SOCIETAT CATALANA DE TRASPLANTAMENT

13º CONGRESO BARCELONA
18-20 MARZO 2015

Rechazo mediado por anticuerpos

AMR (antibody mediated rejection)

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Cardiac allograft rejection

- Hiperacute
- Acute cellular rejection
- Chronic rejection or coronary allograft vasculopathy
- Antibody-mediated rejection AMR “vascular” or “humoral”
- Mixed rejection

Graft injury results from deposition of antibody within the microvasculature of the transplanted heart, most notably in the capillaries

Herskowitz A J Heart Lung Transpl 1987;6:127
Hammond EH J Heart Lung Transpl 1989;8:430

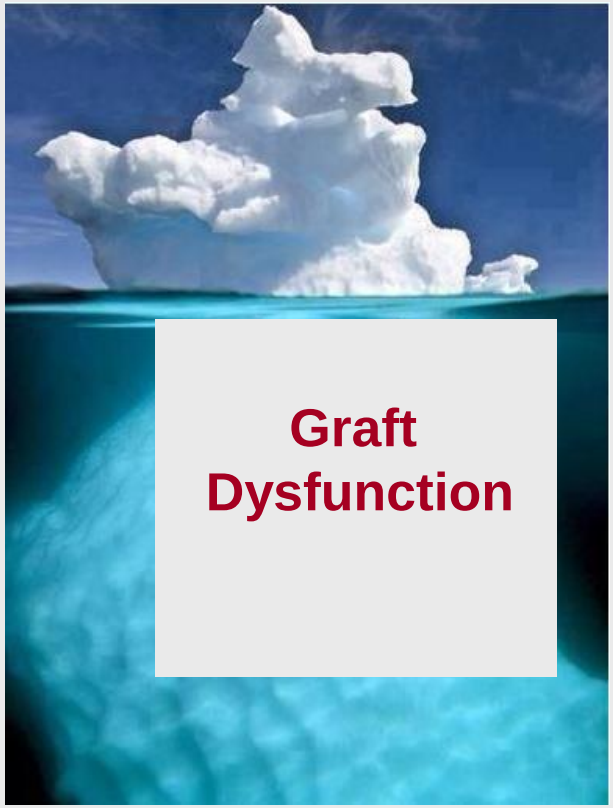
Rechazo mediado humoral o por anticuerpos

- **Disfunción del injerto en ausencia de rechazo agudo por BEM o vasculopatia coronaria significativa**

- **Pueden detectarse anticuerpos circulantes o no**

- **Se trata empíricamente con bolus de corticoides, plasmaferesis y aumento de la inmunosupresión**

Rechazo mediado humoral o por anticuerpos



- Se diagnostica cuando ya hay disfunción del injerto
- Se asocia a una alta mortalidad
- Se están haciendo esfuerzos para detectarlo de forma precoz
- No está claro que tratamiento aplicar
- No hay estudios que avalen el tratamiento

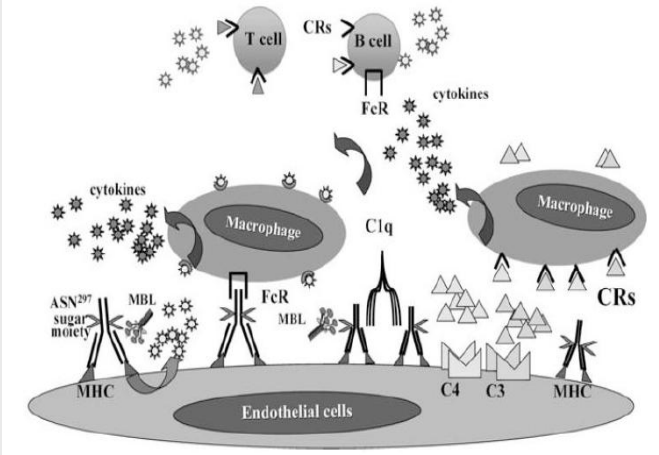
Rechazo mediado por anticuerpos (AMR)

1. Patogénesis del AMR
2. Como diagnosticar el AMR
3. Cuando monitorizar para diagnosticar AMR
4. Pronóstico
5. Cuando tratar el AMR
6. Como tratar el AMR

Patogénesis del AMR

- **Los DSA circulantes se unen a antígenos (MHC Class I or II) presentes en las células endoteliales activando el complemento**

- **El daño en el injerto se debe al depósito de anticuerpos en la microvasculatura del corazón trasplantado, especialmente en el endotelio de los capilares**

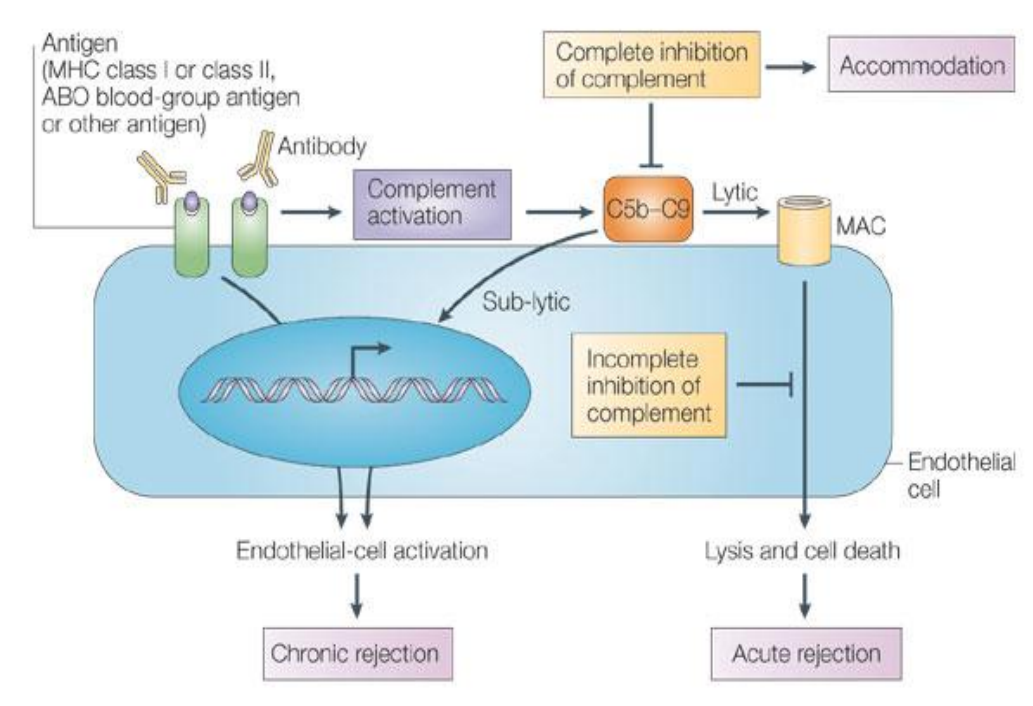


- **La reacción antígeno-anticuerpo junto con la activación del complemento causa una reacción inflamatoria, con activación de citoquinas y células-T, aumento de la permeabilidad celular lo que favorece la necrosis celular y el rechazo**

Herskowitz A J Heart Lung Transpl 1987;6:127
Hammond EH J Heart Lung Transpl 1989;8:430
Wasowska B Immunol Res 2010;47:25

Patogénesis del AMR

- **Cuando la activación del complemento está inhibida, sin causar daño en el injerto, este fenómeno se conoce como acomodación**



- **La activación parcial del complemento puede inducir inflamación endotelial crónica y dar lugar a AMR**

- **La activación de la cascada del complemento genera productos derivados de su activación que pueden propiciar cambios inflamatorios en las células endoteliales**

Colvin R. Nature Reviews Immunology 2005;5:807-817.

AMR - epidemiology

- Can occur both early or late after HT
- The prevalence of AMR is < 5%
- In sensitized patients can be > 20%

Risk factors for AMR

- Patients sensitized to HLA class I or II antigens (LVAD, re-HT)
- Multiple blood transfusions (previous surgery)
- Female gender (previous pregnancy)
- Positive flow cytometric crossmatch
- Development of “de novo” DSAs after HT
- Young age

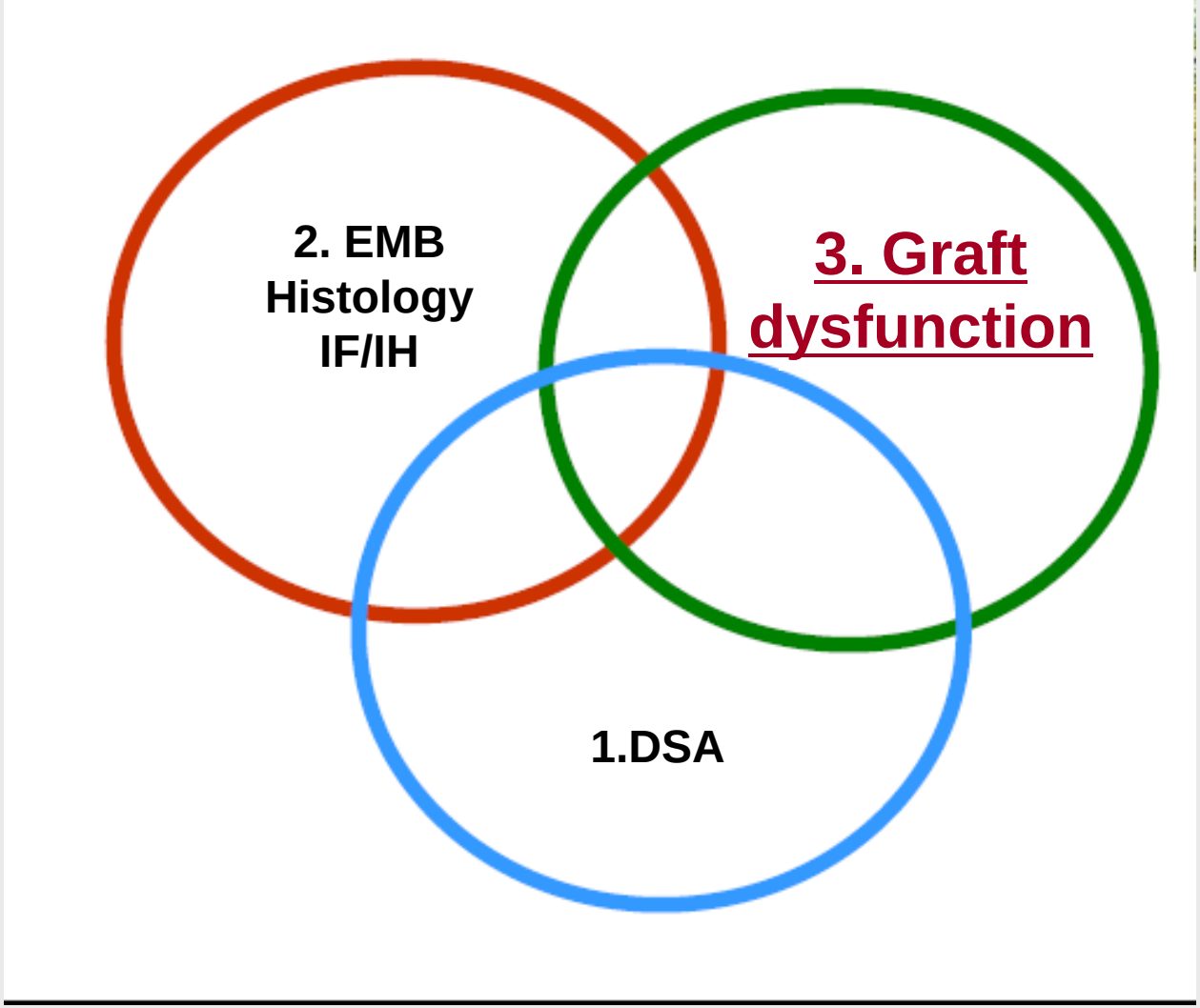
W Uber Am J Transpl 2007;7:2064
Kobashigawa J J Heart Lung Transpl 2011;30:252

Rechazo mediado por anticuerpos (AMR)

1. Patogenesis del AMR

- **Como diagnosticar el AMR**
- Cuando monitorizar para diagnosticar AMR
- Pronóstico
- Cuando tratar el AMR
- Como tratar el AMR

How to diagnose AMR



Kittleson M Curr Opin Organ Transplant 2012;17:551
Chih S J Transplant 2012

Kfoury AG J Heart Lung Transpl 2009;28:781

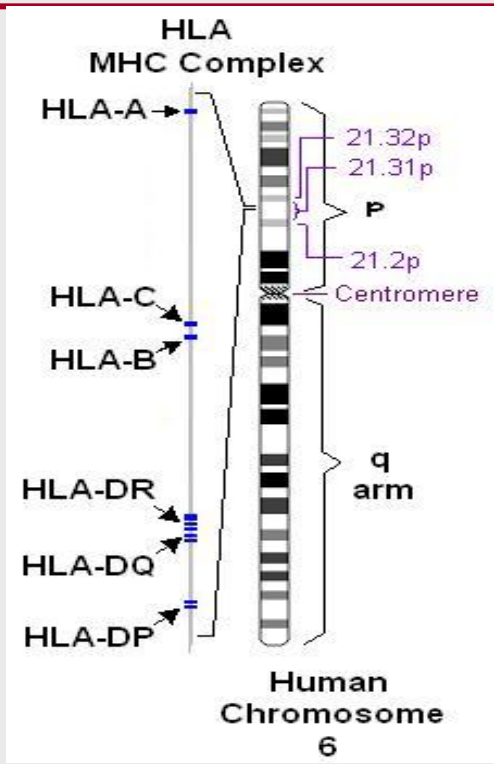
How to diagnose AMR

1. Donor-specific antibodies

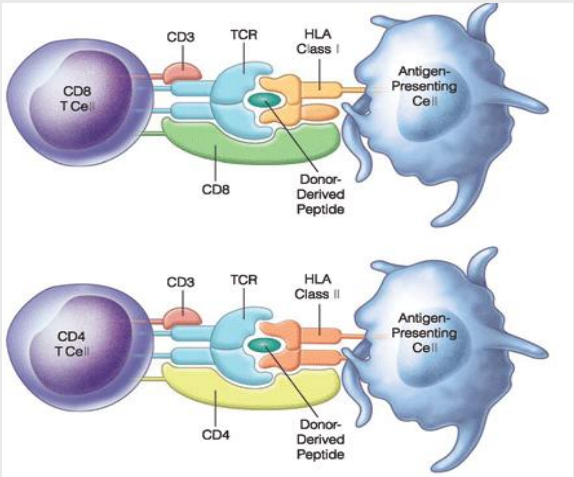
Donor-specific:

- **HLA (Human Leucocyte Antigens) class I (-A, -B, -C)**
- **HLA class II antibodies (-DP, -DQ, -DR)**

- **In absence of detectable HLA AB, non-HLA AB such as MICA, vimentin, others…**



- **Antigen presenting cells to lymphocytes T**
- **Activates immunologic reaction**



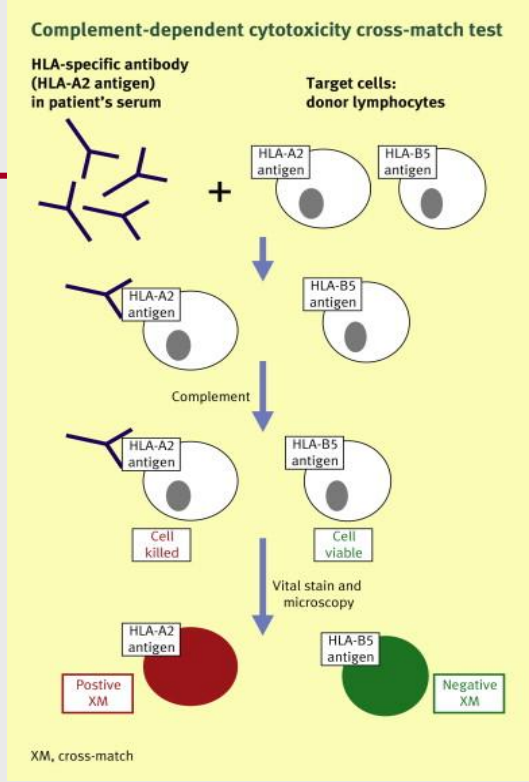
Kobashigawa J J Heart Lung Transpl 2011;30:252
Kittleson M Curr Opin Organ Transplt 2012;17:551
GJ Berry J Heart Lung Transpl 2011;30:601
Tait B Transplantation 2013;95:19

How to diagnose AMR

DSA assessment

- **Cell based panel reactive antiboby (PRA) or flow cytometry**
  - **> 10% positive**
  - **> 80% highly sensitized**

- **Solid-phase immunoassays (SPI): ELISA**
  LUMINEX



AB are tagged with an anti-Ig fluorescent carrier that can be detected by flow cytometry and quantified as MFI (mean fluorescence intensity)
Luminex indicates the presence or absence of HLA antibodies

- **Single-HLA-antigen beads (SAB)**

Kobashigawa J J Heart Lung Transpl 2011;30:252
Kittleson M Curr Opin Organ Transplt 2012;17:551
GJ Berry J Heart Lung Transpl 2011;30:601
Tait B Transplantation 2013;95:19

How to diagnose AMR

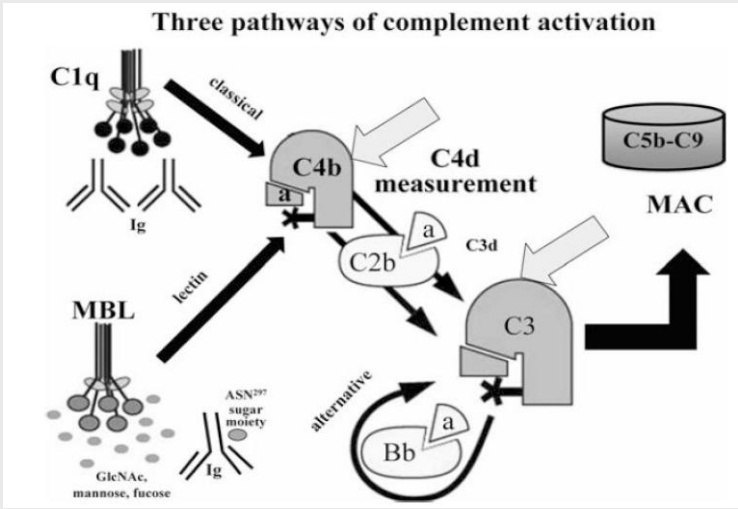
DSA assessment

- Recent studies indicate that the ability of DSA to fix complement may be a better marker of their cytotoxicity

- DSA capable of fixing C1q identify Abs that can initiate complement fixation and potentially activate complement cascade

- However, the detection of DSA has been associated with poor survival independently of their ability to fix complement

- More studies are needed to establish if C1q SPI-assays can be useful to diagnose AMR



- Identification of molecular targets by genomic and proteomic profiling are under investigation

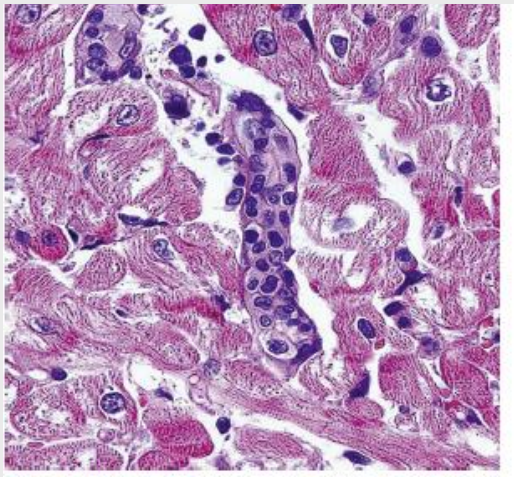
Kittleson M Curr Opin Organ Transplant 2012;17:551
Wasowska B Immunol Res 2010;47:25
Chin C J Heart Lung Transplant 2011;30:158
Zeevi A J heart Lung transplant 2013;32:98

How to diagnose AMR

2. EMB

2.1. Histologic changes

- **Endothelial capillary injury with cell swelling and intravascular macrophages**



- **Interstitial edema and hemorrage**
- **Mixed inflammatory infiltrates**
- **Cell necrosis**
- **Intravascular thrombi**

Berry GJ J Heart Lung Transpl 2011;30:601
Tan C Am J Transplant 2009;9:2075
Kobashigawa J J Heart Lung Transpl 2011;30:252
Revelo M J Heart Lung Transplant 2011;30:144

How to diagnose AMR

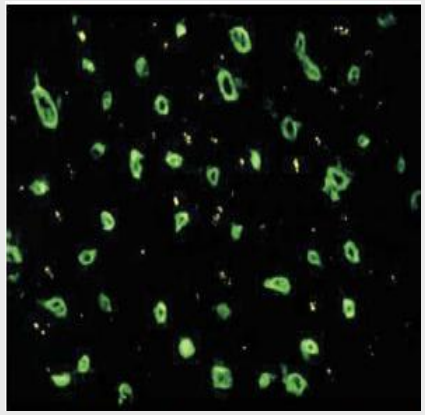
2. EMB

Immunopathology

C4d and C3d are split products of complement that have been identified as markers of antigen-AB interactions

2.1 Immunofluorescence

- **C4d, C3d staining in capillaries**
- **HLA (assessment of capillary integrity)**
- **Optional: Igs, fibrin**



Immunofluorescence + for C4d in capillares

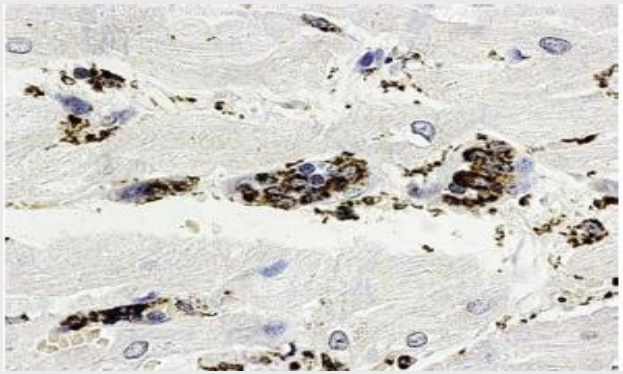
Predictive value using only C4d was 42% and using C4d + C3d 84%

Berry GJ J Heart Lung Transpl 2011;30:601
Tan C Am J Transplant 2009;9:2075
Kobashigawa J J Heart Lung Transpl 2011;30:252
Revelo M J Heart Lung Transplant 2011;30:144

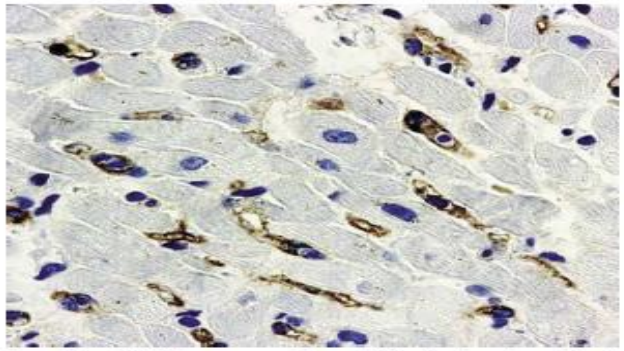
How to diagnose AMR

2.2 Immunoperoxidase

- C4d, CD68 staining
- Optional: C3d, vascular marker (CD34,CD31), CD3, CD20



Immunoperoxidase staining positive for CD68, confirming iv macrophages



Immunoperoxidase staining positive in capillaries for C4d

Interpretation of immunostaining

Only staining of interstitial capillaries

Semiquantitative score of 0 to 3+

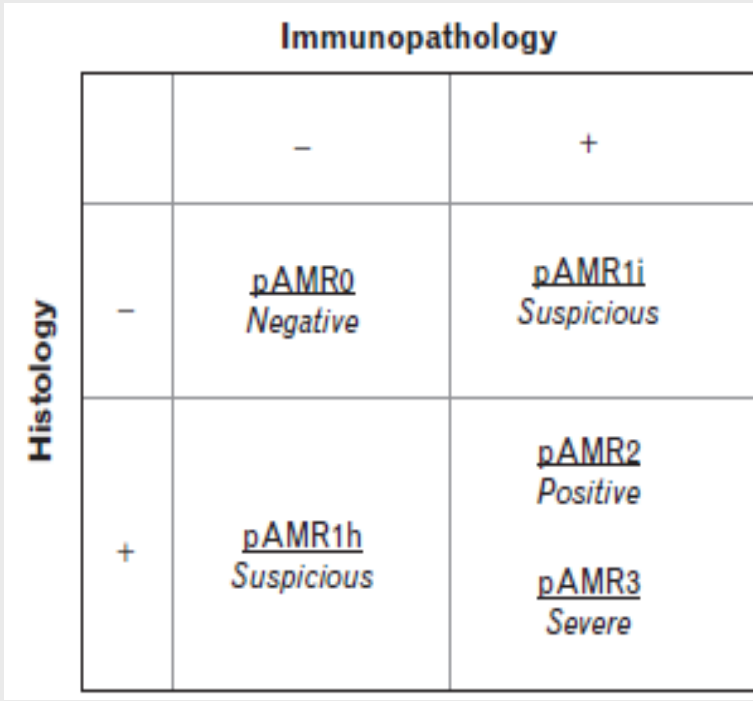
- 0 negative
- 1+ weak focal staining
- 2+ moderate multifocal staining (>50% of capillaries)
- 3+ strong diffuse staining

Immunostaining 2+, 3+ is required for a positive result

Kobashigawa J J Heart Lung Transpl 2011;30:252 Berry GJ J Heart Lung Transpl 2011;30:601

How to diagnose AMR

Grade	Pathologic features of AMR[1]
pAMR 0 Negative for pathologic AMR	Negative histologic and immunopathologic findings
pAMR 1 (H+): Histopathologic AMR	Positive histologic findings alone
pAMR 1 (I+): Immunopathologic AMR	Positive immunopathologic findings alone
pAMR 2 Pathologic AMR	Both histologic and immunopathologic findings
pAMR 3 Severe pathologic AMR	Interstitial hemorrhage, edema, capillary fragmentation, mixed inflammatory infiltrates, endothelial cell pyknosis/karyorrhexis



Without graft dysfunction:

pAMR 0 + DSA latent

pAMR 1 + DSA silent

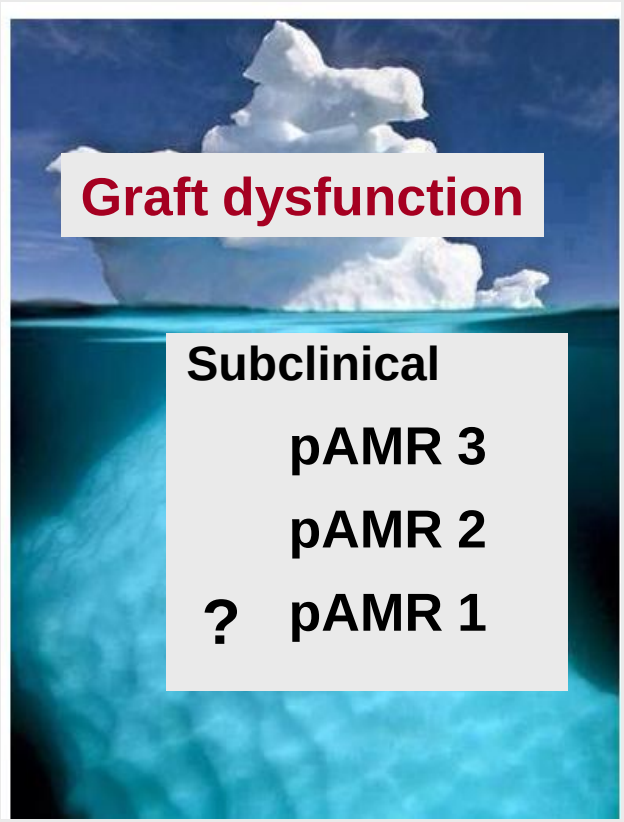
pAMR 2, 3 subclínic graft dysfunction

Kobashigawa J J Heart Lung Transpl 2011;30:252 Kittleson M Curr Opin Organ Transplant 2012;17:551

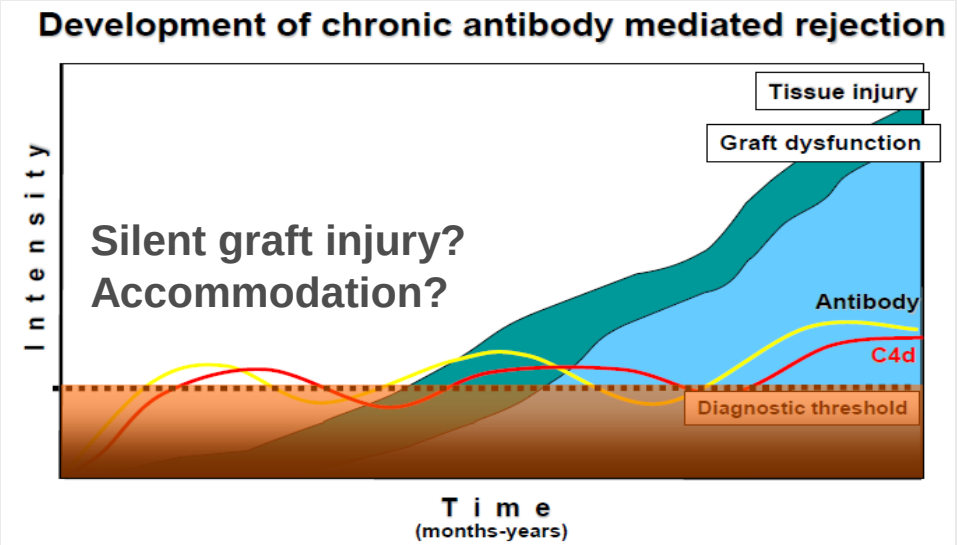
How to diagnose AMR

3. Graft dysfunction

- EF≤45% or ≥25% decrease from baseline
- Severe restrictive physiology
- PCWP >20 mmHg and CI < 2.0 L/min/m^2



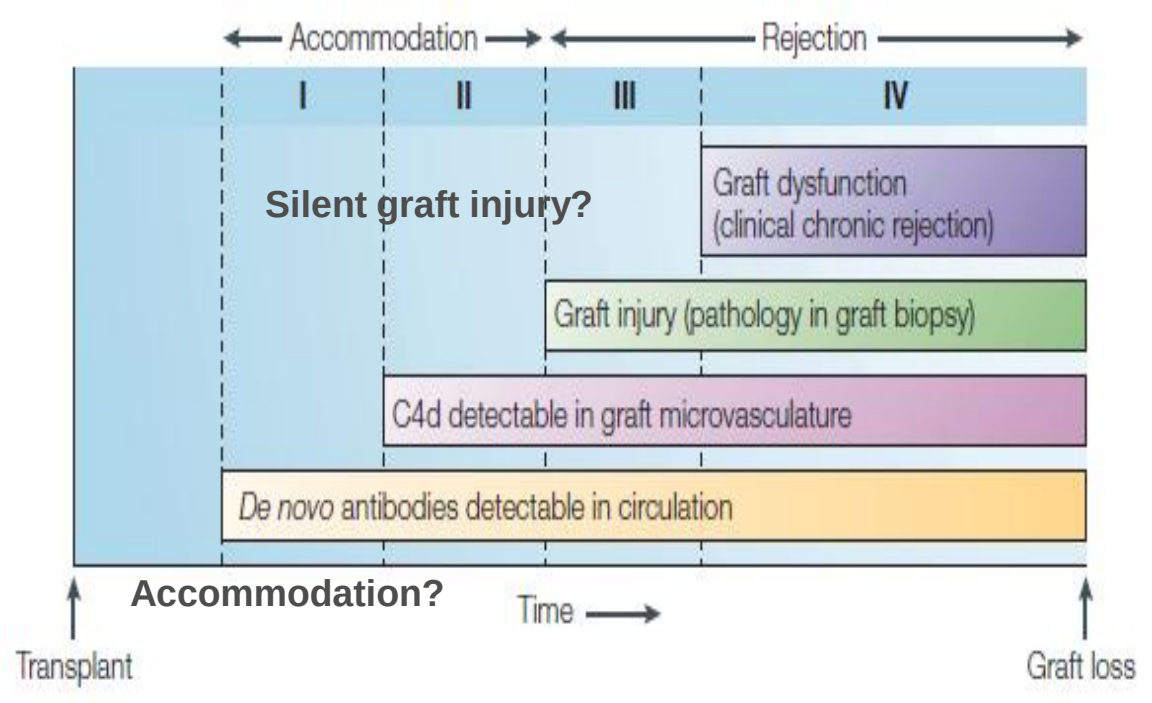
- Asymptomatic
- Clinical heart failure
- Cardiogenic shock



Colvin R. Nature Reviews Immunology 2005: 5, 807-817.

Chih S J Transplant 2012

How to diagnose AMR



Failure to detect circulating DSA does not rule out AMR diagnosis since they may be bound to the graft

Kobashigawa J J Heart Lung Transpl 2011;30:252
Kittleson M Curr Opin Organ Transplant 2012;17:551
Kfoury AG J Heart Lung Transpl 2009;28:781
Michaels PJ J Heart Lung Transpl 2003;22:58

Rechazo mediado por anticuerpos (AMR)

- Patogenesis del AMR
- Como diagnosticar el AMR
- **Cuando monitorizar para diagnosticar AMR**
- Pronóstico
- Cuando tratar el AMR
- Como tratar el AMR

When to monitor for AMR

ISHLT CONSENSUS

Report from a consensus conference on antibody-mediated rejection in heart transplantation

Jon Kobashigawa, MD,[a] Maria G. Crespo-Leiro, MD,[b] Stephan M. Ensminger, MD,[c]

- **Histological AMR evaluation of every EMB**

- **Immunoperoxidase / Immunofluorescent staining**

  at : 2 weeks, 1, 3, 6, 12 months and when clinically suspected.

?

- **DSA at : 2 weeks, 1, 3, 6, 12 months, annually thereafter and when clinically suspected**

- **After a positive EMB, repeat testing until a negative result**

Kobashigawa J J Heart Lung Transpl 2011;30:252

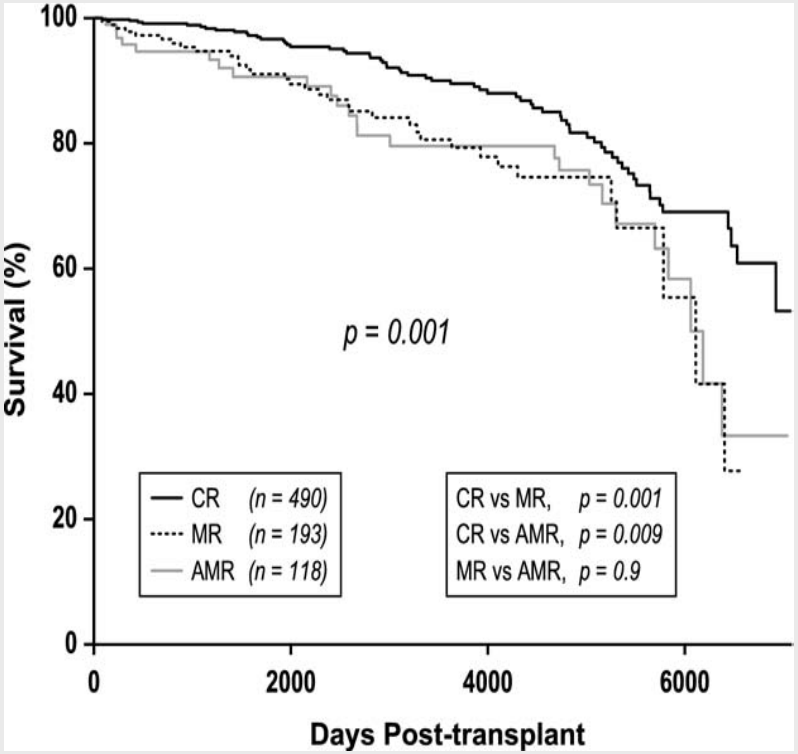
GJ Berry J Heart Lung Transpl 2011;30:601

Rechazo mediado por anticuerpos (AMR)

- Patogenesis del AMR
- Como diagnosticar el AMR
- Cuando monitorizar para diagnosticar AMR
- **Pronóstico**
- Cuando tratar el AMR
- Como tratar el AMR

AMR (antibody mediated rejection)

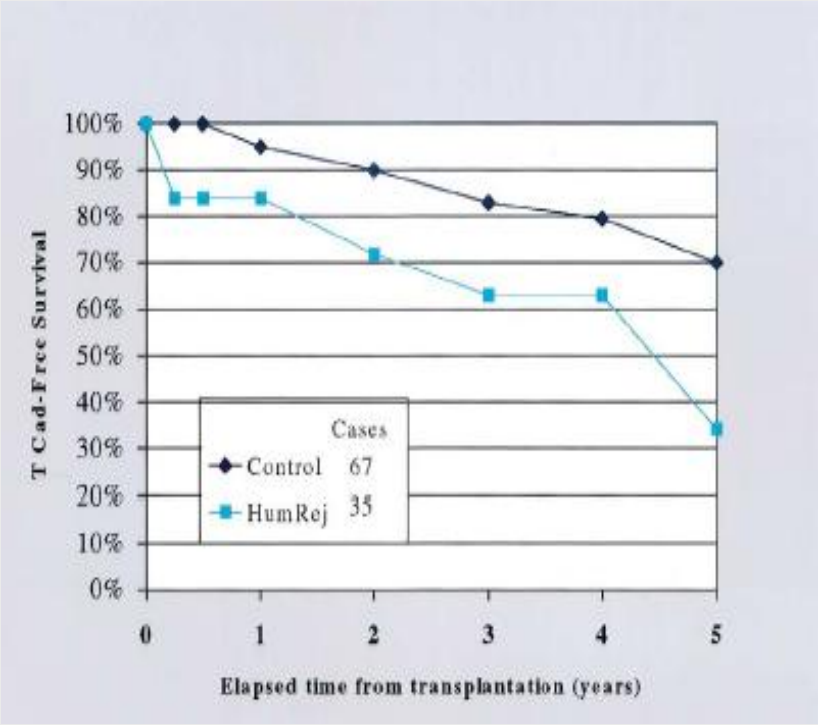
Utah HT program (1985-2004) 869 HT



CV mortality: SD, AMI, CAV, HF, PGF

>3 AMR: complement and Immgl deposits

Endothelial activation, iv macrophages, edema



Accelerated allograft vasculopthy

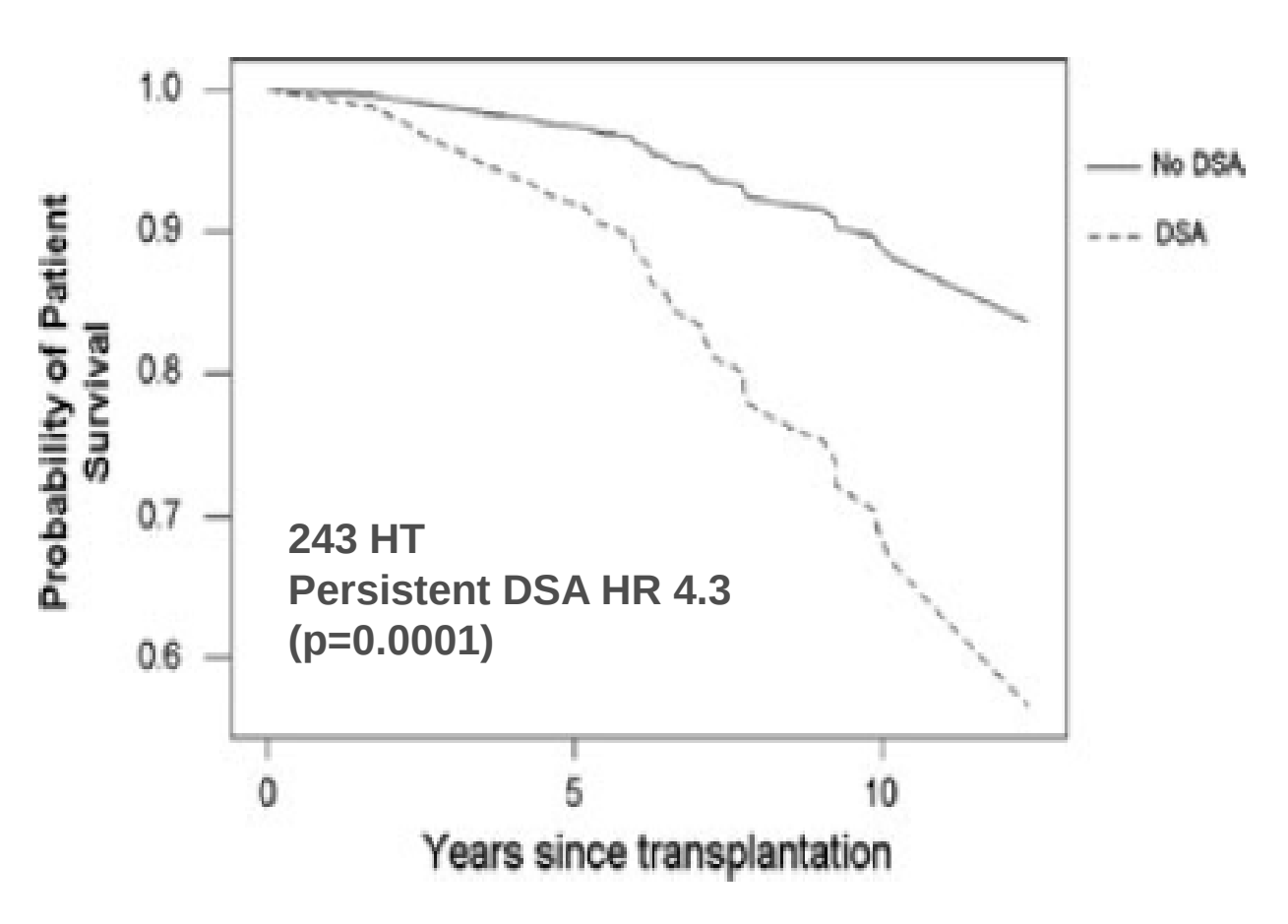
44 HT AMR: Histology and Immunofl.

47% shock, hypotension, low CO, rise in PWP

Kfoury AG J Heart Lung Transpl 2009;28:781

Michaels PJ J Heart Lung Transpl 2003;22:58

AMR - Prognosis



De 243 TC, 57 (23%)presentaron de novo DSA la mayoria HLA clase II

Smith JD Am J Transplant 2011;11:312

AMR - Prognosis

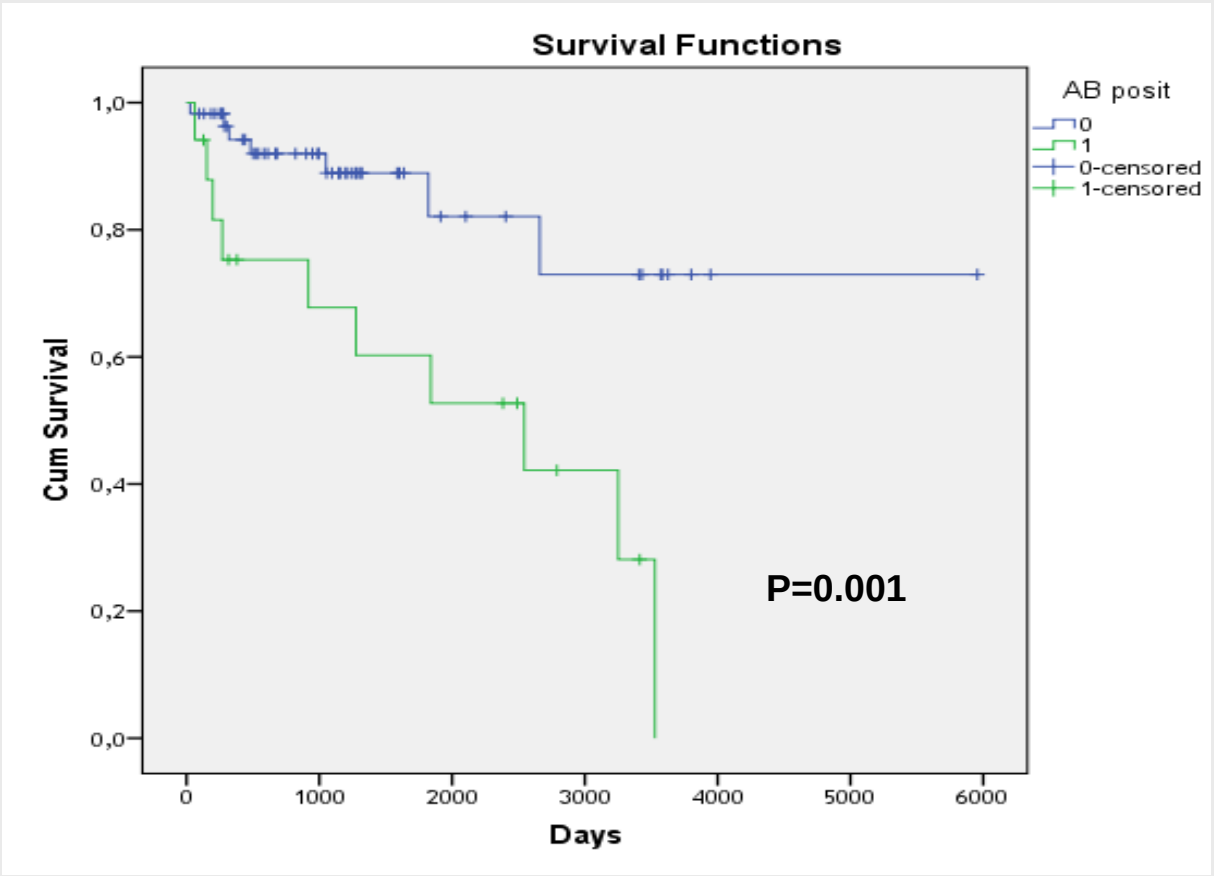
Hospital Sant Pau

77 TC → **17 (22%) con anticuerpos + :** **11 HLA clase II**

(157 muestras)

3 HLA clase I y II

3 HLA clase I



6 Disfunción injerto

1 EVI precoz

3 Exitus

1 Taponamiento car.

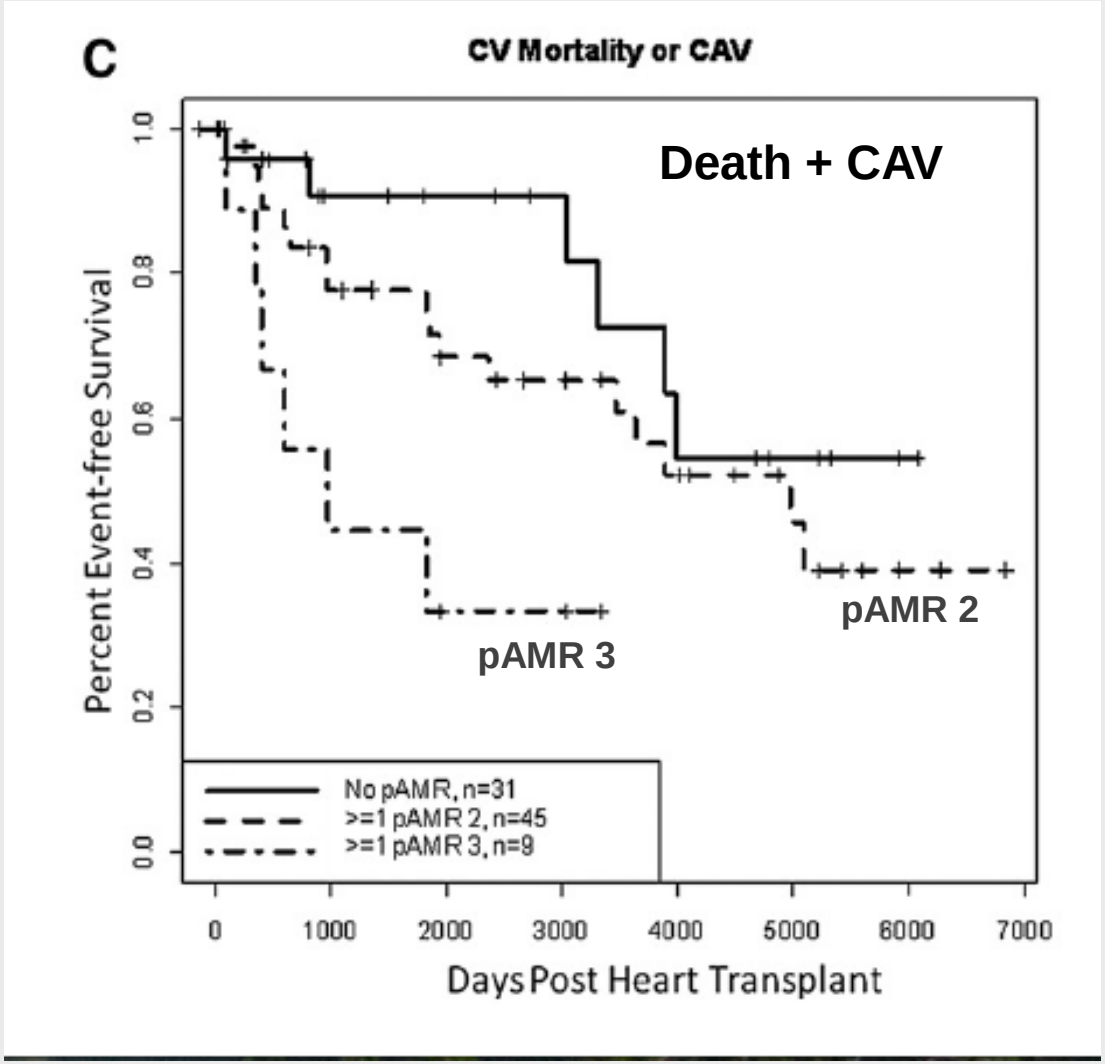
1 Traslado

5 Asintomáticos

59% vs 12%, p=0,0001

5 se han negativizado

AMR - Prognosis



Pediatric HT
1406 EMB
pAMR 2-3 18%

Histology \ Immunopathology	–	+
–	pAMR0 *Negative*	pAMR1i *Suspicious*
+	pAMR1h *Suspicious*	pAMR2 *Positive* pAMR3 *Severe*

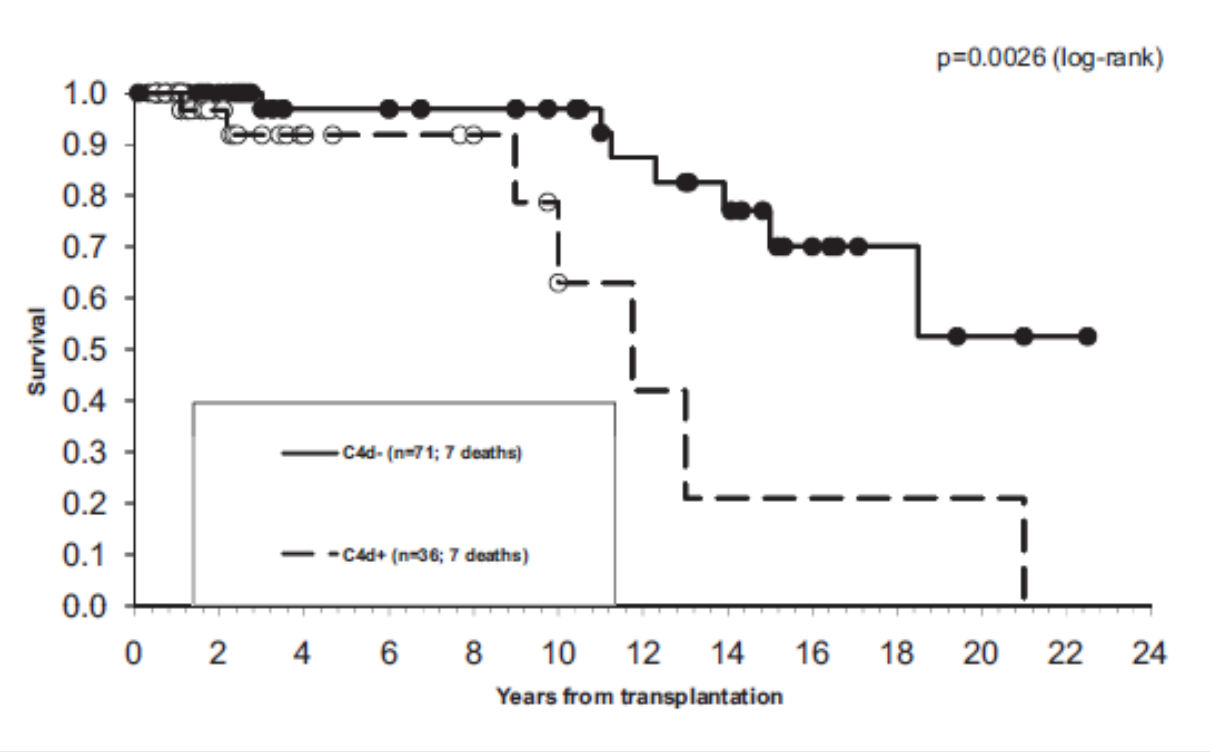
Everitt M J Heart Lung Transplant 2012;31:686, Utah

AMR - Prognosis

985 EMB from 107 HT

C4d + en 36 (34%) of these 57% had graft dysfunction

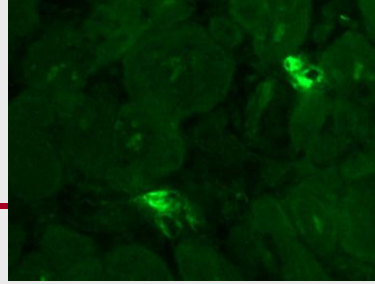
14 DSA (39%)



Fedrigo M Transplant 2010;90:791

Hospital Sant Pau

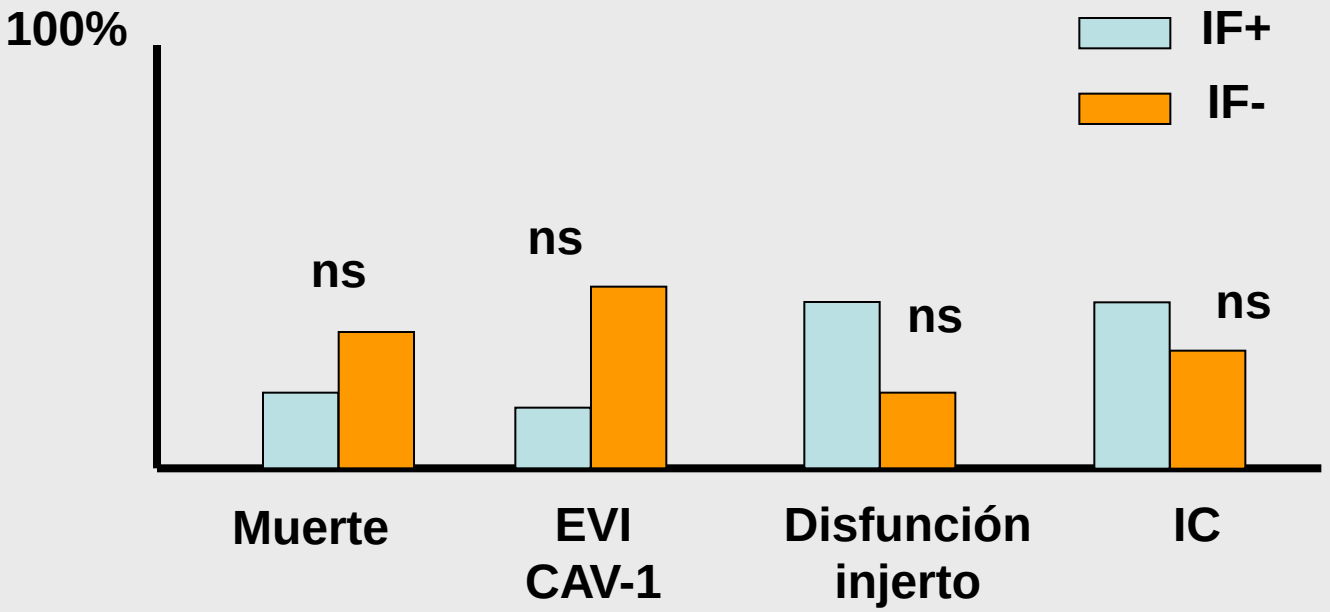
AMR - Prognosis



44 TC análisis Inmunofluorescencia de las BEM (250)

- **25 TC (57%) tenian depositos de C4d o C3 con patrón multifocal o difuso**
- **Seguimiento medio de 2,5 a**

La IF+ (pAMR-1) no se correlacionó con:



Rechazo mediado por anticuerpos (AMR)

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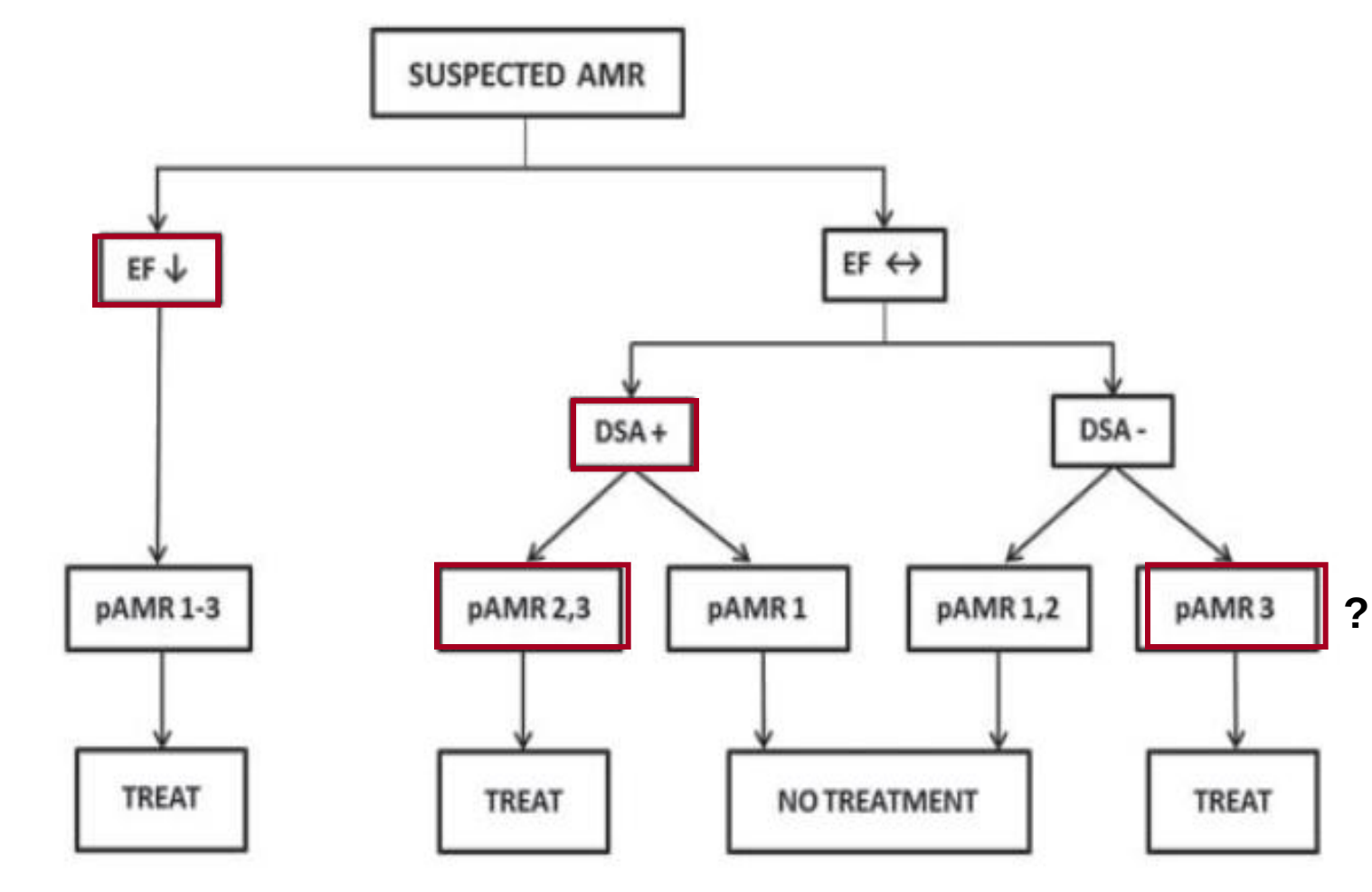
- Pronóstico
- **Cuando tratar el AMR**
- Como tratar el AMR

When to treat AMR after HTx



Chih S Am J Transplant 2013

Rechazo mediado por anticuerpos (AMR)

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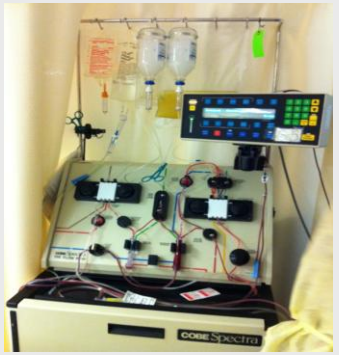
- Pronóstico
- Cuando tratar el AMR
- **Como tratar el AMR**

How to treat AMR

The objectives are: improvement of graft dysfunction, prevention of graft vasculopathy mortality reduction



Depletion

Removal of circulating AB

- **Plasmapheresis:** Plasma exchange
  Double filtration plasmapheresis
- **Immunoadsorption**

Modulation

Blockade of circulating AB

- **B-Lymphocytes suppression** — **Rituximab**
- **Depletion of plasma cells** — **Bortezomib**
- **T-Lymphocyte suppression** — **Bolus steroids**
- **Complement cascade inhibition** — **Eculizumab**
- **Accommodation and tolerance?**

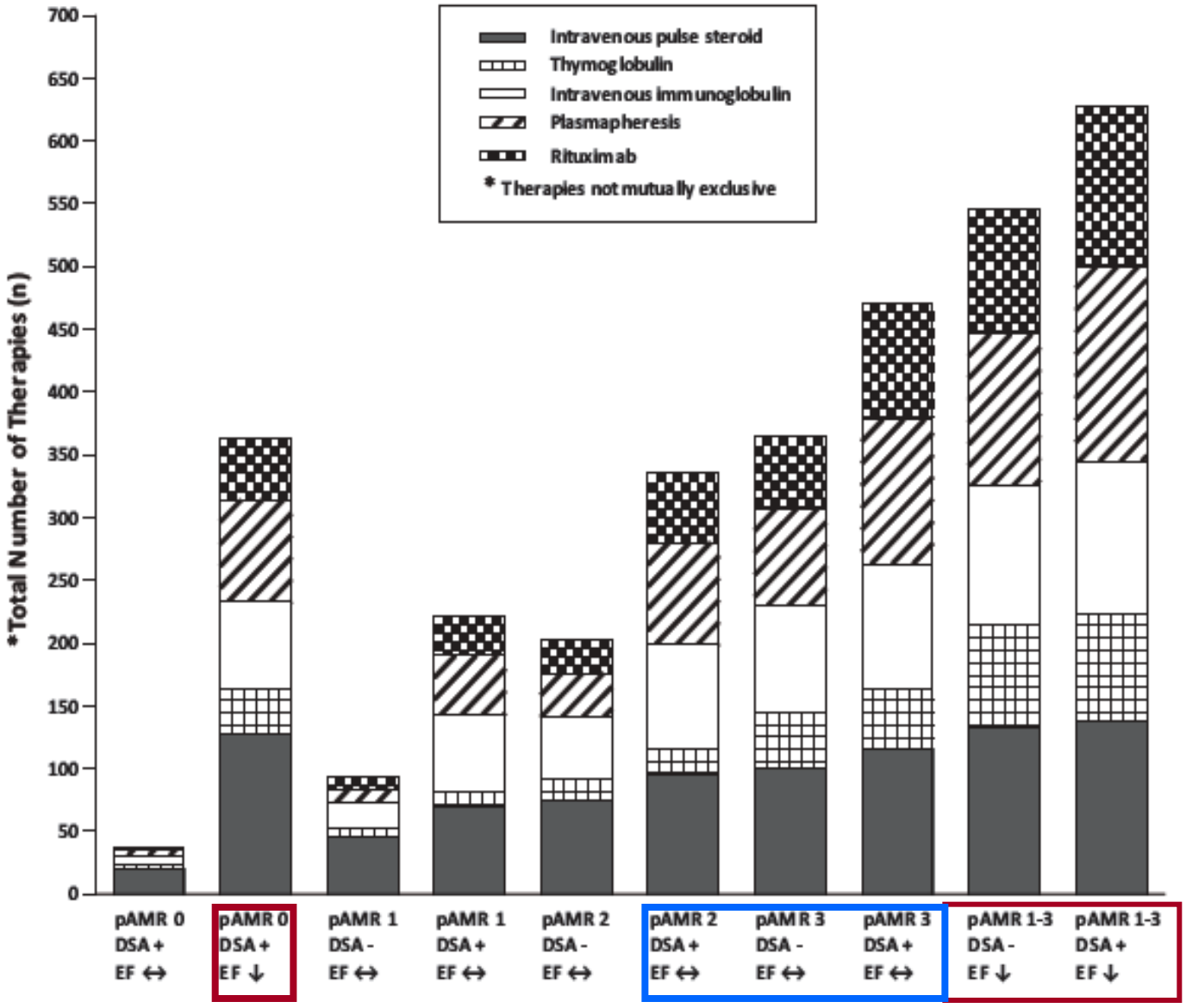
Bartel Transplant 2011;24:1144

MR Costanzo J Heart Lung Transplant 2010;29:914

Chih S Am J Transplant 2013

Nandini N J Heart lung Transplant 2011;30:612

How to treat AMR



Chih S Am J Transplant 2013

Conclusions

- There is lack of information regarding the natural history of AMR without graft dysfunction and when and how to treat these patients

- Treatment may be considered when there is presence of circulating DSA and pAMR 2+ or 3+

- There is general agreement on treating patients with graft dysfunction

- Multidisciplinary teams are also needed (cardiology, pathology, immunology) for better understanding of AMR after HTx

Muchas gracias