

Antibody Mediated Rejection (AMR) in Heart Transplantation Session

Dear Colleagues:

The Heart session at Banff this year will be solely focused on AMR and besides the fact that many of us attending the Banff meeting are also members of the ISHLT, this year we will have a formal representative from the Board of Directors of ISHLT (Dr. Lori West) attending the Heart session of the conference. This session will be Co-chaired by Dr. Adriana Zeevi and myself.

Our aim is to use the current ISHLT definition of Acute Antibody-Mediated Rejection as a starting point and discuss how this definition is working five years after been crafted at ISHLT in 2004 and three years after been published.

ISHLT - Acute Antibody-mediated Rejection of Cardiac Transplants

"The combination of clinical, histologic, and immunopathologic findings as well as demonstration of circulating donor specific antibodies, in the absence of cellular rejection, are recommended to diagnose acute antibody-mediated rejection (Table 1)".

"Table 1. Findings in Acute Antibody-Mediated Rejection of the Heart

1. Clinical evidence of acute graft dysfunction
2. Histologic evidence of acute capillary injury (*a* and *b* are required)
 - a. Capillary endothelial changes: swelling or denudation with congestion
 - b. Macrophages in capillaries
 - c. Neutrophils in capillaries (more severe cases)
 - d. Interstitial edema and/or hemorrhage (more severe cases)
3. Immunopathologic evidence for antibody mediated injury (in the absence of OKT 3 induction) *a* or *b* or *c* are required
 - a. Ig (G,M, and/or A) C3d and/or C4d or C1q (equivalent staining diffusely in capillaries, 2–3)+, demonstrated by immunofluorescence
 - b. CD68 positivity for macrophages in capillaries (identified using CD31 or CD34), and/or C4d staining of capillaries with 2–3+ intensity by paraffin immunohistochemistry
 - c. Fibrin in vessels (optional; if present, process is reported as more severe)
4. Serologic evidence of anti-HLA class I and/or class II antibodies or other anti-donor antibody (e.g., non-HLA antibody, ABO) at time of biopsy (supports clinical and/or morphologic findings)"

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At this juncture, it seems that there is no consensus on: 1. What tests to perform and how to interpret histopathologic patterns of markers. 2. How and when to use serologic data of donor specific antibody. 3. How is dysfunction of the allograft defined/determined to correlate it with histopathologic and serologic findings. Thus, the aim of the heart session of Banff is to focus on these three issues. We will not allocate specific times for discussion of the so called "chronic antibody mediated rejection" as it has not been really defined in heart transplantation by ISHLT. Accordingly we will not discuss the relation of AMR to allograft vasculopathy, as it would be premature at this time.

The Banff Conference will take place from August 9 - 14, 2009 in Banff, Alberta, Canada. (<http://cybernephrology.ualberta.ca/banff/2009/programme.htm>). The heart session will be a concurrent session that will last all day on Wednesday August 12, 2009, and the topics are:

Concurrent Session: Heart : Antibody Mediated Rejection in Heart Transplantation

Chairs: **E Rene Rodriguez (Cleveland) and Adriana Zeevi (Pittsburgh)**

8:00 - 8:05 Introduction and goals for the session. **E Rene Rodriguez and Adriana Zeevi**

8:05 - 8:30 Edmonton results from 100 heart allograft biopsies. **Banu Sis (Edmonton)**

8:30 - 8:50 Markers of Antibody Mediated Rejection and Accommodation in Heart Transplantation.
E Rene Rodriguez

8:50 - 9:30 Antibodies and the cardiac allograft: types, temporal appearance and minim follow-up data points to assess a trend. How to interpret in conjunction with the clinical and pathology team.
Adriana Zeevi, Nancy Reinsmoen (Los Angeles)

9:30 - 9:50 Diagnosis of AMR - controversies and implications from the clinicians (medical –surgical) point of view. **Abdallah Kfoury (Utah)**

10:00 - 10:30 Break

10:30 - 10:45 AMR Current Practice Survey - Europe. **Margaret M. Burke (London) & Annalisa Angelini (Padova)**

10:45 - 11:00 AMR Current Practice Survey - North America. **Marc Halushka (Baltimore)**

11:00 - 11:15 AMR Experience from Birmingham UK. **Desley A.H. Neil (Birmingham, UK)**

11:15 - 11:30 AMR Experience from Boston. **Rex Neal Smith (Boston)**

11:30 - 11:45 AMR Experience from Cleveland. **Carmela D. Tan (Cleveland)**

11:45 - 12:00 Summary of the presentations, and targeted questions to be addressed in the remainder of the heart session **E Rene Rodriguez and Adriana Zeevi**

12:00 - 1:00 Lunch

1:00 - 3:00 Patterns of C4d and C3d deposition in AMR - Reproducibility among centers. Presentation of reproducibility exercise of C4d and C3d staining of the same exact patients in European and North American Centers) (Immunohistochemical and Immunofluorescence) (Open to all participants)

3:00 - 3:30 Coffee Break

3:30 – 4:45 Discussion - Use and interpretation of Serologic data (Correlation with allograft dysfunction and complement deposition) (Open to all attendees)

4:45 - 5:15 Summary of the session

The first part of the session (From 8:00 to noon) is self explanatory, there will be a few short presentations (which will include reports of the aspects relevant to AMR from two independent surveys (one in Europe and one in North America). These morning activities will serve as preamble to the discussion sessions in the afternoon.

The first session in the afternoon will be dedicated to discuss the patterns of complement split products C4d and C3d deposition that appear in the heart and their interpretation.

We will start with the review of results of a short experiment in reproducibility. In this experiment, cardiac pathologists were contacted, asking them if they had a positive autopsy case that clearly was positive for C4d staining in capillary pattern. In turn those who had such case were asked if they could contribute a paraffin block. From those blocks received in Cleveland, we would divide the tissue and create new blocks in which myocardium from control cases and C4d positive cases would be present. These blocks with samples of the exact same cases would be distributed the centers that contributed tissue, so that they, in turn stain these exact same tissues with the

techniques used locally to evaluate C4d and C3d. Stained slides will be scanned in Cleveland and brought to Banff as Aperio files for discussion.

While doing this exercise, we will address technical issues such as monoclonal vs. polyclonal, antigen retrieval methods used, incubation times, sources of antibodies (companies, catalog numbers, when available). Interpretation of the staining patterns, intensity vs. extent (surface area) will be addressed.

In addition, if you have interesting, unusual or difficult cases and would like to send 3-5 PowerPoint slides or glass slides to be scanned ahead of time to rodrigr2@ccf.org, I'll make sure that we can collate a few of these for discussion. Later in the afternoon the use and correlation of DSA with clinical dysfunction and with complement deposition in AMR will be the focus of the discussion.

The issues of reporting AMR findings will also be discussed. Thus by the end of this time block, we hope to have useful conclusions about the use of current, widely available tests in tissue, serum and current clinical practice to make the diagnosis of AMR

The last discussion block will bring up the issue of how DSA testing fits in the workup of a new episode of AMR, its evolution and resolution and will also address the issue of DSA testing during treatment and in persistent/recurrent cases. The role of "chronic" DSA lacks, at this juncture enough experience a many centers to evaluate in a meaningful way, and consequently it will not be discussed in this session.

Practical goals for the heart session:

HOW DO WE MAKE THE PATHOLOGIC DIAGNOSIS OF AMR?

Recommendations for the technical aspects:

1. Who orders the test - is it based on clinical suspicion of the clinicians or histologic screening by the pathologists, any other scenario
2. Which antibodies to use
 - Immunofluorescence
 - Immunoperoxidase
3. Testing schedule - When is testing done - routinely, only during the first 6 weeks
4. Are there false negatives?

Interpretation of tests for complement deposition

1. Which vascular territories to interpret?
2. Is intensity graded? how? do we need it? does it correlate?
3. What is the extent of staining - focal vs diffuse?
4. How often is endothelial swelling and intravascular macrophages seen? severity?
5. Artifacts - interstitial staining, endocardial staining, arterial staining, myocytes?

Reporting

1. How is AMR reported? what is or should be reported as considered positive? ISHLT definition (i.e. histologic markers + complement + DSA + clinical dysfunction)? C4d alone? (there are papers reporting asymptomatic AMR).
2. What are the changes seen after therapy?
How often do you see clear cellular rejection greater than grade 1R along with markers of AMR.

SEROLOGIC ASPECTS IN THE DIAGNOSIS OF AMR

1. When DSA is present, how often do we determine the titers? One time? More than one? Does your center follow the titers to monitor therapy?
2. How often does a drop in DSA titer correlate with improvement in function of the graft? Or changes in the histopathologic markers?
2. Does your center test for Non-HLA antigens? Routinely? Reflexive testing after HLA is negative?

CLINICAL ASPECTS

Could you explain how the cardiology / cardiac surgery team in your center makes the diagnosis of allograft dysfunction (elevated RA pressures, restrictive pattern on echo, drop in ejection fraction (by how much?), decrease in cardiac index, need for inotropic support) What should clinicians do with a positive result? C4d vs C4dC3d positives?
Do you see asymptomatic AMRs? (Not withstanding that the current ISHLT definition of AMR requires clinical dysfunction of the allograft to make the diagnosis).

I hope to see as many of you (pathologists, immunologists, cardiologists and surgeons) as possible to have a constructive session on AMR.

E Rene Rodriguez MD

In preparation for this Heart Session it would be very useful for the participants to have some concrete ideas in your mind about some questions that have been posed by a number of individuals in the last couple of months of email exchanges. (If you feel inclined to answer these, and email the answers to rodrigr2@ccf.org, I'll collate the answers that become available before the meeting). Bear in mind that the results of two surveys (for which the data analysis is not complete as of the time of this email) will be presented during the session:

1. In your center, what sequence of events do you follow most commonly? Do you base your diagnosis of AMR by first looking for histologic markers (endothelial swelling, intravascular macrophages)? Is it more common to receive information about DSA when you receive a biopsy with clinical suspicion? Who makes the diagnosis of AMR? Who orders the pathologic work-up of AMR - pathologists or clinicians? When do you perform immunofluorescence / immunoperoxidase testing for AMR? How often is the test performed after the first 6 weeks? How often is the biopsy positive and there is no allograft dysfunction?
2. Which Immunohistochemical stains (Immunofluorescence or immunoperoxidase) do you use in all (or almost all) your cases to evaluate AMR (IgG, IgM, IgA, Fibrin, HLA DR, C1q, C4d, C3d)
3. In the evaluation of any of the markers in question No. 2 do you use immunofluorescence or immunoperoxidase?
4. If you evaluate a biopsy for the presence of intravascular macrophages, do you do it on H&E only or do you always use a macrophage marker?
5. If you evaluate for complement deposition, which vascular beds do you evaluate? Capillary bed? Arterioles? Small arteries? Venules? Endocardium?
6. When you evaluate a marker do you interpret intensity of the stain? Extent of involvement of the biopsy surface area, Focal vs. Diffuse?
7. How often do you see clear cellular rejection greater than grade 1R along with markers of AMR.
8. Should / Can AMR be graded according to severity?
9. Do you consider the information from serology to render a diagnosis of AMR0 vs AMR1 as suggested by ISHLT? Do you consider the presence or absence of clinical dysfunction of the allograft.
10. Could you explain how the cardiology / cardiac surgery team makes the diagnosis of allograft dysfunction (elevated RA pressures, restrictive pattern on echo, drop in ejection fraction (by how much?), decrease in cardiac index, need for inotropic support)

11. When DSA is present, how often to determine the titers? One time? More than one? Does your center follow the titers to monitor therapy?

12. How often does a drop in DSA titer correlate with improvement in function of the graft? Or changes in the histopathologic markers? Which changes in the pattern - extent and intensity - of staining are seen after therapy for AMR?

11. Does your center test for Non-HLA antigens? Routinely? Reflexive testing after HLA is negative?
