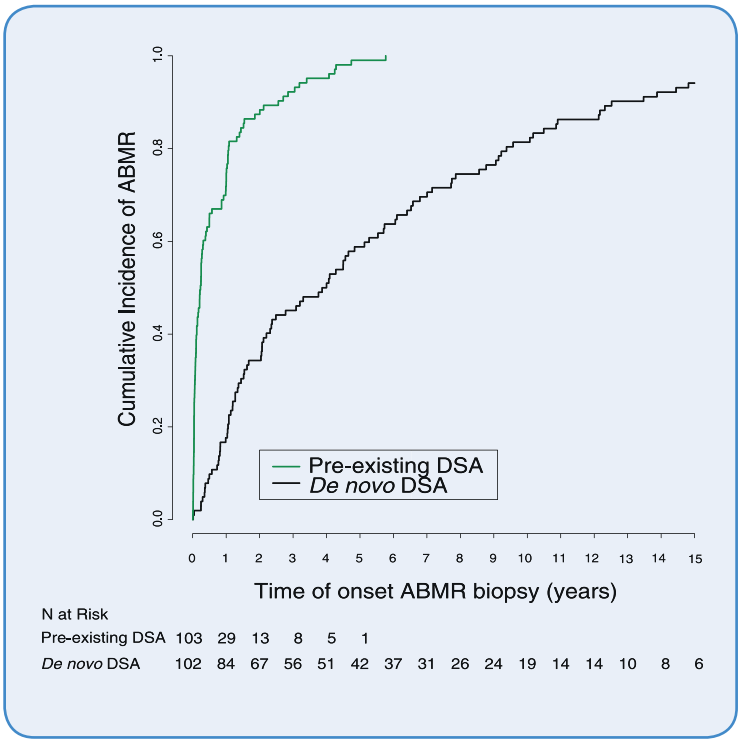


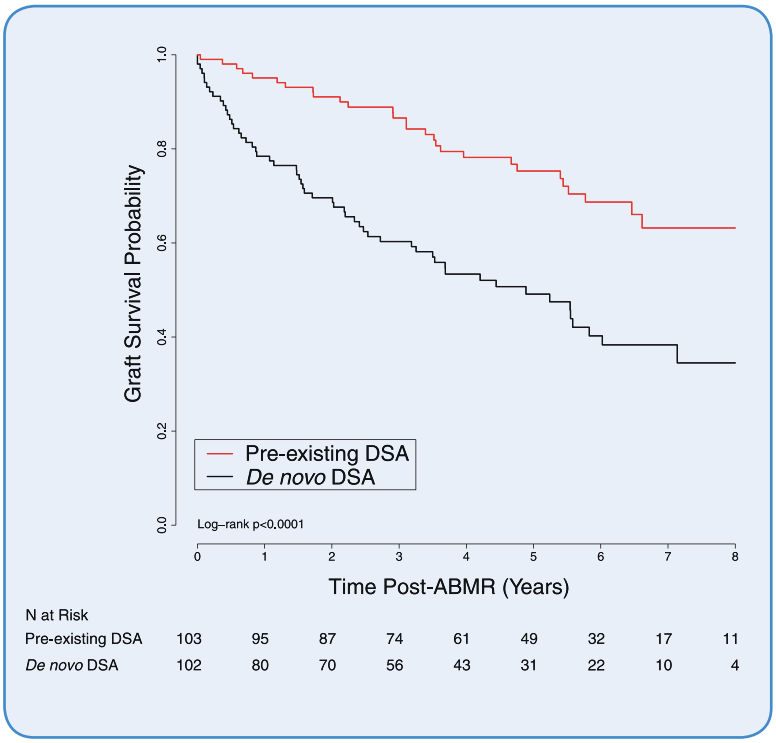
Both clinical and subclinical *de novo* DSA were associated with increase in graft loss. In the subclinical *de novo* DSA group graft loss was delayed. The authors concluded that the association of *de novo* DSA with subsequent graft loss suggests that screening for DSA post-transplant and early intervention could improve graft outcomes.

Wiebe C, et al. Am J Transplant, 2015

ABMR in Pre-existing DSA versus ABMR in *De novo* DSA

ABMR occurs at both a higher frequency and with an earlier onset when DSA is pre-existing as opposed to ***de novo*** DSA (left panel). Furthermore when ABMR occurs as a result of pre-existing DSA, this has a greatly increased negative impact on overall graft survival when compared to post-transplant ***de novo*** DSA formation.



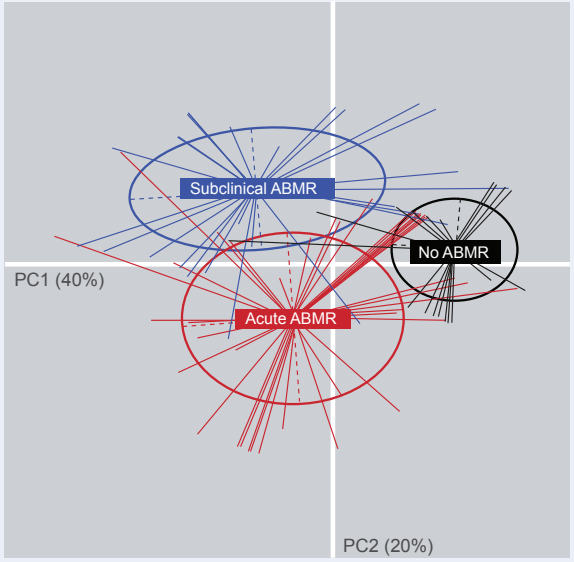


Aubert O, et al. JASN, 2017

IgG Subclasses in Kidney Transplantation

Post-transplant Clinical Impact of IgG Subclasses

In a retrospective study, acute antibody mediated rejection was shown to be associated with the prevalence of DSA of IgG3 isotype. In contrast the presentation of subclinical ABMR was shown to be associated with an increase in IgG4 DSA.



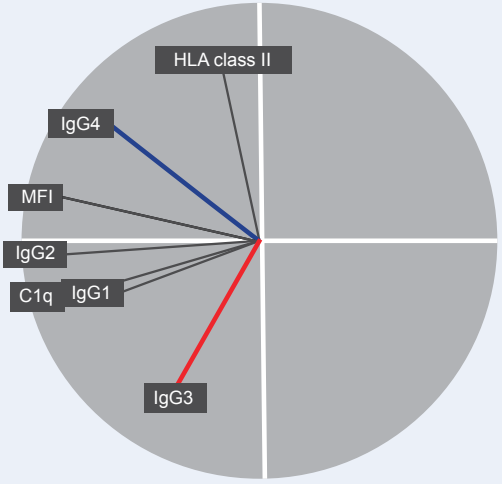


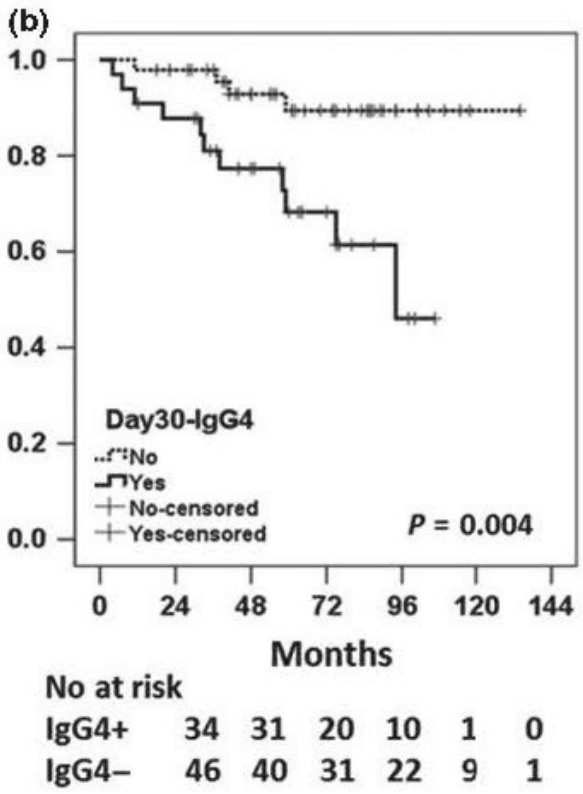
Figure on left: Principal component analysis identified three distinct clinical and histological patterns: acute ABMR (aABMR), sub-clinical ABMR (sABMR), and ABMR-free patients. The patterns are characterized by certain immunodominant DSA features: HLA class specificity, MFI, C1Q binding capacity, and IgG subclasses.

Figure on right: The horizontal axis distinguishes the ABMR-free pattern from antibody mediated injury. The vertical axis segregates aABMR from sABMR.

Lefaucheur C, et al. JASN, 2016

The Detrimental Impact of IgG4

A further retrospective study performed in recipients who received HLA incompatible kidney transplants showed that the presence of IgG4 DSA pre-transplant led to greatly reduced long-term graft survival.

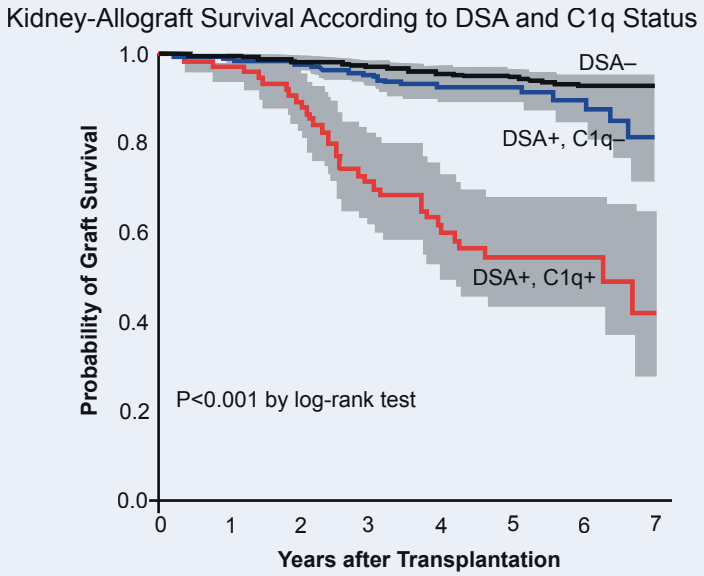


Khovanova et al 2015

Factors Influencing Kidney Transplantation Outcome

Impact of C1q Detection on Kidney Transplantation

The clinical impact of complement binding antibodies on kidney transplant survival was demonstrated by this study, which showed that patients who develop C1q+ DSA after transplantation have significantly poorer graft survival than those patients who only develop C1q- DSA.



No. at Risk

DSA–	700	698	667	612	504	338	164	38
DSA+, C1q–	239	237	227	181	139	80	44	14
DSA+, C1q+	77	75	68	48	37	20	12	5

Loupy A, et al. NEJM, 2013

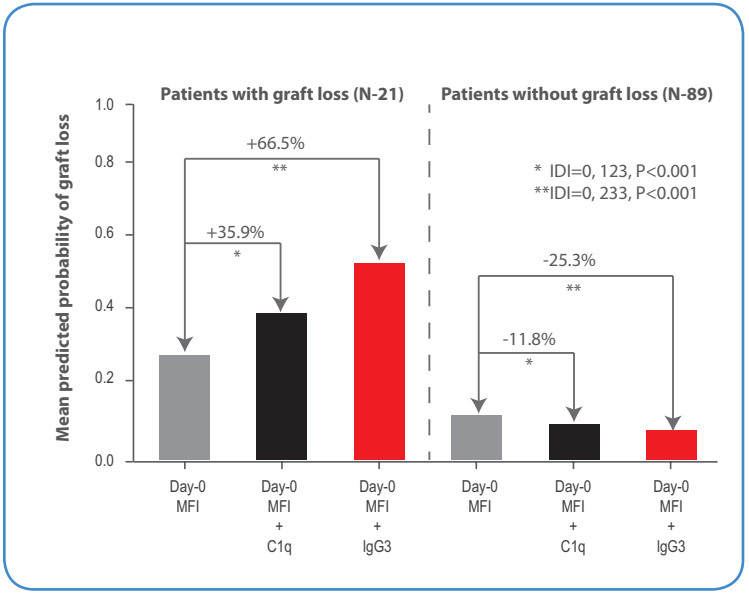
Factors Influencing Kidney Transplantation Outcome

Kidney

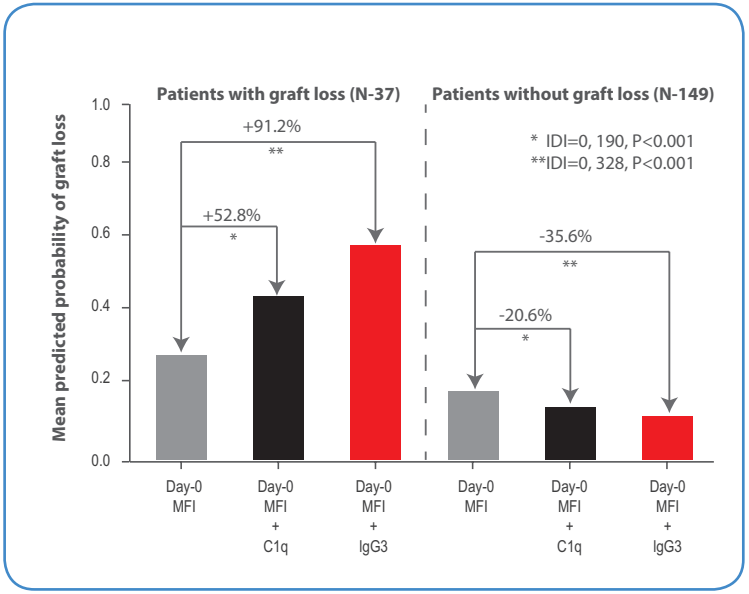
Functional Characteristics of Antibodies: Compounding Effect on Graft Loss

By considering IgG3 and C1q-binding anti-HLA DSA status in addition to MFI Level, calculated risk of allograft loss is better understood. Where DSA is characterized by presence of IgG3 and C1q binding ability, risk of graft loss is greatly increased.

Peri-transplantation



Post-transplantation

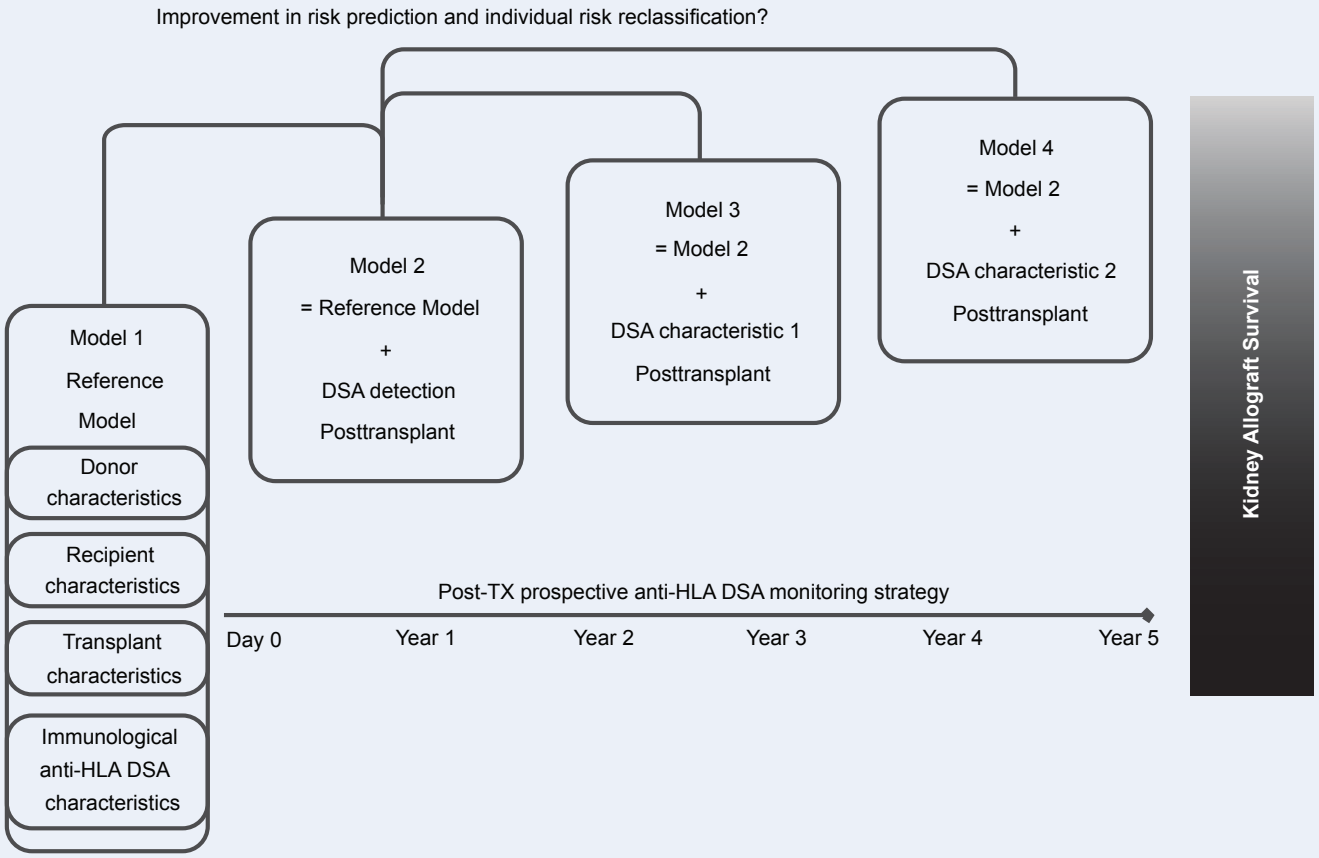


Viglietti D, et al. JASN, 2016

Factors Influencing Kidney Transplantation Outcome

Improving The Model For Risk Stratification

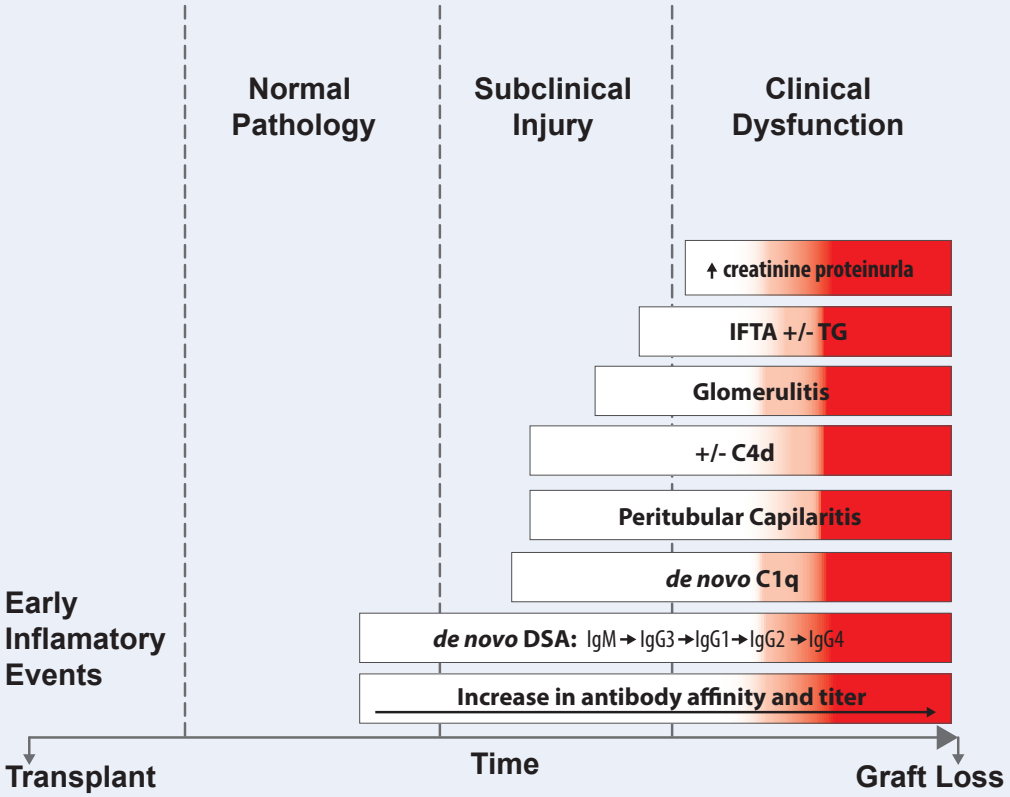
Lefaucheur et al have devised models for risk stratification based upon both donor and recipient characteristics as well as DSA properties. These properties include strength, complement-binding capacity, and IgG subclass composition.



Lefaucheur C, et al. J Immunol Res, 2017

Evolution of the Antibody Immune Response Leading to ABMR

The importance of post-transplant monitoring was also shown by Lefaucheur et al as they demonstrated that *de novo* DSA formation is often one of the earliest biomarkers of the immune response leading to ABMR. DSA is often detectable before any of the clinical indicators, such as peritubular capillaritis, C4d deposition, or increased serum creatinine.

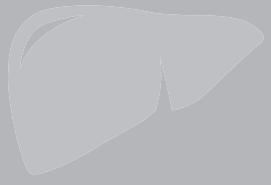


Adapted from: Lefaucheur C, et al. JASN, 2016

Kidney

Heart

Lung

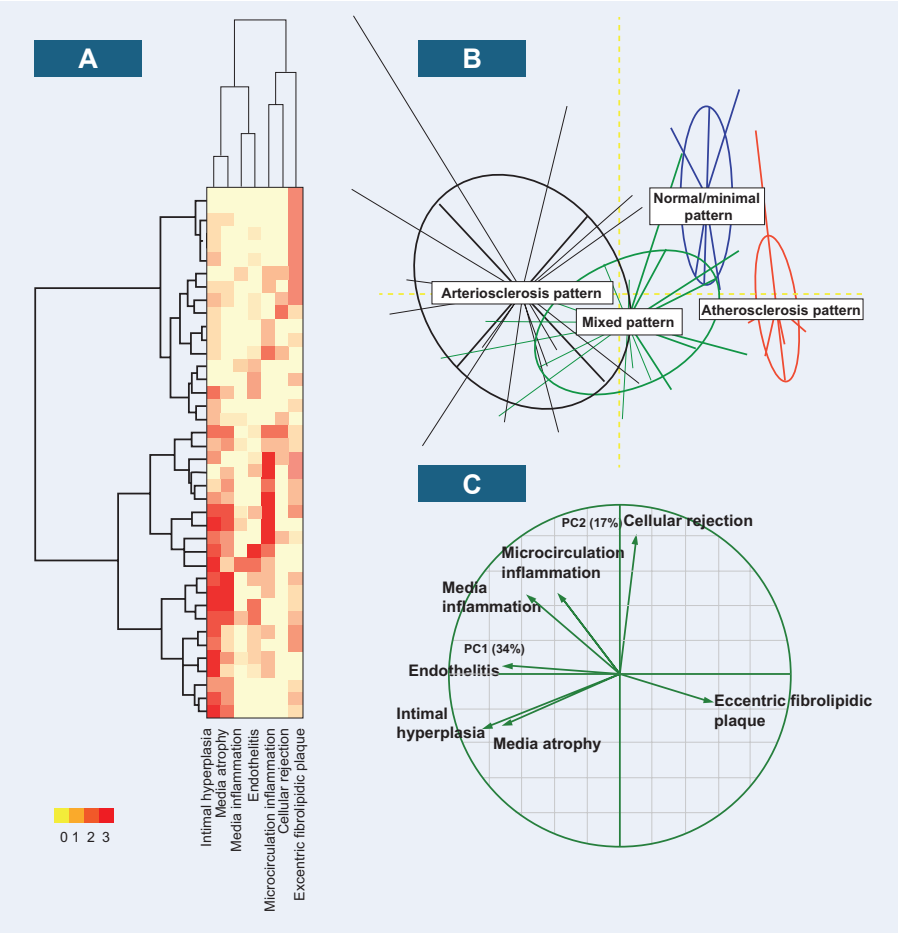


Liver

Bone Marrow

Dynamic Process of ABMR

Recent studies on the role of DSA in ABMR after heart and lung transplantation have shed new light on the mechanisms of rejection and have generated improvements in the use of diagnostic tools for risk stratification. In an assessment of explanted heart allografts for late allograft failure, Loupy et al showed that ABMR may not be the result of a single rejection. Instead, they describe a dynamic process of continuous and indolent ABMR contributing to a chronic form of antibody mediated injury that often leads to late allograft failure.

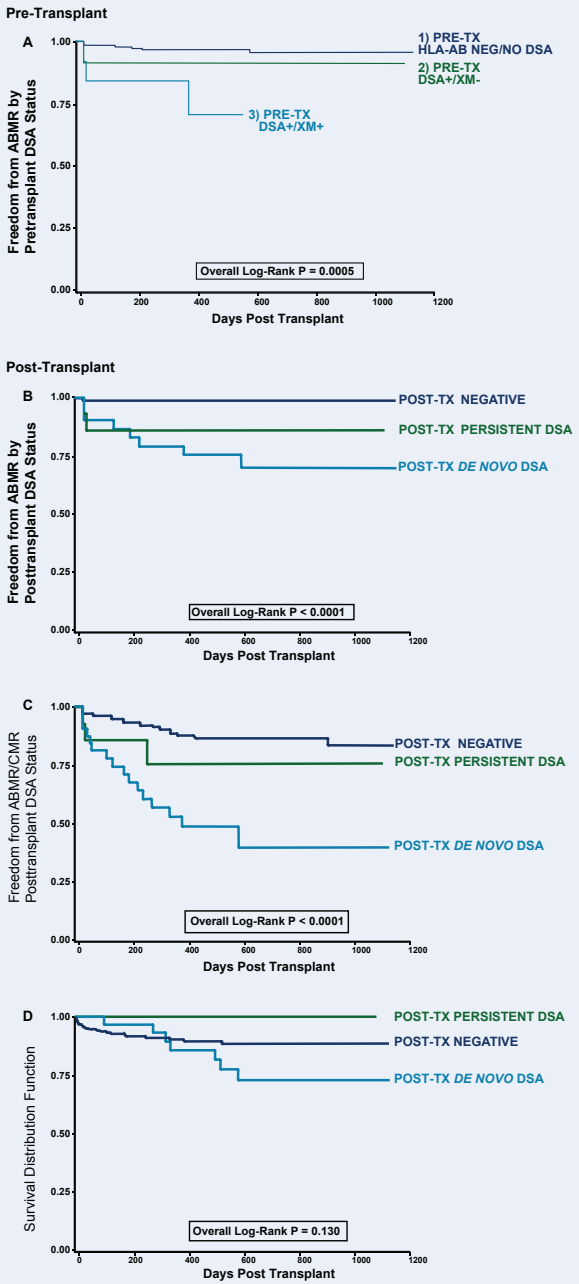


Explanted allograft phenotypes according to cluster, a principal component analysis (PCA). (A) Individual overview of morphological and immunological profiles of failing allografts according to unsupervised cluster analysis. **(B)** Unsupervised PCA of failing allografts. **(C)** The correlation circle interpreting the meaning of the PC axis shows the correlation and anti-correlation between various parameters.

Loupy A, et al. Am J Transplant, 2016

ABMR in Heart Transplantation

Freedom from Antibody-Mediated Rejection by DSA Status



Comprehensive Antibody Testing and Heart Transplantation

Reinsmoen et al devised a risk stratification process to prioritize unacceptable antigens. Four antibody detection methods were employed: (1) Single antigen beads, (2) SAB at 1:8 serum dilution, (3) C1q SAB, and (4) CDC panel to allow for strategic prioritization of UA assignment across DSA barriers. This led to survival rates comparable to DSA negative heart transplant recipients.

Although there was no difference in overall survival based on DSA pre-transplant status in 3 years, patients that developed de novo DSA had a higher incidence of ABMR and Cellular Mediated Rejection (CMR). Those results suggest that post-transplant antibody monitoring is critical for applying immunosuppressive therapies early enough to decrease worse impact on graft outcome.

(A) Freedom from ABMR by pre-transplant DSA status. **(B)** Freedom from ABMR, **(C)** freedom from ABMR/CMR and **(D)** overall graft survival by post-transplant DSA status. (Adapted from: Reinsmoen, et al)

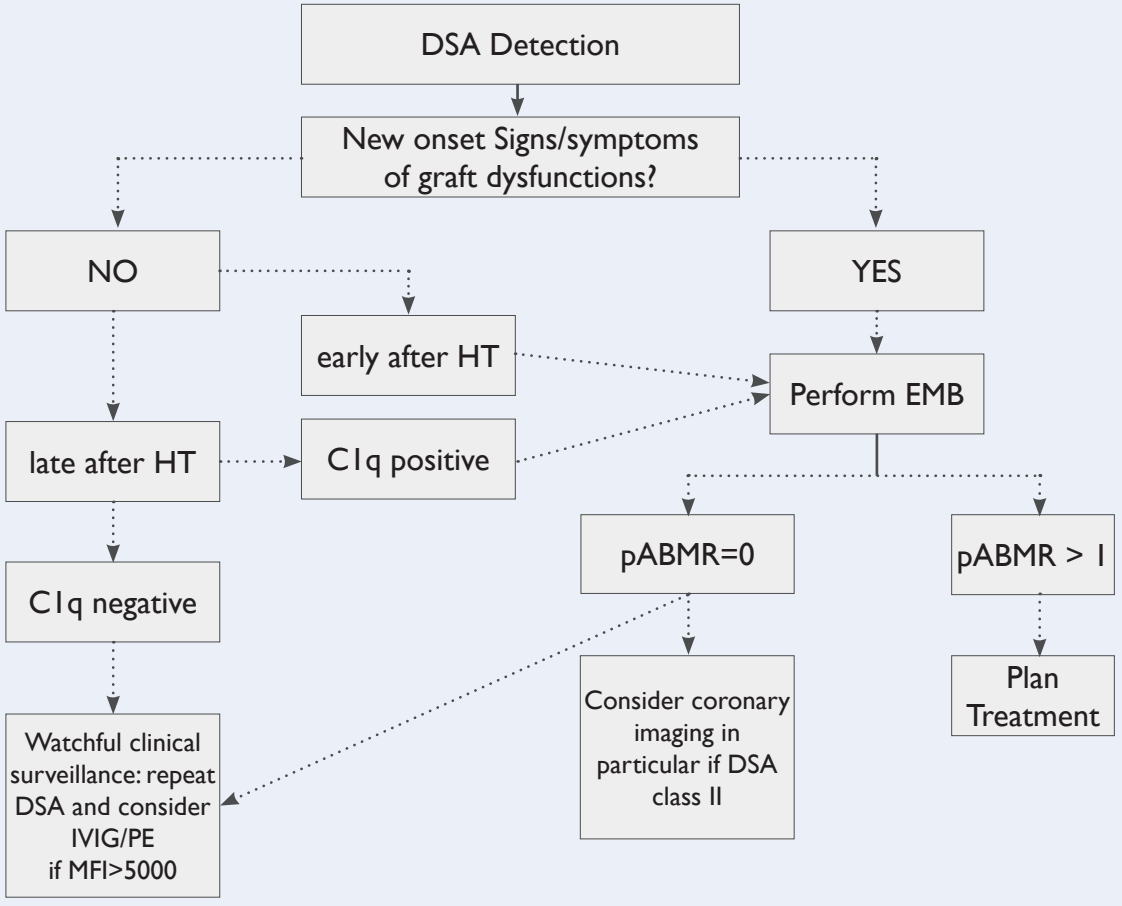
The Management of Antibodies in Heart Transplantation: An ISHLT Consensus

- "Solid-phase assays, such as the Luminex SAB assay, are recommended to detect circulating antibodies.
- Standardization of operating procedures and manufacturing processes for solid-phase assays is needed to decrease the inter- and intra-laboratory variability of assay results, so that they may be used in multicenter clinical trials.
- Patients at risk for sub-optimal outcome post-transplant are defined as having a PRA >10% or donor-directed antibodies at the time of transplantation.
- Post-transplantation monitoring for DSA should be performed at 1, 3, 6, and 12 months post-operatively in accordance with ISHLT guidelines. Patients who are low risk should be monitored annually for DSA after the first year. Sensitized patients should be monitored more frequently
- DSA testing should be performed for any patient presenting with symptoms or signs of graft dysfunction.
- DSA with graft dysfunction and restrictive physiology should be considered for treatment.
- DSA that remain at higher dilutions, C1q+ DSA antibodies, DSA that persist, and DSA arising late after transplantation have been associated with adverse outcomes. Further research is required to determine whether treating antibodies in these situations would improve outcomes.
- The clinical significance of antibodies against non-HLA antigens such as MICA are equivocal and require further validation.
- First-line therapies for desensitizing patients include IVIg, plasmapheresis, immunoadsorption, and rituximab.
- Randomized, controlled trials are needed to assess the benefit of treatment in both pre- and post-transplant sensitized patients. Ideally, centers would agree on and use the same desensitization protocols so that data derived from treated patients would be comparable.
- Other future studies will include: identifying which antibodies require treatment; whether sensitization in MCS device patients requires different treatment approaches; assessing the role of non-HLA antibodies; and validating the suspected causal link between antibodies and CAV.
- An antibody registry is suggested to assist in the facilitation of research."

Kobashigawa J, et al. J Heart Lung Transplant, 2018

ABMR in Heart Transplantation

Decision-Making Algorithm Following Donor-Specific Antibodies Surveillance



Manfredini V, et al. Curr Opin Organ Transplant, 2017

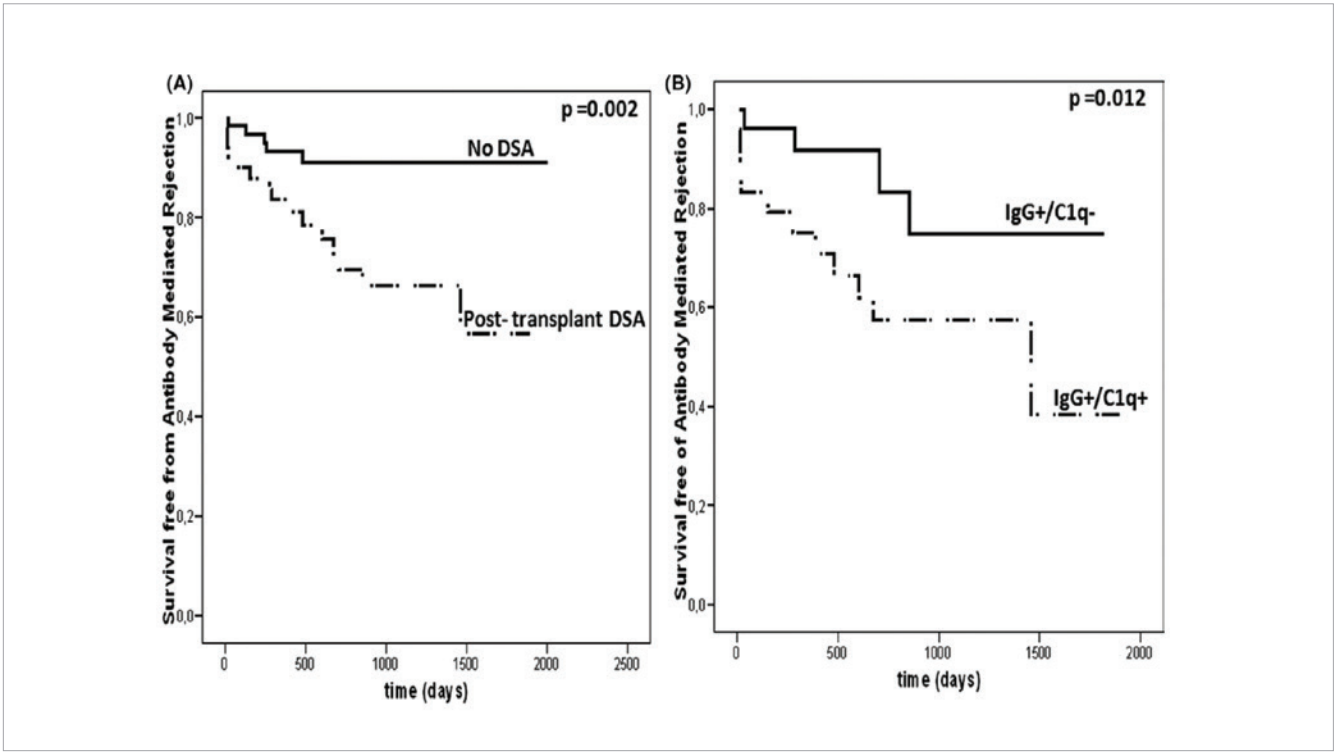
Decision-Making Algorithm Following Donor-Specific Antibodies Surveillance (continued)

"When [DSA formation] is within the first year after transplantation, [it is] more often associated with acute rejection responding to treatment. Late-onset donor-specific antibodies, in particular when not associated with complement binding activity, may lead to chronic injury, initially difficult to diagnose, that may express with CAV development. This may justify not performing endomyocardial biopsy [EMB] in asymptomatic patients with late-onset donor-specific antibodies, but may support the need for a low-toxicity therapy such as intravenous immunoglobulins. The association of donor-specific antibodies with pathological antibody-mediated rejection findings on the other hand, justifies specific treatment, in particular if associated with signs of graft dysfunction."

Manfredini V, et al. Curr Opin Organ Transplant, 2017

Monitoring DSA Post-Heart Transplantation: The Impact of C1q

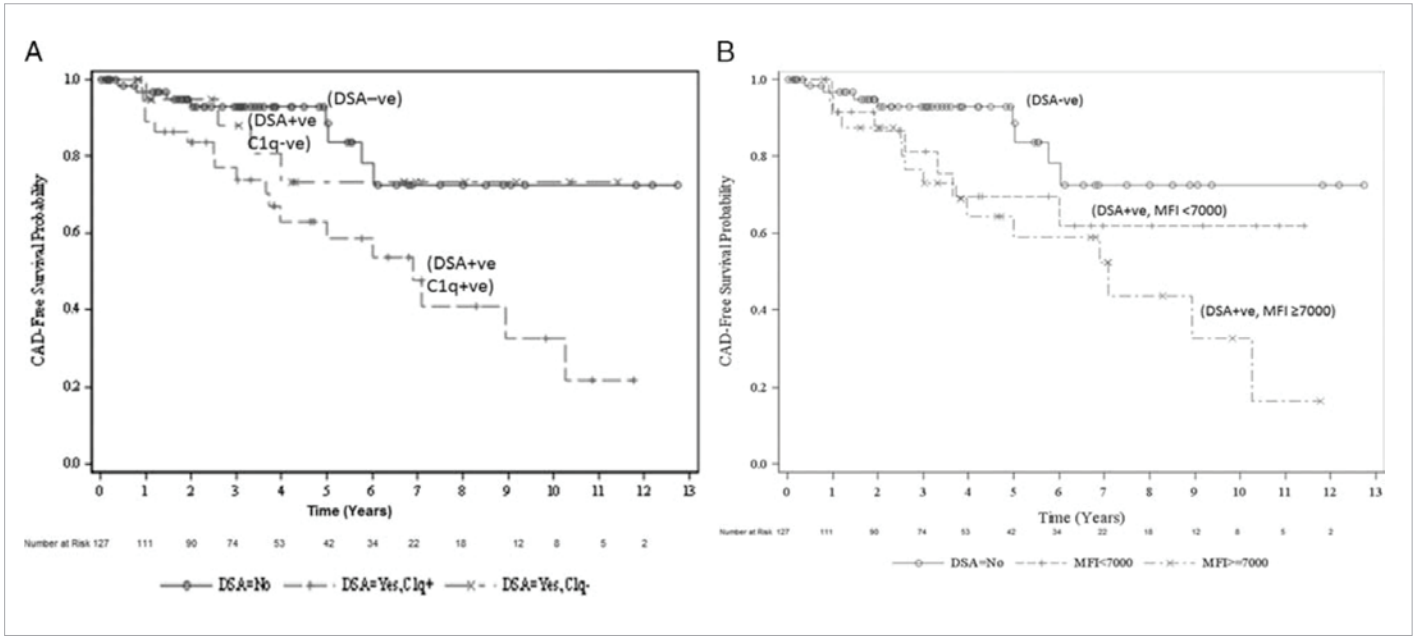
The clinical utility of post-transplant DSA monitoring was demonstrated in a recent study of 121 heart transplant recipients. Fifty-two patients developed post-transplant DSA, and this cohort showed greatly increased rates of ABMR (p=0.02). The presence of C1q-fixing antibodies also showed a trend towards a higher incidence of ABMR, albeit not quite reaching statistical significance.



Farrero Torres M, et al. Clin Transplant, 2017

C1q-Binding *De Novo* DSA After Heart Transplantation

The authors evaluated a population of 127 pediatric heart transplanted patients. Fifty-nine (46.4%) patients developed ***de novo*** DSA, of those 37 had C1q+DSA. C1q-binding DSA was an independent risk for the development of coronary artery vasculopathy (CAV) identified by multivariate analysis (Hazard ratio = 3.25; 95%CI 1.33-7.93; p=0.0095). DSA strength of 7000 MFI or greater better correlated with C1q-positivity, but independently was not associated with CAV. The authors concluded that close monitoring of DSA strength in MFI and C1q-binding may be useful for identifying patients at risk for the development of CAV.



Das B B, et al. Transplantation, 2018

Comprehensive Antibody Testing and Heart Transplantation

Cited publications from the last 6 years (2010–present) showing the impact of HLA antibody on heart transplantation in pediatric recipients.

Reference	Number of patients (study period)	Method	DSA	GS	AMR	CAV	Comments
Rossano et al. (31)	3,534 (October 1987–May 2004, follow-up through May 2008), UNOS database	CDC-PRA/XM most commonly used	PRA >10% = 387 (11%); 9% XM+	Median graft survival PRA >10% = 7.1 y PRA 1–10% = 9.6 y PRA 0% = 9.8 y			Decreased long-term GS in patients with PRA >10%
Irving et al. (38)	59, mean post-Tx follow-up 5.1 y (range 0.7–18.5 y)	Luminex screen/SAB	*N* = 4 (7%): 1 transient Class I, 3 persistent Class II	DSA+: 1/4 functioning, 2/4 retransplanted, and 1/4 died (7 y post-Tx)	DSA+: 2/4 (50%); non-DSA+: 1/15 (7%); no Ab: 5/40 (13%)	DSA+: 3/4 (75%); non-DSA+: 1/15 (7%); no Ab: 3/40 (7.5%)	Severe cellular rejection (≥3R) *n* = 3 (5.1%), all DSA–
Chin et al. (42)	18 (June 2007–February 2009)	CDC-XM, SAB, SAB-C1q, Flow CXM	SAB-IgG DSA: Pre-Tx 61.1%, Post-Tx 55.5%; SAB-C1q DSA: Pre-Tx 21.4%, post-Tx 35.7%	94% (1 y), 82% (2 y)	Within 1st month: *n* = 5 (27.7%), all post-Tx SAB-C1q+ DSA		SAB-C1q assay may better predict early AMR
Mahle et al. (32)	1,904 (January 1993–December 2008) Pediatric Heart Transplant Study Group	CDC-PRA most commonly used	PRA ≥ 10% = 397 (15.8%); PRA ≥ 50% = 189 (7.6%)	1 y patient survival: PRA ≥ 50%, 73 vs. 90% for PRA <10%		No CAV association with pre-Tx Ab	No association of PRA with time to 1st rejection or CAV
Ho et al. (16)	108 (January 2000–December 2009)	CDC-PRA, SAB	PRA >10% Class I = 9% Class II = 14%	87% GS in CDC– vs. 33% CDC+ after 7 y			Correlation between AMR and presence of CDC- or SPA-detected DSA
Scott et al. (34)	101 (2004–2008)	CDC-PRA, FLOW		PRA >25% decreased GS vs. patients with PRA <25%	*n* = 12: 33% with PRA >80% vs. 13% with PRA <80%		
Peng et al. (44)	60 (October 2005–January 2011)	FLOW-PRA, SAB, 183 paired DSA and C4d			6 (3/6 XM+)		Correlation between C4d+ in EMB and DSA >6,000 MFI
Daly et al. (58)	134 (January 1998–January 2011)	CDC-AHG PRA, Luminex SAB; XM+ patients received preoperative plasmapheresis + IVIG	12 XM+ (9%) T+/B+ = 8 T–/B+ = 2 T+/B not tested = 2	No significant difference in GS for XM+ (*n* = 3, 25%) vs. XM– (*n* = 12, 10%)	1 yr post-Tx: XM+ = 6 (50%), XM– = 2 (2%) ($p < 0.001$)		Serious infection higher in XM+ vs. XM– (50 vs. 16%, $p = 0.005$); shorter time to 1st infection in XM+ ($p = 0.001$)
Asante-Korang et al. (35)	70 (January 2005–July 2013)	Luminex PRA, SAB, Flow-XM; desensitization performed in patients with PRA >10%	PRA >10% = 14 (20%)	Overall patient survival: 92.9% in sensitized group vs. 80.4% in non-sensitized	Freedom from AMR or rejection grade ≥2R/3A: 71.4% in sensitized vs. 64% in non-sensitized	Freedom from CAV: 93% for sensitized vs. 91% in non-sensitized	12/14 high PRA patients had reduced Ab levels following desensitization; no significant differences in outcomes between desensitized patients and those with no Ab

(*Continued*)

Mangiola M, et al. Front Immunol, 2017

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Reference	Number of patients (study period)	Method	DSA	GS	AMR	CAV	Comments
Chen et al. (36)	25 (January 2008–June 2010)	PRA and SAB, 195 samples	12/25 dnDSA	No impact short-term survival			Majority of dnDSA within 1 y
Irving et al. (47)	108 (1996–2009)	SAB, 691 samples	43 DSA (58% persistent) Class I = 30% Class II = 47% Class I + II = 23%	9/14 with graft loss had persistent DSA		9/10 with CAV DSA+; 6/9 DSA persistent	Persistent DSA associated with poor outcome and CAV
Godown et al. (39)	121 (1987–2014), mean follow-up 4.1 y	Flow, Luminex, all were XM–	dnDSA: 40 (33%) Class I = 24% Class II = 50% Class I + II = 26%				Multiple factors influence DSA development; DSA seen more frequently in patients with prior sensitizing events
Ware et al. (43)	66 (January 2009–September 2013)	SAB	27 DSA+ (4 XM+)	No impact	DSA level associated with pAMR2, 3	No impact	Negative predictive value of DSA testing for absence of pAMR
Tran et al. (37)	105 (January 2002–December 2012, follow-up 0.13–10.8 y)	SAB (5 times first year and yearly after)	45 (43%) DSA Class I = 20% Class II = 62.2% Class I + II = 17.8%	5 y GS 72.4% DSA– vs. 21% DSA+		CAV 36% DSA+ vs. 13% DSA–	DSA+ had 2.5 times more rejection events per year compared to DSA–
Thrush et al. (40)	1,596 (January 2010–December 2014), Pediatric Heart Transplant Study database	Unknown		33 deaths (16%) post-AMR development; short-term patient/GS lower for patients with treated AMR ($p = 0.004$, $p = 0.001$, respectively); patient survival post-AMR diagnosis: 88% 1 y, 77% 3 y	179 (11%), freedom from AMR: 88% 1 y, 82% 3 y		AMR often concurrent with ACR

Ab, antibody; ACR, acute cellular rejection; AHG, anti-human globulin; AMR, antibody-mediated rejection; C1q, complement component 1q; C4d, complement component 4d; cAMR, clinical AMR; CAV, cardiac allograft vasculopathy; CDC, complement-dependent cytotoxicity; XM, crossmatch; DSA, donor-specific HLA antibodies; dnDSA, de novo donor-specific HLA antibody; EMB, endomyocardial biopsies; GS, graft survival; HR, hazard ratio; IF, immunofluorescence; pAMR, pathologic AMR; post-Tx, posttransplant; PRA, panel-reactive antibodies; pre-Tx, pretransplant; SAB, Luminex single antigen bead assay; SPA, solid phase assays; y, year(s); MFI, mean fluorescence intensity; HLA, human leukocyte antigens.

Mangiola M, et al. Front Immunol, 2017

Comprehensive Antibody Testing and Heart Transplantation

Cited publications from the last 6 years (2010–present) showing the impact of HLA antibody on heart transplantation in adult recipients.

Reference	Number of patients (study period)	Method	DSA	GS	AMR	CAV	Comments
Gandhi et al. (19)	85 (August 2006–January 2010)	CDC-AHG PRA/XM, Flow XM, SAB	All CDC XM–; DSA+ (MFI >1,500), *n* = 11 (13%): Class I = 2, Class II = 6, Class I + II = 3		(*n* = 80 for biopsy) AMR: 7/11 DSA+		CMR ≥ 1R/1A: 9/11 DSA+ vs. 48/69 DSA–/weak; DSA MFI >1,500 associated with increased incidence of AMR and CMR
Smith et al. (14)	243 (October 1995–July 2004)	SAB (8.8 ± 2.5)	57 dnDSA Class II = 48 (42/48 DQ)	Poor GS $p = 0.0001$ (HR = 4.35)		29% 5 y; 55% 10 y	DnDSA risk for poor GS and CAV
Ho et al. (16)	950 (January 1995–December 2009)	CDC T and B, SAB (mean number of sera tested per patient = 24 ± 9)	221 dnDSA 1 y, 118 dnDSA >1 y, 460 no HLA-Ab	GS 52%, $p < 0.005$; GS 48%, $p < 0.001$; GS 70%	23		DSA and non-DSA increased in rejection
Loupy et al. (29)	196 (1985–2009)	SAB	20 very late rejection (VLR >7 y)			CAV grade VLR, 2.06 vs. 0.76 in control	VLR associated with severe CAV
Hodges et al. (15)	762 (November 2005–August 2011)	Luminex Screen, SAB	15 AMR (14/15 dnDSA)	1.8 y mean survival after AMR treatment	15		Late cardiac AMR with dnDSA
Zeevi et al. (20)	15 (8 pediatric, 7 adult)	SAB, SAB-C1q	35 DSA in 14 patients: Class I = 4, Class II = 2, Class I + II = 8		1st month post-Tx: 7/7 cAMR+ are DSA+/C1q+; 4 cAMR-free, DSA+/C1q– ($p < 0.005$)		Persistent C1q+ DSA post-Tx associated with early clinical AMR
Potena et al. (11)	173 (2000–2005)	CDC/PRA, Luminex Screen	Pre-Tx 32 Ab+ Class I = 28, Class II = 16, Class I + II = 12	Survival 65% for Ab+ 82% for Ab–	9/37 with biopsy were HLA-Ab+, pAMR >2		
Raess et al. (13)	272 (1989–2010)	CDC-PRA/XM, Luminex screen, SAB, SAB-C1q	DSA 26 (9.6%), Class I = 14, Class II = 5, Class I + II = 7, C1q+ DSA = 2	Overall survival: 80% (1 y), 68% (5 y) SAB Class I DSA+: 62% (1 y), 50% (5 y) SAB Class I DSA–: 87% (1 y), 73% (5 y)	Fatal pAMR = 6, all ≤1 month post-Tx	(*n* = 245) CAV-free survival: 96% (1 y), 86% (5 y)	ACR-free survival: 38% (1 y), 30% (5 y); pre-Tx HLA Ab status affected short-term survival but had no effect on long-term survival/rejection
Topilsky et al. (27)	51 (January 2004–December 2009)	SAB; Flow XM for 30 patients	All CDC-XM–; DSA+ 17 (33%): Class I = 4, Class II = 11, Class I + II = 2			36 (71%) with Grade 1 CAV	CAV analysis done for patients with only Class II DSA; pre-Tx Class II DSA may give higher risk of accelerated CAV: DSA+ 100% vs. DSA– 64.2% at 4 y
Tible et al. (22)	111 (October 2009–September 2010)	SAB, 150 paired DSA and EMB	47/150 DSA+, Class I = 40.4%, Class II = 40.4%, Class I + II = 19.2%		37		MI and CD68 associated with DSA+
Frank et al. (28)	109 (February 1996–June 2011)	SAB, 330 paired DSA and EMB	51/112, Class I = 5, Class II = 26, Class I + II = 20			24 (22%): 40% DSA+, 13% DSA–	33% with CAV pre-Tx DSA+; Class II DSA, IF C4d+, and MI high risk for failed allograft with CAV

(*Continued*)

Mangiola M, et al. Front Immunol, 2017

Continued

Reference	Number of patients (study period)	Method	DSA	GS	AMR	CAV	Comments
Coutance et al. (24)	20 (November 2006–February 2013)	Luminex Screen, SAB	19/20 tested were dnDSA+	50% after 1 y	Late AMR (>1 y post-Tx)		Prognosis for late AMR poor despite aggressive therapy
O'Connor et al. (12)	12,858 (June 2004–March 2013); UNOS database	CDC-PRA, Flow-PRA	PRA ≥ 10%, Class I: CDC+ = 227, Flow+ = 2,243, Class II: CDC+ = 126, Flow+ = 2,218	PRA ≥ 10%: HR = 1.24 (95% CI 1.12–1.36)			Percent Ab+ patients increased from 2005 to 2011 as use of flow increased; pre-Tx PRA ≥ 10% by Flow associated with increased risk of graft loss
Svobodova et al. (21)	264 (April 2005–December 2012; mean follow-up 39 months, range 19–66)	CDC-PRA/XM; SAB, SAB-C1q	DSA = 28 (11%): Class I = 18, Class II = 3, Class I + II = 7, C1q+ DSA = 4	90% (1 y), 79% (5 y)	19 (7%)	31 (12%)	74 patients (28%) with 83 instances of ACR grade ≥ Banff 2; pre-Tx DSA and elevated peak CDC-PRA were strongest predictors of AMR
Frank et al. (23)	44 (2005–2011)	SAB-C1q paired with EMB C4d stain	C1q+ DSA in 82% with graft dysfunction	18/44 died or retransplanted	16/17 C4d+ IF had C1q+ DSA; 24 C1q+ DSA were C4d-IF		Better concordance of C4d+ IF with C1q DSA as compared to IgG DSA
Loupy et al. (25)	40, failing grafts	SAB			AMR = 19		
Clerkin et al. (26)	689 (January 2004–December 2013, follow-up through October 2015)	Luminex SAB and/or CDC screen	Overall: *n* = 29 (42.6%); early AMR: *n* = 22 (51.1%); late AMR: *n* = 7 (28.0%)	Decreased post-AMR survival in patients with late vs. early AMR: 80 vs. 93%, 1 y; 51 vs. 73%, 5 y ($p < 0.05$)	*n* = 68 (9.9%): 43 early (<1 y post-Tx), 25 late (>1 y post-Tx)	No difference in prevalence early AMR vs. late AMR ($p = 0.51$); accelerated *de novo* CAV in late AMR + graft dysfunction (50% at 1 y, HR = 5.42, $p = 0.009$)	Graft dysfunction increased in late AMR group (56.0 vs. 25.6%, $p = 0.01$)

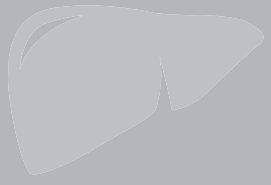
Ab, antibody; ACR, acute cellular rejection; AHG, anti-human globulin; AMR, antibody-mediated rejection; C1q, complement component 1q; C4d, complement component 4d; CAV, cardiac allograft vasculopathy; CDC, complement-dependent cytotoxicity; CMR, cell-mediated rejection; XM, crossmatch; DSA, donor-specific HLA antibodies; dnDSA, de novo donor-specific HLA antibody; EMB, endomyocardial biopsies; GS, graft survival; HR, hazard ratio; IF, immunofluorescence; MFI, mean fluorescence intensity; MI, microcirculation inflammation; pAMR, pathologic AMR; post-Tx, posttransplant; PRA, panel-reactive antibodies; pre-Tx, pretransplant; SAB, Luminex single antigen bead assay; VLR, very late rejection; y, year(s); HLA, human leukocyte antigens.

Mangiola M, et al. Front Immunol, 2017

Kidney

Heart

Lung

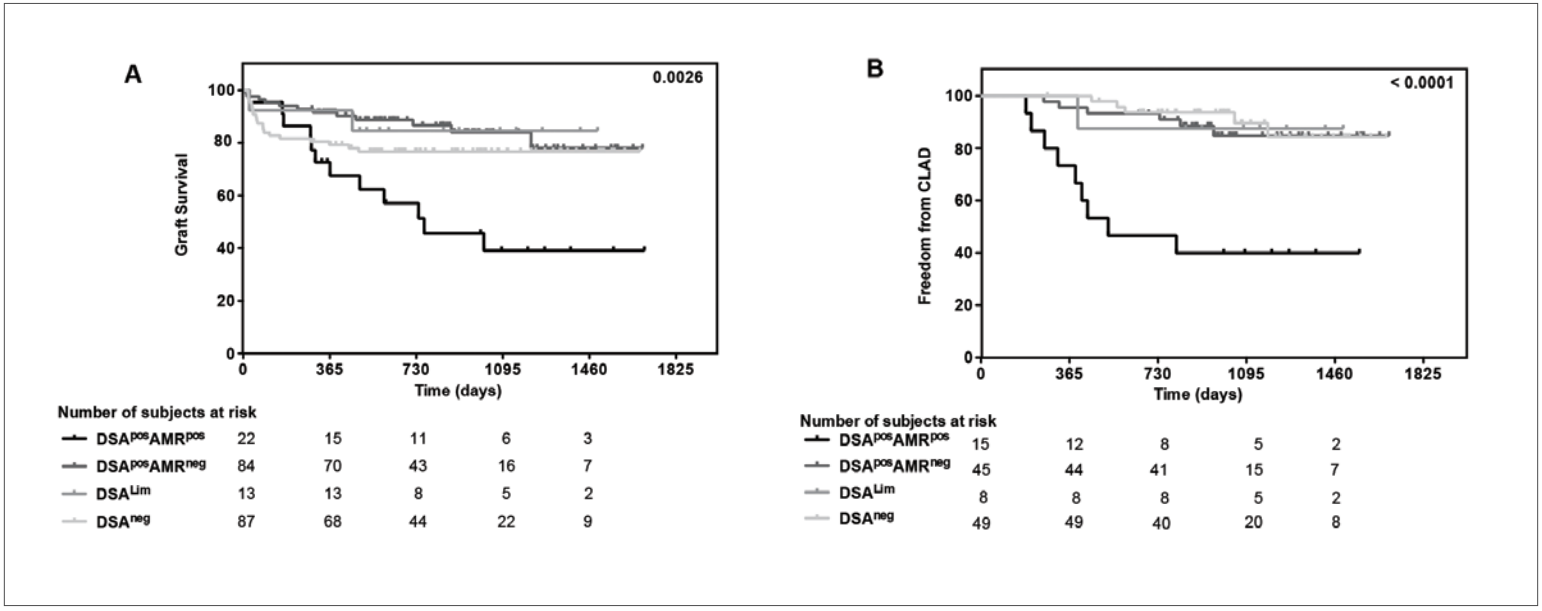


Liver

Bone Marrow

DSA Monitoring in Lung Transplantation

Despite the recognition of ABMR as a cause of allograft dysfunction in lung transplantation, the criteria for its diagnosis is not well established. A consensus report was recently published by the International Society of Heart and Lung Transplantation (ISHLT) in order to initiate criteria for ABMR diagnosis after lung transplantation and provide more consistency between study results, improving the knowledge and data for the field. The role of DSA in hyperacute rejection after lung transplantation was first reported 20 years ago. By frequently monitoring DSA with Single Antigen beads and C4d staining to prospectively diagnose ABMR, a recent study by Roux et al established an association of ABMR diagnosis in the presence of DSA with the occurrence of chronic lung allograft dysfunction and allograft loss.



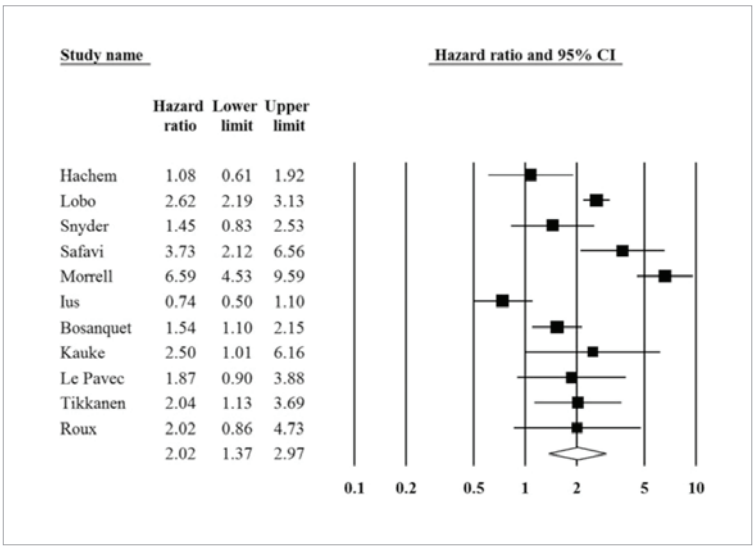
A. Graft Survival according to antibody mediated rejection (ABMR) and donor specific antibodies (DSA) status. B. Freedom from chronic lung allograft dysfunction (CLAD) according to antibody mediated rejection (ABMR) and donor specific antibodies (DSA) status. DSALIM – DSA limited (DSA with positivity in only one Single Antigen Beads test with MFI between 500-1000).

Reproduced from: Roux A, et al. Am J Transplant, 2016

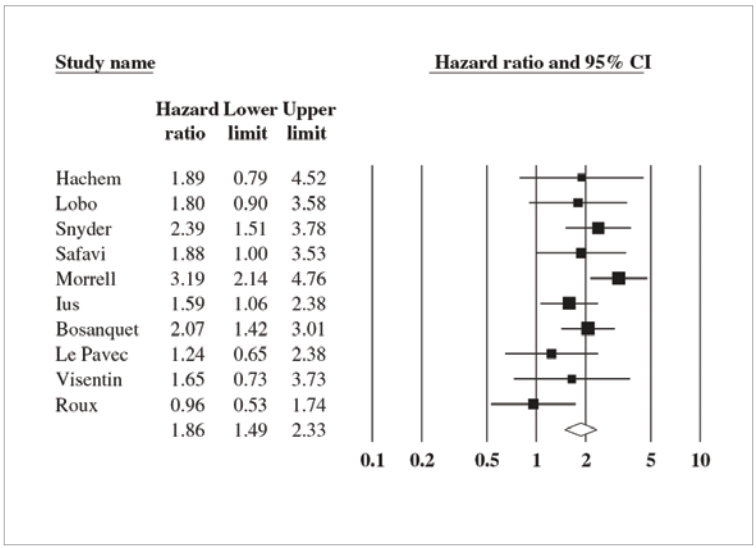
ABMR in Lung Transplantation

Meta-analysis of the Relationship Between *De Novo* DSA and CLAD

In this systematic review, the authors tried to identify the source of heterogeneity in the identification of pre- and post-lung transplant HLA antibodies in the literature. They found substantial center-to-center variability in the approach to detect HLA antibodies. Centers that performed screening test plus single antigen beads specific tests showed more relevance in antibodies detected to development of chronic lung allograft dysfunction and mortality than centers that performed only single antigen tests.



*Hazard ratio, HR=2.02, 95%CI:1.37-2.97, p<0.001, I2=87.6, Q=81.1, p<0.001

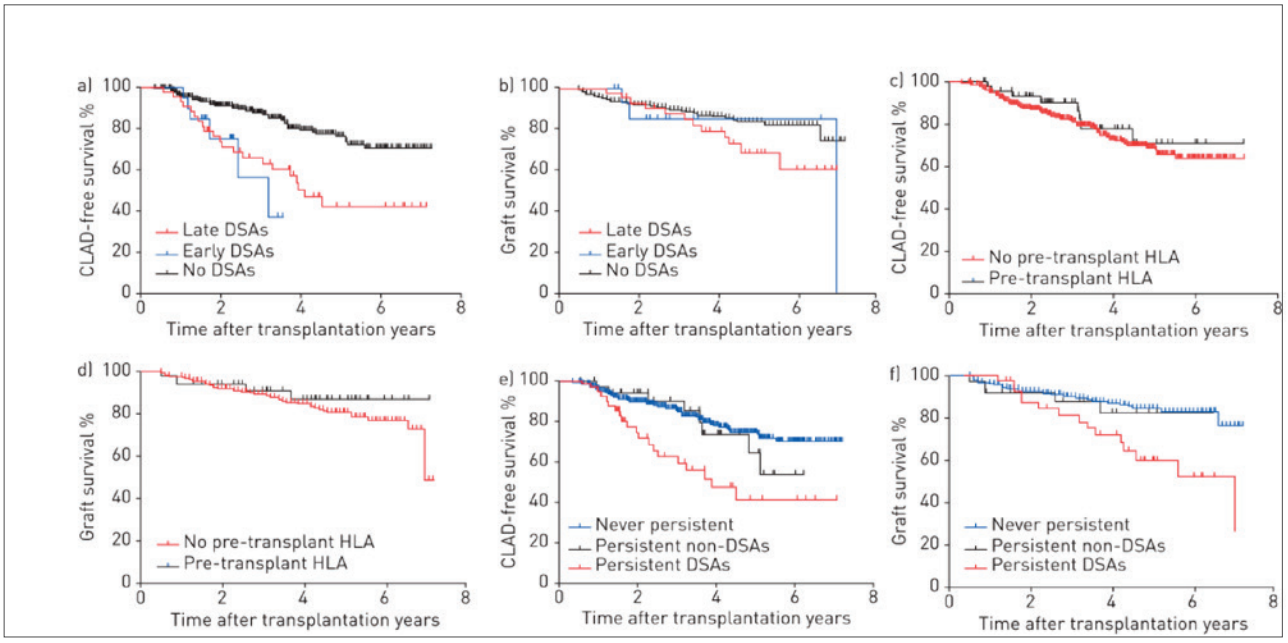


*Hazard ratio, HR=1.86, 95%CI:1.49-2.33, p<0.001, I2=40.7, Q=15.2, p=0.09

Courtwright A, et al. HLA, 2018

Persistent DSA Leads to Reduced Graft Survival

Verleden et al evaluated the association of anti-HLA antibodies, CLAD, and graft survival in a cohort of 362 lung transplanted patients. The analysis was stratified according to DSA status, persistence and timing of antibodies. Sixty-one patients had DSA and this was associated with CLAD and graft loss. Both persistent DSA (HR=3.386, 95%CI:1.928-5.948, p<0.0001) and transient DSA (HR=2.998, 95%CI: 1.406-6.393, p=0.0045) were associated with shorter CLAD-free survival. However, only persistent DSA (HR=3.071, 95%CI: 1.632-5.778, p=0.0005) was associated with graft loss.

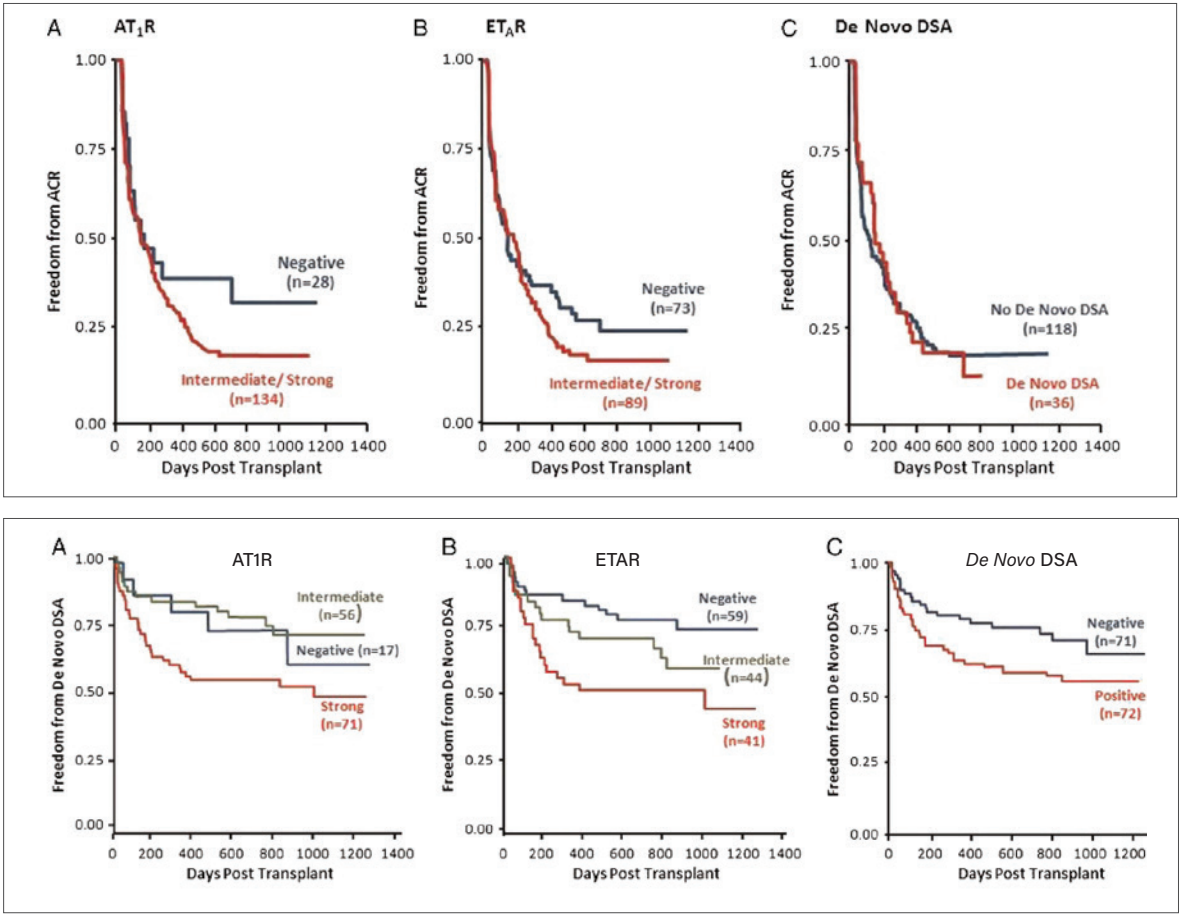


Verleden SE, et al. Eur Respir J, 2017

ABMR in Lung Transplantation

Impact of Non-HLA Antibodies in Lung Transplantation

A recent study has also shown the impact of non-HLA antibodies in lung transplantation when detected in the presence of HLA-directed DSA. In addition to HLA DSA, detectable antibodies to angiotensin type 1 receptor (AT1R) and endothelin type A receptor (ETAR) led to significant increases in ABMR in a cohort of 162 lung transplant recipients.



Reinsmoen NL, et al. Transplantation, 2017

Kidney

Heart

Lung

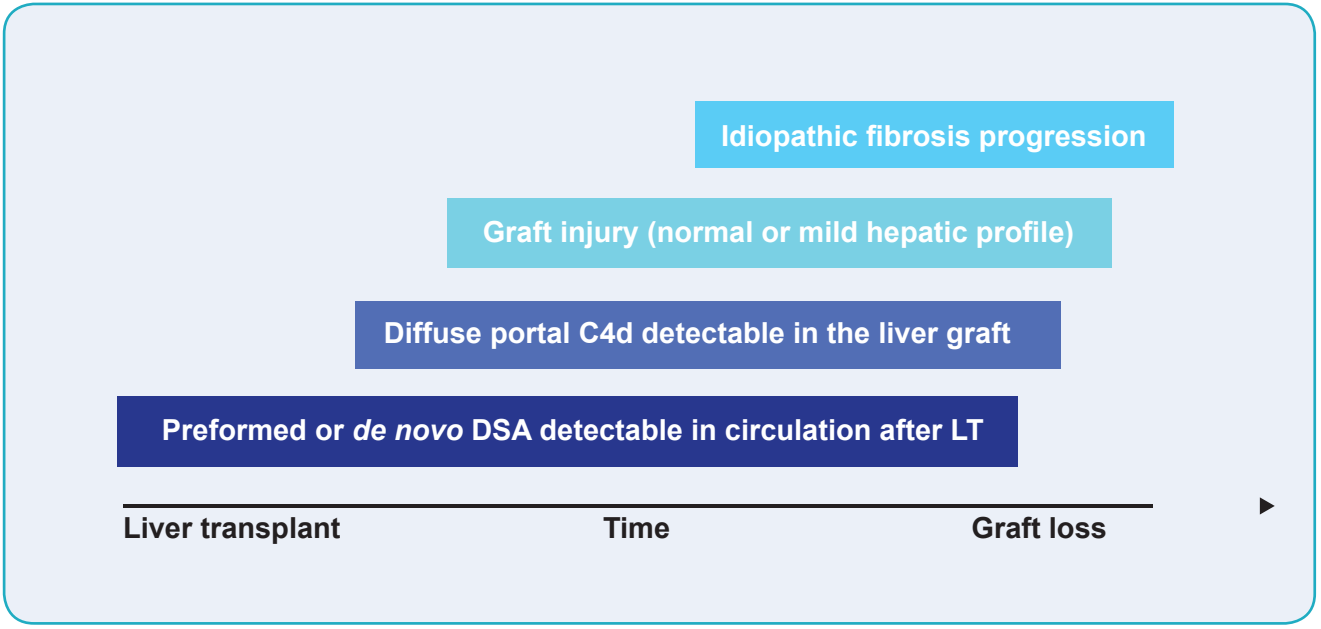
Liver

Bone Marrow

ABMR in Liver Transplantation

Hypothetical Model of Liver Transplant Damage

HLA antibodies have historically not been considered a major risk factor in liver transplantation. However, in this hypothetical model of idiopathic fibrosis progression post-liver transplant, the presence of preformed or *de novo* DSA is considered a potential effective early biomarker.

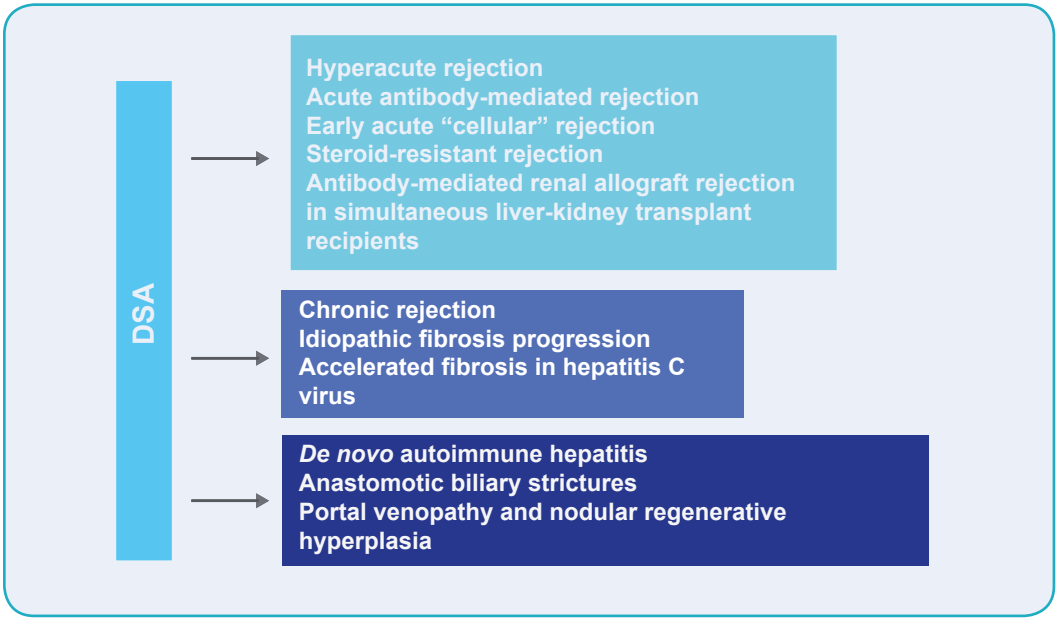


Hypothetical chain of events for idiopathic fibrosis progression.

Adapted from: Cuadrado A, et al. World J Gastroenterol, 2015

Importance of HLA Antibodies in Liver Transplantation

The recognition of ABMR in liver allografts has been an important subject of discussion. Although liver allografts are relatively resistant to ABMR compared to other solid organs, findings from the last decade have demonstrated that they are still susceptible. Early acute ABMR is rare occurring in highly sensitized recipients, representing less than 1% of all liver transplants. However, chronic ABMR in the setting of *de novo* HLA class II DSA presents an incidence between 8-15% and has been associated with specific characteristics of antibodies that could be easily identified with Solid Phase Antibody testing. Better characterization of those DSA phenotypes in randomized controlled trials may identify other potential associations of DSA with liver transplantation outcomes.



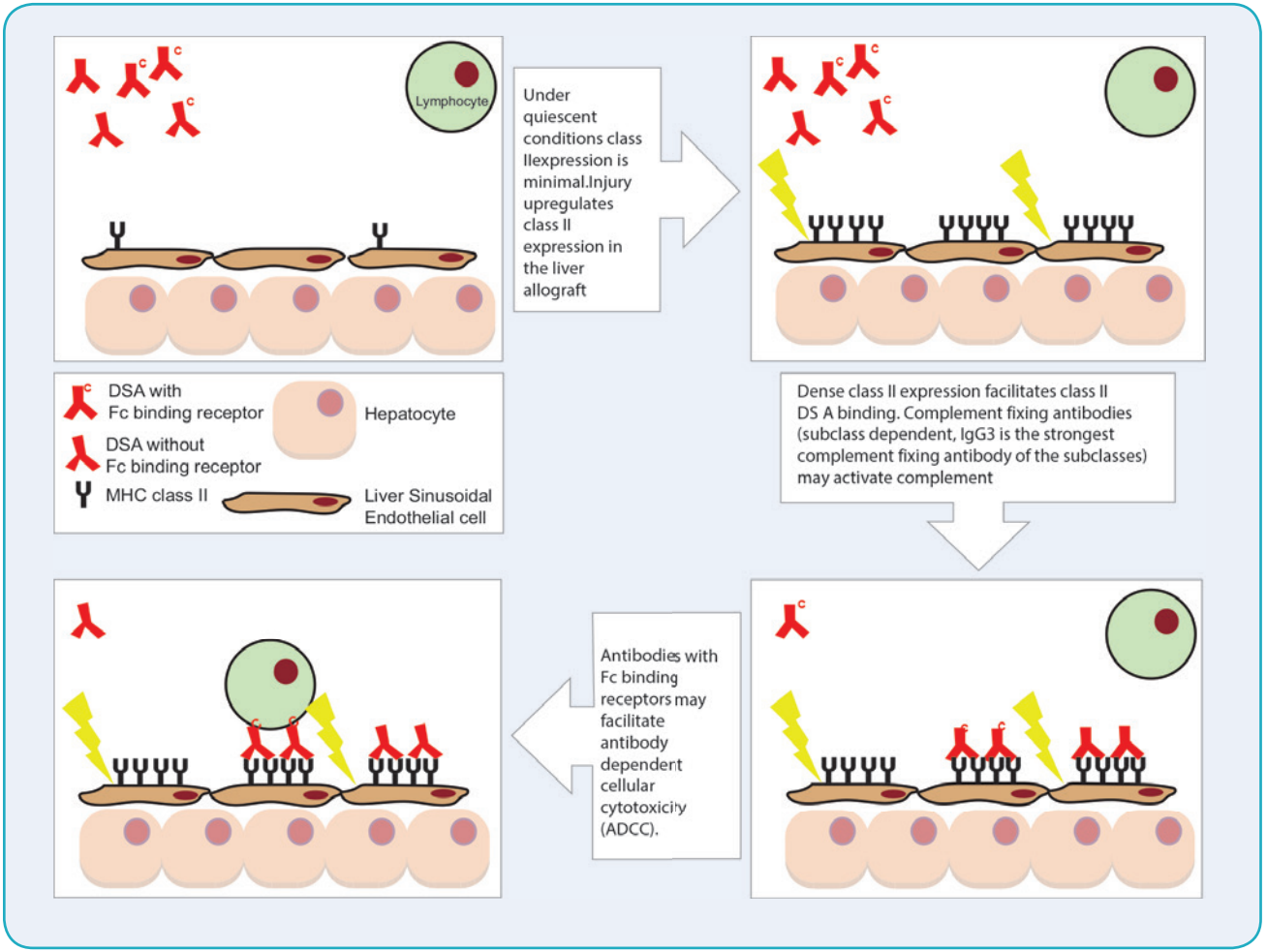
Potential associations of anti-HLA DSA with outcomes in liver transplantation.

Adapted from: O'Leary JG, *et al.* Am J Transplant, 2014 and Cuadrado A, *et al.* World J Gastroenterol, 2015

ABMR in Liver Transplantation

The Two-Hit Hypothesis of Liver Allograft ABMR

The two-hit hypothesis of ABMR in liver transplantation is different for acute and chronic rejection. Acute ABMR requires preformed high titer HLA class I DSA and is usually associated with marginal donors. Chronic ABMR occurs in the presence of HLA class II DSA and presents an injury that increases HLA class II expression in the organ.



Reproduced from: Kim PT, et al. Curr Opin Organ Transplant, 2016

ABMR in Liver Transplantation

Liver

Summary of recent studies of de novo DSA on clinical outcomes ABO-compatible liver transplantation.

Reference	Study design	Sample size	Prevalence of de novo DSA	Study findings
Kaneku et al. (2013) [13]	Retrospective	749 adult	8.1% at 1 year	(i) Presence of DSA associated with inferior patient and graft survival (ii) Almost all de novo DSA were against HLA class II antigens (majority DQ) (iii) Risk of de novo DSA formation increased by low calcineurin inhibitor levels and the use of cyclosporine (versus tacrolimus)
Grabhorn et al. (2015) [17]	Retrospective	43 pediatric	33% in stable recipients; 68% in chronic rejectors	(i) Higher rate of de novo DSA among pediatric LT recipients with chronic rejection (ii) Antibodies predominantly against HLA class II antigens
O'Leary et al. (2015) [15]	Retrospective	749 adult	8% at 1 year	(i) IgG3 subclass DSA-positive patients at highest risk for death (ii) IgG3-negative, DSA-positive patients still had inferior outcomes compared to DSA-negative patients
Wozniak et al. (2015) [16]	Cross-sectional	50 pediatric	54%	(i) Younger age associated with presence of DSA (ii) Nontolerant patients more likely to have DQ DSA (61%) compared with stable (20%) and tolerant (29%) patients (iii) DQ DSA associated with de novo autoimmune hepatitis and late acute rejection
Del Bello et al. (2015) [18]	Prospective	152 adult	14%	(i) Younger age, low exposure to calcineurin inhibitors, and noncompliance were risk factors for de novo DSA emergence (ii) Nine of 21 (43%) DSA-positive recipients developed acute rejection (iii) No differences in patient or graft survival with DSA presence
Levitsky et al. (2016) [19]	Retrospective analysis of an observational cohort study	195 adult (129 LDLT, 66 DDLT)	5.4% in LDLT; 6.1% in DDLT	(i) No differences in the prevalence of de novo DSA between LDLT and DDLT recipients (ii) Presence of DSA was an independent risk factor for graft failure in LDLT and DDLT

LDLT, living donor liver transplantation; DDLT, deceased donor liver transplantation.

Cheng E, J Immunol Res, 2017

Liver

ABMR in Liver Transplantation

Summary of reported cases of acute antibody-mediated rejection following ABO-compatible liver transplantation.

Reference	Age/gender	Onset of graft dysfunction	DSA detection method	Type of DSA	DSA specificity	AMR treatment	Clinical outcome
Rostron et al. (2005) [22]	23/F	POD 6	Luminex SAB	Preformed	Bw6	Steroids, MMF, PP, IVIG	Alive with functioning graft
Wilson et al. (2006) [23]	36/F	4 years	Luminex SAB	De novo	DR52	Steroids, MMF, PP, IVIG, Rituximab, ATG	Alive with functioning graft
Watson et al. (2006) [24]	50/F	POD 5	Flow cytometry SAB	Preformed	B7	Steroids, MMF, PP, IVIG, Rituximab	Death
Kamar et al. (2009) [25]	49/F	POD 10	Luminex SAB	Preformed	A2, DR7	Steroids, MMF, PP, Rituximab, OKT3	Death
	39/F	POD 6	Luminex SAB	Preformed	A2, A24, B27, DR4	Steroids, PP, Rituximab	Alive with functioning graft
Kozlowski et al. (2011) [26]	N/A	POD 5	Flow cytometry or Luminex SAB	Preformed	3 DSA (specificity not provided)	Steroids, PP, IVIG, Rituximab	Death
	N/A	POD 7		Preformed	A30, A74, B7, B45, DR15, DR51, DQ7	Steroids, PP, IVIG, Rituximab, ATG	Death
	N/A	POD 7		Preformed	4 DSA (specificity not provided)	Steroids, PP, IVIG, Rituximab, OKT3	Retransplant, alive
Paterno et al. (2012) [27]	62/F	POD 8	Luminex SAB	De novo	DR13, DR15, DR51, DR52	Steroids, OKT3, ATG, Bortezomib	Alive with functioning graft
	28/F	POD 452	Luminex SAB	De novo	DQ2, DQ6	Steroids, PP, Rituximab, ATG, Bortezomib	Alive with functioning graft
	53/F	POD 6	Luminex SAB	Preformed	B51, Cw2, DQ7	Steroids, ATG, Bortezomib	Alive with functioning graft
Kheradmand et al. (2014) [28]	43/F	POD 1	Luminex SAB	Preformed	B35, B51, DR4, DR53, DQ8	Steroids, PP, IVIG, Rituximab, ATG	Alive with functioning graft
Wozniak et al. (2016) [29]	22 mo/M	POD 45	Luminex SAB	De novo	B44, DQ2	Steroids, MMF, IVIG, Rituximab	Alive with functioning graft
	3/F	POD 13	Luminex SAB	N/A	A1, DQ5	Steroids, MMF, IVIG	Alive with functioning graft
	19 mo/F	POD 8	Luminex SAB	Preformed & de novo	Cw7, Cw17, DR4, DR53, DQ8	Steroids, MMF, IVIG, Rituximab	Alive with functioning graft
	11/M	POD 7	Luminex SAB	De novo	DR53, DQ8	Steroids, MMF, PP, IVIG, ATG, Bortezomib	Alive with functioning graft
	6 mo/M	POD 38	Luminex SAB	De novo	DQ7, DQ9	Steroids, MMF, PP, IVIG, Rituximab	Retransplant, death
	3/M	POD 7	Luminex SAB	Preformed	A1, B8, Cw7, DR17, DQ2, DP1	Steroids, MMF, PP, IVIG, Rituximab, Bortezomib, Eculizumab	Alive with functioning graft

N/A, not available/information not provided; SAB, single antigen bead-based testing; PP, plasmapheresis; IVIG, intravenous immunoglobulin; ATG, antithymocyte globulin.

Cheng E, J Immunol Res, 2017

Kidney

Heart

Lung

Liver

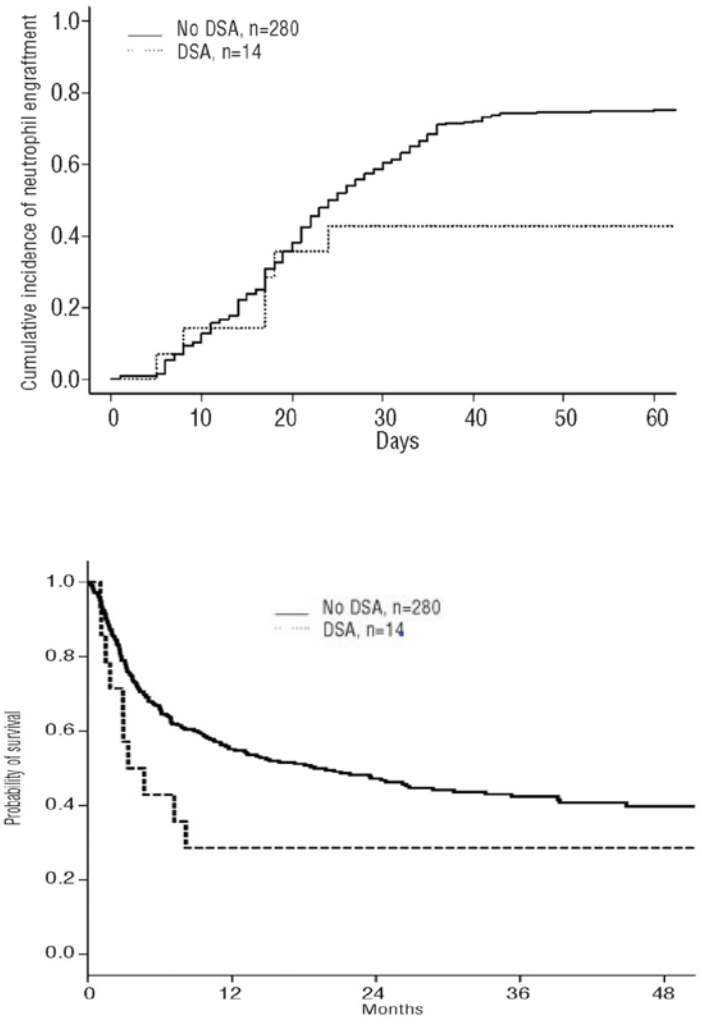
Bone Marrow

DSA and Stem Cell Transplantation

A number of recent studies have indicated that donor-specific anti-HLA antibodies (DSA) are an immunologically important barrier against successful engraftment of donor haematopoietic stem cells. Such DSAs can have a negative impact on graft survival.

Reference	Sample Size	Study Findings
Ciurea et al 2009	28	DSAs are associated with a high rate of graft rejection in patients undergoing haploidentical stem-cell transplantation.
Spellman et al 2010	115	Presence of DSA is significantly associated with graft failure
Takanashi et al 2010	386	Pre-transplant DSA testing should be performed prior to cord blood donor selection
Ciurea et al 2011	592	The presence of anti-DPB1 directed DSAs is associated with graft failure in MUD HSCT
Cutler et al 2011	73	The use of cord blood units where DSA is present should be avoided
Yoshihara et al 2012	79	Donors should be selected based upon thorough evaluation of DSA status
Ruggeri et al 2013	294	Where possible avoid the selection of donor units to which the patient has preformed DSA.
Chang et al 2015	345	DSA testing should be incorporated into HSCT donor selection algorithms
Ciurea et al 2015	122	Patients with DSA (>5000 MFI) and complement binding antibodies have increased risk of primary graft failure

Preformed DSA Leads to Decreased Neutrophil Engraftment and Overall Patient Survival



Detrimental impact of preformed DSA on both neutrophil engraftment (top graph) and probability of patients survival (lower graph).

Ruggeri et al, Haematologica, 2013

Haploidentical HSCT: The Importance of HLA Antibody Detection

Screening for anti-HLA antibodies is of particular importance in haploidentical transplants where the degree of HLA mismatching is increased.

The formation of these antibodies shows a higher prevalence in multiparous females compared with males.

European Society for Blood and Marrow Transplantation (EBMT) Consensus Guidelines 2018

The following recommendations were made for the Detection and Treatment of Donor Specific Anti-HLA Antibodies (DSA) in Haploidentical Hematopoietic Cell Transplantation:

1) DSA testing (by Luminex platform and/or cell-based assays) be performed in all candidate patients for haploidentical (or HLA mismatched) donor transplants;
(2) If DSA > 1,000 MFI, C1q testing and/or cell-based assays must be done to further assess the risk to the allograft;
(3) DSA testing should be incorporated in donor selection prior to transplantation.

ONE LAMBDA

A Thermo Fisher Scientific Brand

Product Sheet

LABScreen™

PRA Screening and Specificity Assignments Utilizing Flow Analysis Technology

Key Benefits

Proven Accuracy

- Distinguishes HLA Class I and Class II antibodies
- Contains a purified single antigen or a defined pool of HLA antigens, includingrare alleles
- Eliminates false positive reactions due to non-HLA antibodies or auto antibodies
- Single antigen assay identifies negative or safe antigens, even for high PRA patients
- Detects IgG antibodies

Premier Automation

- Based on Luminex® xMAP® technology
- Provides software-driven data acquisition

Maximum Consistency

- High reproducibility
- Delivers reaction-to-reaction consistency

PRA Screening and Specificity Assignment Utilizing Advanced Flow Analysis Technology

LABScreen reagents are powered by Luminex xMAP technology, a microbead platform used to deliver multiplex antibody assays. This antigen-bead based assay allows for a precise determination of antibody profiles against HLA and MICA. The proven reliability of LABScreen's consistency, high sensitivity and robustness for PRA screening has gained rapid momentum in the transplant community.

The LABScreen product line consists of color-coded microbeads coated with purified HLA Class I, Class II and MICA antigens. The beads are analyzed using Luminex xMAP multiplex technology.

Antibody Detection

Product Sheet

LABScreen Single Antigen

Single antigen assays provide a unique solution to the dilemma presented by high PRA patients. In these patients, antibody reactive to one or more dominant epitopes can mask the presence of additional antibody specificities. These specificities can now be identified by single antigen technology.

LABScreen Single Antigen Supplement

A single antigen panel designed to screen antibodies against HLA antigens found in higher frequencies among certain ethnic populations.

LABScreen Single Antigen MICA

A single antigen panel designed to identify MICA antibodies.

LABScreen PRA

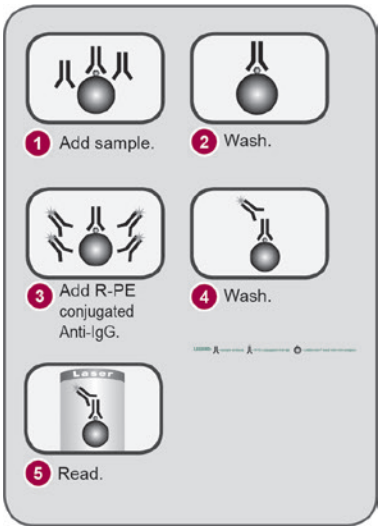
Determines percent PRA and identifies antibody specificities using HLA antigens purified from different cells. HLA Class I and Class II PRA tests may be used separately or together.

LABScreen Mixed

Tests for the presence of HLA Class I and Class II antibodies, as well as MICA antibodies, with a single tube protocol. Well suited for monthly patient antibody screens in both low and high throughput laboratories.

Principle

Detection by R-Phycoerythrin Conjugated Antibody



Ordering Information

Product	Tests	Cat. No.
For In Vitro Diagnostic Use. (Unless otherwise stated.)		
LABScreen™ Single Antigen Class I		
LABScreen™ MICA Single Antigen - Group 1	25 tests	LSMICA001
LABScreen™ Single Antigen HLA Class I - Combi	25 tests	LS1A04
LABScreen™ Single Antigen HLA Class I Supplement - Group 1	25 tests	LS1ASP01
LABScreen™ Single Antigen Class II	25 tests	
LABScreen™ Single Antigen HLA Class II - Group 1		LS2A01
LABScreen™ Single Antigen HLA Class II Supplement - Group 1	25 tests	LS2ASP01
LABScreen™ PRA		
LABScreen™ PRA Class I	25 tests	LS1PRA
LABScreen™ PRA Class II	25 tests	LS2PRA
LABScreen™ PRA Class I & II	25 tests	LS12PRA
LABScreen™ Mixed		
LABScreen™ Mixed Class I & II	100 tests	LSM12
Ancillary Products		
LABScreen™ Negative Control	20 tests	LS-NC
PE Conjugated Goat Anti-Human IgG	1000 tests	LS-AB2

Product Sheet

C1qScreen™

Detection by PE Conjugates Anti-C1q

Key Benefits

- Identifies complement binding antibodies
- Internal positive control bead included
- Robust software analysis

Build a Better Profile Quickly

C1qScreen combines the sensitivity of Luminex® solid phase technology with the specificity of anti-HLA single antigen detection for the detection of complement binding donor specific antibody (DSA).

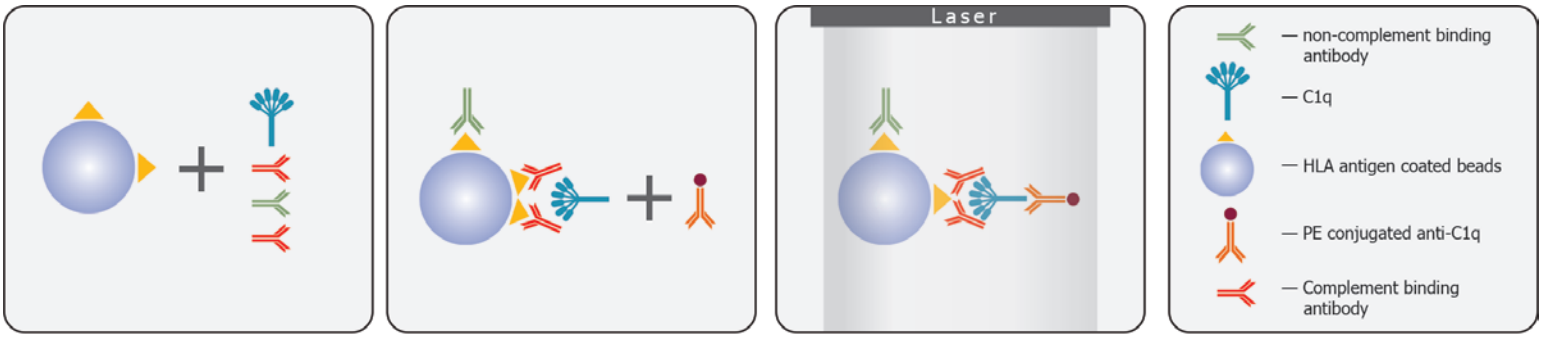
Complement component (C1q) bound by the antigen-antibody complex is detected with an R-phycoerythrin (PE) labeled anti-C1q antibody. Using our Luminex®-based LABScan™ 100 or LABScan3D™ flow analyzer, fluorescence intensity is measured to indicate the relative amount of antibody bound to the sample.

In addition to the internal C1q Positive Control Bead already included, Positive Control Serum for Class I and Class II and negative control serum are also available (each sold separately).

C1qScreen - A Reliable Tool

Investigate complement fixing antibody's possible involvement in Antibody Mediated Rejection. With C1qScreen the presence of complement binding (C1q) antibodies can be identified and monitored in sera, providing information for building better antibody profiles.

Principle



1. Add C1q to HI serum sample
2. Add PE conjugated anti-C1q
3. Binding and detecting
Legend

Ordering Information

Product	Tests	Cat. No
For In Vitro Diagnostic Use. (Europe Only)		
C1qScreen™	25 tests	C1Q
C1qScreen™ Class I Positive Control	20 tests	C1QS-PC1
C1qScreen™ Class II Positive Control	20 tests	C1QS-PC2
C1qScreen™ Negative Control Serum	20 tests	C1QS-NC
For Research Use Only. Not for use in diagnostic procedures.		
C1qScreen™	25 tests	PEC1Q
C1qScreen™ Class I Positive Control	20 tests	C1Q-PC1
C1qScreen™ Class II Positive Control	20 tests	C1Q-PC2
C1qScreen™ Negative Control Serum	20 tests	C1Q-NC

Product Sheet

LABScreen™ Autoantibody

Autoantibody Detection

Key Benefits

- Characterize and monitor autoantibody targets
- Multiplexing capability – simultaneously detect up to 33 targets
- Negative and positive control sera available

Premier Automation

- Minimal training required
- Use existing instrumentation (LABScan 100/200 and LABScan3D)
- Test up to 96 samples in under 4 hours
- Analyze results with Fusion Research software

Expanding your Antibody Detection Repertoire

Mounting evidence suggests the importance of testing autoantibodies1,2. Employing single antigen bead technology, the LABScreen Autoantibody assays now allow you to characterize and monitor a broad range of autoantibody targets in human sera.

Following a familiar workflow and analysis algorithm, these assays can be easily integrated into your laboratory workflow without the need for new instrumentation or extensive retraining.

Expand your antibody detection capabilities and get the answers you need.

LABScreen Autoantibody

The LABScreen Autoantibody assays provide a convenient way to characterize and study the autoantibody repertoire in human sera. Target antibodies can now be identified by single antigen bead technology.

Group 1 – 32 targets
Group 2‡ – 1 target
Group 3 – 6 targets

LABScreen Autoantibody Negative Control Serum

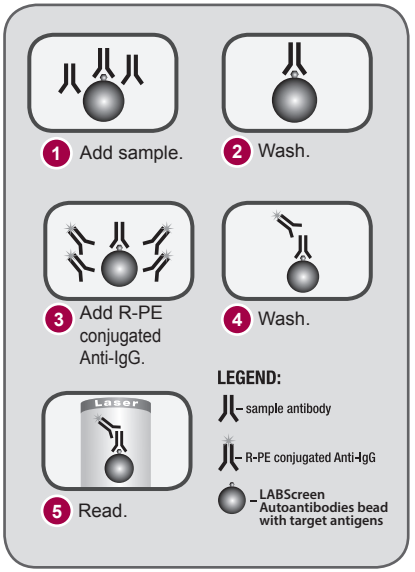
A negative control serum used as an indicator of the non-specific background signal of LABScreen Autoantibody Groups 1, 2, and 3 beads when reacting with a serum sample that does not contain the specified target antibodies.

LABScreen Autoantibody Positive Control Serum (for Group 1 and 2)

A positive control serum used as an indicator of the target-specific signal of LABScreen Autoantibody Group 1 and 2 beads when reacting with a serum sample that contains the specified target antibodies.

‡Note: Groups 1 and 2 can be combined in a single run.

Principle



Ordering Information

Product	Tests	Cat. No.
For Research Use Only. Not for use in diagnostic procedures		
LABScreen Autoantibody Group 1	25 tests	LSAUT1
LABScreen Autoantibody Group 2	25 tests	LSAUT2
LABScreen Autoantibody Group 3	25 tests	LSAUT3
LABScreen Autoantibody Negative Control Serum (for Group 1, 2, 3)	10 tests	LSAUT-NC
LABScreen Autoantibody Positive Control Serum (for Group 1 and 2)	10 tests	LSAUT-PC

LABScreen Autoantibody
Antibody Detection

Features

- Characterize and monitor autoantibody targets with Single Antigen Bead technology
- Multiplexing capability – simultaneously detect up to 33 targets
- Familiar workflow: minimal training required
- Compatible with LABScan 100/200 and LABScan3D
- Test up to 96 samples in under 4 hours
- Analyze results with Fusion Research software

General Description

The LABScreen Autoantibody assays now allow you to characterize and monitor a broad range of autoantibody targets in human sera.

Coverage by Researched Organs

Kidney/Pancreas	Heart/Lung	HSCT	Experimental	
Group 1				
Vimentin	Enolase	CHAF1B	FLRT2	PRKCH
AGT	CD36	FLRT2	IFIH1	LMNB
PECR	Myosin		AURKA	CXCL10
CXCL11	HNRNPK		PPIA	ARHGDIB
CXCL9	Tubulin		EIF2A	GDNF
Agrin			LMNA	GAPDH
IFNG			PRKCZ	TNFA
PLA2R				
PTPRN				
REG3A				
Group 2				
LG3 (Perlecan)				
Group 3				
Collagen I	Collagen VC		ollagen II	
Collagen III				
Collagen IV				
Fibronectin				

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