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## Meeting report

# Report of the 2022 Banff Heart Concurrent: Focus on non-human leukocyte antigen antibodies in rejection and the pathology of “mixed” rejection



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## ABSTRACT

The Banff Heart Concurrent Session, held as part of the 16th Banff Foundation for Allograft Pathology Conference at Banff, Alberta, Canada, on September 21, 2022, focused on 2 major topics: non-human leukocyte antigen (HLA) antibodies and mixed rejection. Each topic was addressed in a multidisciplinary fashion with clinical, immunological, and pathology perspectives and future developments and prospectives. Following the Banff organization model and principles, the collective aim of the speakers on each topic was to

- Determine current knowledge gaps in heart transplant pathology
- Identify limitations of current pathology classification systems
- Discuss next steps in addressing gaps and refining classification system

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## 1. Non-human leukocyte antigen antibodies in heart transplantation

### 1.1. Clinical perspective

Although the landscape of antibody-mediated rejection (AMR) due to antihuman leukocyte antigen (HLA) donor-specific antibodies (DSAs) has been well defined in heart transplantation, the picture of non-HLA cardiac rejection remains blurry.<sup>1–4</sup> AMR without circulating anti-HLA DSA may be just as deleterious for graft loss and progression of cardiac allograft vasculopathy as AMR with anti-HLA DSA.<sup>5</sup> However, the accurate evaluation and prognostic implications of non-HLA antibodies in AMR remain challenging owing to imprecise definitions and criteria and limited epidemiologic data. At a high level, the imprecision in definitions arise from a lack of validated laboratory assays for assessing the presence and attributes of non-HLA antibodies and a lack of understanding about how these antibodies mediate graft injury (making histologic criteria hard to define). This paucity of robust data directly affects treatment options.

The clinical decision to consider non-HLA antibody testing is a complex one. It is most straightforward in the setting of a patient with clinical graft dysfunction and histopathologic evidence of AMR in a biopsy but the absence of detectable DSA in the serum. Interpreting the results of non-HLA antibody testing is also complicated and should consider that some of detected antibodies may be “innocent” and not necessarily the cause of graft dysfunction.

A 3-step diagnostic process is proposed for non-HLA AMR (Fig. 1). First, the presence of microvascular inflammation should be confirmed, and alternative (nonrejection) explanations for this such injury should be excluded (eg, early posttransplant injury).<sup>6,7</sup> Gene expression patterns, as a companion tool of pathology, could bring valuable information in this regard but the data are less mature than for those of kidney allografts.<sup>8–11</sup> Second, thorough exclusion of anti-HLA DSA is mandatory. Current and past DSA testing should be reviewed because the absence of circulating anti-HLA DSA at one particular point in time (eg, clinical rejection) should not be considered as a sufficient criterion to exclude anti-HLA DSA as a cause of rejection. This is not because the HLA antibody is present and undetectable but to

consider the possibility of problems with the sample or assay run. Third, immunologic exploration for the presence of circulating non-anti-HLA antibodies (eg, multiple antibodies and endothelial crossmatching)<sup>12,13</sup> should yield some evidence of an antibody capable of inciting rejection. It is recognized that such testing may not be readily available for routine testing in most immunology, immunogenetics, and histocompatibility laboratories and they may add cost and lead to delays in clinical decisions. However, the refinement of the diagnosis of non-HLA cardiac rejection must depend on thorough and systematic efforts to better understand this condition.

Finally, it shall also be underlined that beyond antibody (anti-HLA and/or non-HLA) binding to endothelial cells of graft vasculature, other immunologic mechanisms can trigger the development of similar histologic lesions (ie, microvascular inflammation). Indeed, the inability of graft endothelial cells to provide HLA class I-mediated inhibitory signals to recipient circulating natural killer (NK) cells triggers their activation, which in turn promotes endothelial damage, leading to chronic vascular rejection in the absence of DSA.<sup>13,14</sup> Of note, this pathophysiologic mechanism can either operate alone or synergize with low levels of DSA to accelerate the development of vascular damages.<sup>15</sup> Analysis of HLA-I typing of donor/recipient pair and KIR receptor genotyping allow identifying recipients at risk of this missing self-induced NK cell activation.<sup>16</sup>

However, it is important to note that although this process is meant to define AMR solely due to non-HLA antibodies, many studies have shown that anti-HLA and non-HLA antibodies are often found together and even synergize increase the severity of AMR.<sup>12,17</sup> So, separating HLA and non-HLA antibodies in real-world clinical practice may be an artificial and oversimplified approach. Well-annotated cohorts of patients with and without anti-HLA DSA are needed to fully understand this overall picture and the full histologic presentation.

### 1.2. Pathology perspective

AMR (whether due to HLA or non-HLA antibodies) encompasses heterogeneous clinical and pathologic manifestation. These range from canonical cases with graft dysfunction, de

1. Confirm microvascular injury by histopathology, immunopathology, and/or gene expression (and exclude other non-rejection causes of injury)
2. Confirm the absence of circulating anti-HLA DSA, throughout the entire post-transplant course (not just at one point in time, such as a current rejection episode), while also recognizing anti-HLA DSA and non-HLA antibodies may coincide and are not mutually exclusive
3. Confirm the presence of non-HLA antibody(ies) or (antibody-independent) missing-self pathways capable of causing rejection (antibody assays, endothelial crossmatching, complete HLA-I typing of donor and recipient, KIR receptor genotyping, etc.)

**Figure 1.** Diagnostic process for non-HLA antibody-mediated cardiac allograft rejection.

novo DSA, and histologic and immunophenotypic evidence of AMR (pAMR2) to more challenging cases that have some but not all these features. In recent years, drawing from renal transplant experience, an emerging type of AMR due to non-HLA antibodies has come into the spotlight in both clinical and research realms. Recent literature suggests that the presence of these non-HLA antibodies is an independent or synergistic risk for graft dysfunction or graft loss, and different antibodies may have different weight on clinical significance.<sup>12,18</sup>

As we have learned more from the serology data about non-HLA AMR, it is still unclear whether endomyocardial biopsies (EMBs) from patients with non-HLA AMR can be distinguished from those seen in patients with HLA-DSA-mediated AMR. In the limited case reports and case series,<sup>19,20</sup> most of the non-HLA AMR EMBs demonstrated various degrees of microvascular changes but with a tendency toward negative C4d staining (or possibly perimyocytic or sarcolemmal staining patterns). It is also unclear whether non-HLA antibodies to different antigens are associated with different histologic features in EMBs. More studies are required to identify clinically applicable and validated assays/testing modalities, in both serology and histology, to reliably diagnose orthotopic heart transplant (OHT) patients with non-HLA AMR.

In summary, non-HLA AMR is a phenomenon recognized in a subgroup of cardiac transplant recipients experiencing graft dysfunction. Additional studies (multidisciplinary and multicenter) with well-defined cohorts and clinical outcomes are required to form a consensus on how to diagnose, treat, and monitor these patients.

### 1.3. Immunologic perspective

Because allograft tissues include both non-self-antigens and common antigens shared among our species, the host immune responses to graft implantation may be both alloimmune and autoimmune in nature (Table 1). The most studied of these on non-HLA antibodies include those reactive against angiotensin II receptor type 1 (AT1R), Vimentin, and the highly polymorphic MHC class I-related chain A (MIC-A). MIC-A is arguably a true anti-HLA DSA,<sup>21</sup> but has historically been considered non-HLA. Antiendothelial cell crossmatching may also detect non-HLA antibodies to yet-to-be-determined antigenic targets.

An early report from University of California–Los Angeles found the frequency of non-HLA antibodies among 19 heart transplant patients with AMR to be 43%.<sup>22</sup> Based on these data,

they implemented routine testing for non-HLA antibodies (MIC-A, anti-EC, and ATR1) for all patients with graft dysfunction but no demonstrable HLA DSA, at their center.

Despite this and several other reports over the past 2 decades, the role that non-HLA antibodies play in cardiac allograft rejection and transplant outcomes remains unclear.<sup>4,5,12,18–20,22</sup> Major challenges to be overcome include understanding of the following: (1) non-HLA antigen expression in allografts, (2) pathophysiology of non-HLA antibody binding-mediated graft injury, and (3) non-HLA antibody assay performance characteristics (sensitivity/specificity). Whether a different set of diagnostic criteria (apart from HLA-DSA-mediated AMR or acute cellular rejection [ACR]) are needed for non-HLA AMR, and the efficacy of the limited AMR-directed therapies in patients with non-HLA antibodies are also unanswered questions.<sup>12</sup>

Insights into autoimmune non-HLA antibodies can be gained from patients supported mechanically with ventricular assist devices (VADs) before transplant. Implanting these devices exposes sequestered myocardial antigens, which may trigger an immune response that augments after transplantation. In 15 VAD-supported patients experiencing primary graft dysfunction shortly after cardiac transplantation, 11 (73%) seroconverted to AT1R positive. AT1R antibody strength of >10 U/mL after VAD placement correlated with reduced cardiac output and primary dysfunction after transplantation. Older age and longer time on the waiting list exacerbated this effect (M. Hickey, E. DePasquale, S. Shah, D. Vucicevic, J.Q. Zhang, N.M. Valenzuela, M. Deng, E.F. Reed, unpublished data, 2022). At least 1 case of hyperacute rejection with a high titer anti-ATR1 antibody has also been reported; that patient was supported by 2 VADs before transplant.<sup>20</sup>

Graft rejection is a mechanism whereby previously sequestered antigen may be presented to the recipient immune system in a proinflammatory setting. This phenomenon could occur in tertiary lymphoid structures that develop within rejected grafts.<sup>23,24</sup> Accumulating evidence indeed suggests that these ectopic dysfunctional germinal centers are the site where the breakdown of self-tolerance occurs.<sup>25,26</sup> This may be yet another mechanisms whereby non-HLA antibody formation can injure the graft.

As the antiendothelial crossmatch data suggest, there are certainly other clinically important non-HLA antigen targets to be discovered. Immunogenetic and proteomic approaches to identifying new non-HLA antibodies hold promise in this regard. Butler et al<sup>12</sup> reported using stored sera from cardiac allograft

**Table 1**  
Autoimmune and alloimmune non-HLA antibodies in heart transplantation.

	Target	Mechanism	Prevalence (%)	Associations
MIC-A	MIC-A	Allograft	10	Retransplantation patients
Anti-AT1R	AT1R	Autograft	>50	Hypertension, endothelial injury
Vimentin	Vimentin	Autograft	10	Cardiac allograft vasculopathy
Antiendothelial	Unknown	Autograft/allograft	25	History of vasculitis

AT1R, angiotensin II receptor type 1; MIC-A, MHC class I-related chain A.

recipients with AMR and positive endothelial cell crossmatch but without evidence of HLA DSA or MIC-A. These sera were hybridized to protein microarrays containing full-length human proteins. Bioinformatic analysis of the protein microarrays identified 366 antigens with positive antibody reactivity. Gene ontology analysis further narrowed these to 19 candidate plasma membrane antigens and 10 autoantigens. Synthetic beads with these 19 antigens and 48 other non-HLA antigens identified from published literature were then used in high throughput testing using sera from 2 cardiac transplant cohorts.<sup>12</sup> Through this work, 4 novel potentially significant protein targets of non-HLA antibodies were described: EMCN, DEXI, LPHN1, and SSB. This expands the list of putative clinically significant non-anti-HLA antibodies such as LIMS1, myosin, and protein kinase C.<sup>27</sup>

A similar discovery-oriented effort by Gates et al<sup>28</sup> reported experience using a novel “shotgun” immunoproteomic approach. Sera from heart transplant patients with AMR but no HLA DSA and control sera from nonrejecting patients were subject to antidonor heart affinity chromatography columns and captured proteins analyzed by quantitative proteomics. A large number (155) of non-HLA antigen targets were identified. About one-third of these were to highly prevalent antigens. Increased antibody strength was seen in patients with acute rejection compared with controls with 33 of the antibodies and <10% of the antibodies overlapped between those 2 groups overall. Furthermore, using immunofluorescence, they demonstrated increased expression of the specific antigen targets correlating with antibody strength and rejection in biopsies, and in vitro studies showed endothelial activation due to incubation with some of these antibodies.<sup>28</sup> So, the list of non-HLA antibody antigen targets is likely to continue to expand.

Of course, when it comes to AMR, HLA DSA and non-HLA antibodies are not mutually exclusive, and they may co-occur and potentially even exhibit synergistic effects. This remains largely unexplored because once a DSA is detected, rejection is attributed to this without further consideration of the role non-HLA antibodies may be playing.

In summary, the strength of evidence in support of testing for non-HLA antibodies remains low, mostly owing to the limited availability of validated assays (and consequent limited patient data regarding non-HLA antibodies). Currently existing evidence suggests the following: (1) pretransplant sensitization through VAD support, previous transplant, and positive DSA increase the risk of rejection and graft failure after heart transplantation and (2)

non-HLA antibodies at high strength can cause immediate early graft dysfunction, in the absence of HLA DSA (and more indolent presentations of AMR later on).

#### 1.4. Opportunities for collaborative exploration

- Develop/validate standardized diagnostic tests and reference sera to understand specificity and prevalence of non-HLA antibodies.
- Evaluate the prevalence and role of novel specific non-HLA antibodies (eg, EMCN, DEXI, and SSB) in graft injury, rejection, and outcomes (retrospectively and prospectively).
- Perform prospective clinical trials to determine the evolution of non-HLA antibodies and their role in rejection and clinical phenotype.
- Assess the efficacy of existing (and develop new) therapeutic interventions for non-HLA antibodies to improve transplant outcomes.

## 2. “Mixed rejection” in heart transplantation

### 2.1. Clinical perspective

Mixed rejection (MR) is a term used to describe the co-occurrence of pathologic features of cellular rejection (CR) and AMR in the same heart transplant biopsy. Estimates of its prevalence vary widely.<sup>29,30</sup> The concept of MR is addressed in the International Society for Heart and Lung Transplantation (ISHLT) working formulation for AMR,<sup>7</sup> with the recommendation that each biopsy be evaluated and classified separately for these processes. However, because some of the histologic features may overlap and variation in thresholds for classifying the various features exist, comparing data on MR between centers and countries remains challenging.

The risk of MR is believed to be highest during the first year posttransplant.<sup>31</sup> In retrospective single-center studies, MR was associated with a high rate of persistence on subsequent biopsies and an increased risk of cardiovascular mortality.<sup>30</sup> Patients with MR showed prognosis comparable with patients with AMR but worse than patients with CR. The higher the rejection grade of both ACR and AMR, the higher the cardiovascular mortality.<sup>31</sup>

Table 2 compares data (from 4 centers) on the prevalence of MR, and Table 3 summarizes trends with MR across severity of rejection grades for AMR and CR.

From these data, it appears that, in recent years, there has been a general decline in the prevalence of MR and indeed in all rejection patterns (with the possible exception of CR in Padua). Regarding severity, milder grades of rejection, by far, predominate

**Table 2**

Prevalence of cardiac MR (and other rejection types).

	Interval	% MR (biopsies)	% MR (patients)	% AMR (biopsies)	% CR (biopsies)
Utah	1984-2014	7.8	29.0 <sup>a</sup>	24.1	9.4
Utah	2015-2022	3.6	8.3	11.7	19.1
Cedars Sinai	2012-2018	2.5	5.6	6.4	3.0
Paris (La Pitié)	2012-2020	0.5	1.8	1.3	4.8
Padua	2012-2022	1.2	12.0	1.6	19.3

AMR, antibody-mediated rejection; CR, cellular rejection; MR, mixed rejection.

<sup>a</sup> Defined as >2 biopsies showing MR.

**Table 3**  
Mixed rejection according to AMR/CR grade.

Utah (2015-2022, n = 18,900 biopsies)			
pAMR /ACR	1R	2R	3R
1(H+)	1038 (5%)	147 (1%)	17 (<1%)
1(I+)	990 (5%)	70 (<1%)	4 (<1%)
2	682 (4%)	110 (1%)	10 (<1%)
3	88 (<1%)	1 (<1%)	61 (<1%)
Padua (2012-2022, n = 62 patients)			
pAMR /ACR	1R	2R	3R
1(H+)	12 (21%)	4 (6%)	0 (0%)
1(I+)	28 (45%)	4 (6%)	1 (2%)
2	8 (12%)	1 (2%)	0 (0%)
3	1 (2%)	1 (2%)	1 (2%)
Pitié-Salpêtrière (n = 90 patients)			
pAMR /ACR	1R	2R	3R
1(H+)	76 (84%)	4 (5%)	0
1(I+)	4 (5%)	2 (2%)	0
2	3 (3%)	1 (1%)	0
3	0	0	0

ACR, acute cellular rejection; AMR, antibody-mediated rejection; CR, cellular rejection.

in MR. In addition, severe AMR (pAMR3) seems to be more strongly associated with milder CR than severe (3R) CR.

The presence (and strength) of circulating anti-HLA DSA should be considered in the diagnostic workup of MR. On the one hand, the presence of circulating DSA may highlight the significant role of AMR in the mixed rejection process in case of minimal histologic signs of AMR. On the other hand, the evaluation of the AMR-related rejection in severe ACR may be challenging, and the consideration of the presence of anti-HLA DSA may be crucial to guide aggressive therapy.

Concerning treatment, ISHLT guidelines recommend aggressive treatment based on cytolytic therapy and high-dose intravascular corticosteroids when allograft dysfunction (hemodynamic compromise) is present.<sup>32,33</sup> AMR-directed therapies should be considered for these patients as well. For more subclinical MR, the treatment should follow the algorithm for CR with consideration for additional intravenous immunoglobulin (added in the revised 2022 guideline). The poor prognosis associated with MR raise the question of whether severe forms of MR is worse than either AMR or CR alone and warrants even more aggressive therapy than would be given for either alone.

## 2.2. Pathology perspective

As mentioned previously, the pathologic features of CR and AMR should be evaluated independently in heart transplant

biopsies, following the practice other solid organ transplants. A separate scheme or scoring system for MR is not needed.

Histologically, there are some gray areas that can present a challenge in separating AMR from CR. These include the former 1990 ISHLT 1B or busy B pattern of CR in which diffuse interstitial inflammation without clustering of lymphocytes or mononuclear cells into larger aggregates is seen. This appearance can closely resemble the diffuse-activated intravascular mononuclear cell appearance of AMR (Fig. 2). Similarly, there is morphologic overlap at the severe end of the spectrum - 3R / pAMR3 (Fig. 3).

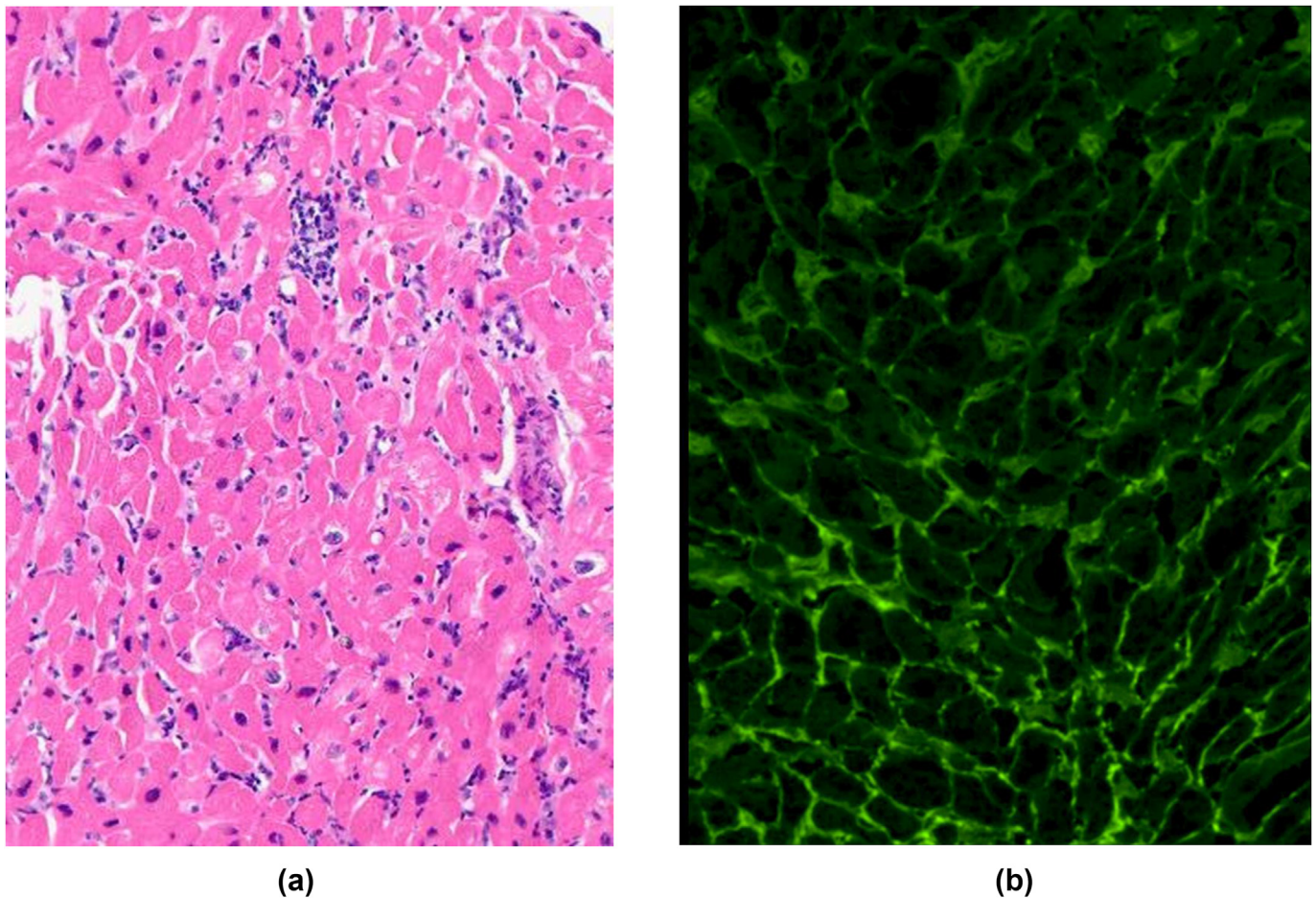
Immunophenotyping studies have also shown areas of overlap between CR and AMR. CD68<sup>+</sup> macrophages (or monocytic lineage cells), intravascularly, not only are a hallmark feature of AMR but also present in significant numbers in many cases of CR, albeit mostly in the interstitium. CD3<sup>+</sup> and even CD8<sup>+</sup> T cells can be demonstrated in cases of otherwise-typical AMR as well.<sup>34</sup>

A more precise pathology evaluation could provide valuable data to guide clinicians in the treatment of MR. Giving a sense of the extent of microvascular inflammation and presence of features such as vasculitis, in addition to the ISHLT working formulation grading, has been suggested. Obviously, a complementary biomarker-driven approach may allow the refinement of the diagnosis of MR, particularly by providing insights into the respective roles of ACR and AMR in the process with a more continuous quantitative estimate of biological activity in the ongoing rejection process.<sup>35</sup> Providing information on disease activity and allograft injury may help clinicians to tailor the treatment of MR, particularly for subclinical rejection cases.

## 2.3. Molecular perspective

The integration of precision genomics and transcriptomics has the potential to revolutionize the surveillance of cardiac allograft rejection, particularly in the context of MR. The development of monitoring supporting tools that combines cutting-edge technologies with routine and standard of care histology/immunopathology, while also being cost effective, quick, and compatible with paraffin tissue samples, is the goal in this field.

To improve the diagnosis of cardiac rejection, tissue-based gene expression techniques have been developed as a support tool for routine pathology. Whole-transcriptome profiling identify molecular characteristics of rejection and add mechanistic insights, although there are still challenges in the way of its clinical implementation.<sup>8,9</sup> Gene expression profiling from formalin-fixed paraffin-embedded heart allograft biopsies is a promising approach for feasibly providing a molecular diagnosis. The consensus-approved Banff Human Organ Transplant (B-HOT) panel coupled to targeted transcriptome technology of nCounter Nanostring was designed to allow solid organ allograft gene expression analysis affordable and reproducible.<sup>3</sup> However, large-scale studies are needed to validate the B-HOT panel for heart transplantation monitoring, addressing potential diagnostic discrepancies to reach the integration of histology assessment with molecular biopsy features. Ongoing multicenter studies are being conducted on well-phenotyped cohorts of heart-transplanted patients to validate the B-HOT panel and identify the optimal heart rejection-associated gene sets and related diagnostic algorithms. Further applications and refinements



**Figure 2.** Overlapping features of AMR and CR in this example of “mild” mixed rejection. (A) Intravascular activated mononuclear cells (and endothelial) cells within interstitial capillaries and perivascular and interstitial lymphocyte aggregates are seen in hematoxylin and eosin staining. (B) Complement (C4d) deposition is seen in both capillary walls and a “perimyocytic” or sarcolemmal staining pattern. On the severe end of the spectrum, pAMR3 and 3R CR show many overlapping features, such as diffuse interstitial inflammation, vasculitis, and fibrin thrombi. Evidence of complement deposition or DSA in serum may be needed to help distinguish AMR from CR in such cases (Fig. 3). AMR, antibody-mediated rejection; CR, cellular rejection.

of the formalin-fixed paraffin-embedded–based targeted transcriptome approach combined with a targeted transcriptome approach has the potential to elucidate the pathophysiologic mechanisms co-occurring in challenging cases as MR, while correlating with clinical and histologic assessments. A synergistic strategy aimed at optimizing the detection of injury and rejection in heart transplantation, facilitating therapeutic decision-making, and ultimately enhancing the long-term survival of the graft.

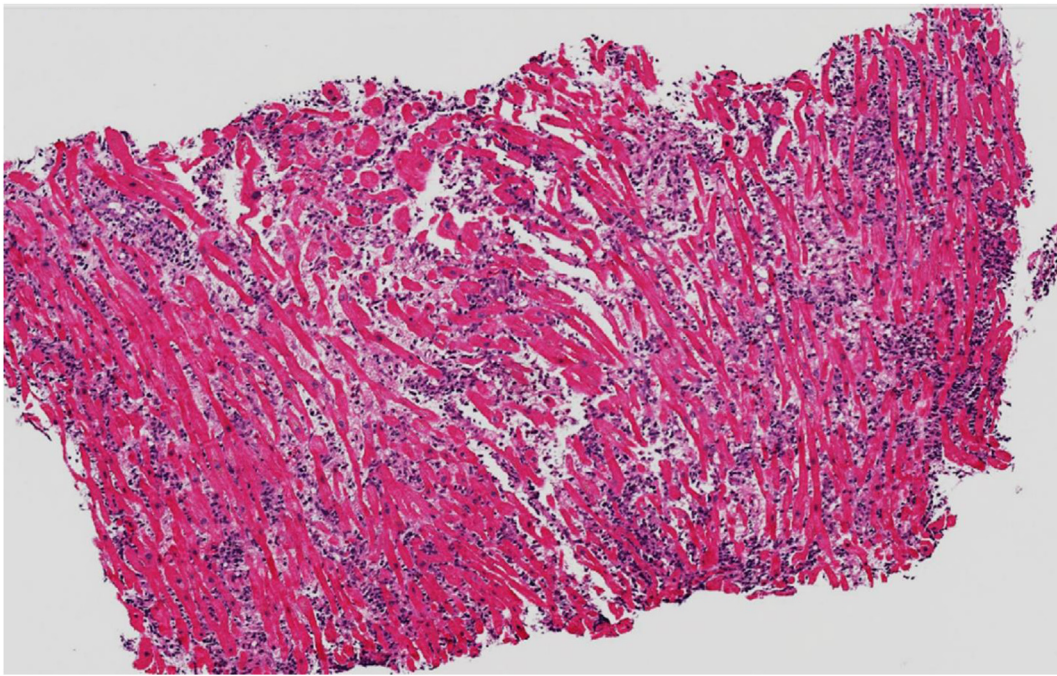
#### 2.4. Immunology perspective

Immunologically speaking, the MR paradigm affords an interesting glimpse into the space between the cell-mediated and antibody-mediated arms of adaptive immunity and even into potential interplay between innate and adaptive immunity. There is certainly evidence for crosstalk between these broad divisions of the immune system, and many of these may come into play with MR.

It is important to note that current immune suppression strategies for all solid organ transplants do not directly suppress B

cells. Rather, their activities against B cells occur indirectly by preventing their activation by T cells, particularly CD4 T-follicular helper cells.<sup>36,37</sup> Other T cell subsets, such as effector naïve, suppressor, central memory, and effector memory T cells, also play important roles in the crosstalk between the arms of the immune system. This is particularly intriguing, given the data from kidney transplant studies demonstrating that nonadherence to immunosuppression is strongly correlated with and precedes development of de novo DSA.<sup>38</sup> A careful look at pathology feature data from a kidney transplant cohort with AMR from Lyon also reveals that in cases that fully manifest an AMR phenotype, there are almost always more subtle (borderline) features of T cell-mediated rejection in the background.<sup>39</sup> Again, this suggests some degree of crosscommunication between B cell and T cell divisions of immunity.

Perhaps even more intriguing is recent work examining secondary lymphoid follicles and tertiary lymphoid organs in allograft. This was a major focus of the last Banff Concurrent session in 2019.<sup>40</sup> These are believed to represent parenchymal ectopic germinal centers whose formation recapitulates the embryonic



**Figure 3.** Overlapping features of AMR and CR in this example of “severe” mixed rejection. There is diffuse involvement of the entire biopsy with both intravascular and extravascular infiltrates. Myocyte necrosis and edema are also prominently seen in hematoxylin and eosin staining. AMR, antibody-mediated rejection; CR, cellular rejection.

genesis of lymphoid organs and the spleen.<sup>23</sup> Nodular lymphoid aggregates (especially subendocardial-Quilty lesions) have long been recognized in cardiac allografts, but their significance has been elusive. Recent studies of transplant tertiary lymphoid organs have shown that, although they may appear to be identical morphologically, their net immune effect may be either regulatory/inhibitory or activating regarding cell-mediated and antibody-mediated injury to the graft.<sup>41,42</sup> In other words, 2 identical-appearing Quilty lesions could have completely opposite effects on the immune system, graft function, and transplant survival. This may help explain the enigmatic nature of Quilty studies over several decades with some reporting an association with worse outcomes and others suggesting more neutral effects. More precise immunophenotyping will be necessary to refine our understanding of this, and the possibility that these tertiary lymphoid organs may be yet another connection between the 2 arms of the immune system and possibly important in the context of MR.

### 2.5. Opportunities for collaborative exploration

- Continue to gather prevalence and biopsy phenotype data from patients with MR and systematically collect treatment details and outcomes (perhaps a Banff MR registry?).
- Prospective clinical trials to determine response of MR to treatment strategies.
- Further validate and refine the B-HOT–based molecular profiling assay by testing more cohorts, particularly those including biopsies showing MR.
- In-depth phenotyping of cases with prominent Quilty effect (tertiary lymphoid organs), especially from cohorts with disparate outcomes, to identify regulatory/suppressive versus activating patterns (using spatial omics?).

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