Banff 2022 Kidney Commentary: Reflections and Future Directions

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Abstract. In September 2022, in Banff, Alberta, Canada, the XVIth Banff meeting, corresponding to the 30th anniversary of the Banff classification, was held, leading to 2 recent publications. Discussions at the Banff meeting focused on proposing improvements to the Banff process as a whole. In line with this, a unique opportunity was offered to a selected group of 16 representatives from the pathology and transplant nephrology community, experts in the field of kidney transplantation, to review these 2 Banff manuscripts. The aim was to provide an insightful commentary, to gauge any prospective influence the proposed changes may have, and to identify any potential areas for future enhancement within the Banff classification. The group expressed its satisfaction with the incorporation of 2 new entities, namely "microvascular inflammation/injury donor-specific antibodies–negative and C4d negative" and "probable antibody-mediated rejection," into category 2. These changes expand the classification, facilitating the capture of more biopsies and providing an opportunity to explore the clinical implications of these lesions further. However, we found that the Banff classification remains complex, potentially hindering its widespread utilization, even if a degree of complexity may be unavoidable given the intricate pathophysiology of kidney allograft pathology. Addressing the histomorphologic diagnosis of chronic active T cell–mediated rejection (CA TCMR), potentially reconsidering a diagnostic-agnostic approach, as for category 2, to inflammation in interstitial fibrosis and tubular atrophy and chronic active T cell–mediated rejection was also an important objective. Furthermore, we felt a need for more evidence before molecular diagnostics could be routinely integrated and emphasized the need for clinical and histologic context determination and the substantiation of its clinical impact through rigorous clinical trials. Finally, our discussions stressed the ongoing necessity for multidisciplinary decision-making regarding patient care.

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INTRODUCTION

In September 2022, in Banff, Alberta, Canada, the XVIth Banff meeting, corresponding to the 30th anniversary of the Banff classification, was held. This event led to the 2 recent publications: "The Banff 2022 Kidney Meeting Report: Re-appraisal of microvascular inflammation and the role of biopsy-based transcript diagnostics"[1] (also referred to as article no. 1) and the more general article "Banff 2022 Kidney Meeting Work Plan: Data-driven refinement of the Banff classification for renal allografts"[2] (also referred to as article no. 2). Article no. 1 summarizes the meeting highlights which formed the basis for the most recent changes to the Banff classification, namely the introduction of 2 new entities in the "antibody-mediated changes and microvascular inflammation (MVI)" category (category 2) and the results of discussions held around the use of molecular diagnostics. Article no. 2 synopsizes discussions centered on other aspects of kidney transplant pathologies such as T cell–mediated rejection (TCMR), in particular, chronic active TCMR (CA TCMR), activity and chronicity indices, digital pathology, xenotransplantation, clinical trials, and surrogate endpoints. Furthermore, it provides updates on the different Banff working groups including current activities and objectives and guidance for future directions.

The Banff meetings provide an environment for intense (and often lively) discussions, but subsequent changes to the Banff classification may sometimes seem arbitrary and perhaps not always clearly data-driven or consensual. With this in mind, discussions at the last Banff meeting focused on proposing improvements to the Banff process as a whole (Tables 1 and 2 of article no. 1). To strengthen this further, the findings from a post-Banff 2022 survey sent to the kidney session attendees (65 respondents), was used as a basis to write the initial report. This was followed by a period of open consultation and interactive discussion whereby the content of the reports was reviewed by the transplant community via the open-access platform of the *American Journal of Transplantation*, before peer review and subsequent publication.

In line with this, a unique opportunity was extended to a select group of representatives from the pathology and transplant nephrology community. These individuals, recognized as experts in the field of kidney transplantation, were invited to review these 2 Banff manuscripts with the aim of providing an insightful commentary. Their perspectives were sought to gauge any prospective influence the proposed changes may have, as well as to identify any potential areas for future enhancement within the Banff classification. It must be acknowledged that because of the "open" review process of the 2 Banff manuscripts, some of those invited to write this commentary also participated in reviewing the Banff manuscripts and are therefore listed as co-authors on either one or both of these papers. Although this in itself may have some added benefit, it could also be seen as potential bias. However, it should be noted that none of the authors of the present article were centrally involved in the writing and finalization of the Banff manuscripts.

MAIN TEXT

A panel of 16 experts was assembled consisting of 8 pathologists and 8 transplant nephrologists with equitable representation of sex and variable geographic location (United States, Canada, and Europe: specifically, France, Poland, Austria, the Netherlands, and the United Kingdom). The participants received a questionnaire to complete and attended 2 virtual meetings the purpose of which was to delve into significant topics raised in the Banff manuscripts. The synopses of these discussions are summarized in this report and the main areas of improvement are reported in Table 1. Key take-home messages summarize the issues raised in this report are also briefly outlined in Table 2. This concerted effort represents the initial steps toward developing a mechanism to generate a more structured response to the latest Banff Kidney Reports with the overall aim of enhancing its diagnostic and research utility. In the future, we aspire to broaden the panel to a wider spectrum of Banff users worldwide, transcending geographical boundaries beyond North America and Europe.

TABLE 1.

Summary of the main areas for improvement

Main areas for improvement	Proposed solution
• Improve worldwide adoption of the Banff classification	• Extending feedback to pathologists and transplant nephrologists from nonexpert centers and low-/middle-income countries by creating a blog on the Banff website
• Improve reproducibility	• Develop Banff image repository • Develop automated algorithms, with free application • Creating a blog for feedback/commentary • Update of image repository and algorithms
• Insufficient evidence for chronic active TCMR category	• Consider a diagnosis-agnostic approach (as in MVI), to stimulate research aimed at shedding light on the different pathophysiologic processes leading to i-IFTA
• Insufficient evidence for integrating molecular tools in the routine practice	• Conduct interventional parallel-group trials in the setting of specific situations to evaluate the added value of molecular diagnostics (eg, borderline TCMR lesions to guide steroid treatment)
• Insufficient evidence for integrating noninvasive tools as routine tests in clinical practice	• Include as topic in the next Banff meetings • Clearly define the role of noninvasive tools in clinical practice, that is, screening vs diagnostic
• Pathologic condition/situations not covered by the Banff classification (Table 2)	• Include at the next Banff meeting discussions to provide guidance for the interpretation of such situations

i-IFTA, inflammation in interstitial fibrosis and tubular atrophy; MVI, microvascular inflammation/injury; TCMR, T cell–mediated rejection.

TABLE 2.

Take-home messages and proposed future directions

Discussed topics	Take-home messages	Proposed future directions
Alterations to category 2 "antibody-mediated lesions and microvascular inflammation"	• Positive move with creation of a new entity "MVI, C4d negative, DSA negative" • Introduction of the "probable AMR" entity • New framework for the diagnosis of category 2. Figure 2 in manuscript no. 1 attempts to simplify category 2 through visual representation • Elimination of acute tubular injury and arterial intimal fibrosis of new onset as histologic criteria	• Specifically study the new "MVI C4d negative, DSA negative" category in terms of pathophysiology (including non-HLA DSA, missing-self, monocytes allorecognition), prognosis and treatment.
Complexity of the Banff classification	• 15 changes made to category 2 described in supplementary Table S3 of manuscript no. 1 • Complexity may prevent worldwide dissemination and reproducibility	• Develop automatic algorithm for Banff interpretation, provided that these algorithms are updated with the Banff changes • Develop an image repository to illustrate the lesions and improve reproducibility • Update Banff website which is the only reference repository of the most updated Banff classification https://banfffoundation.org/central-repository-forbanff-2019-resources-3/.
Chronic active TCMR	• Insufficient evidence for this diagnosis because of lack of specificity • No study clearly proving that therapeutic intervention could prevent graft loss or renal function decline	• Proposition of a diagnostic-agnostic approach as for category 2 which better reflects its uncertain pathogenesis • Study the impact of treatment on larger series
Molecular diagnostics	• The use of molecular diagnostics raises more questions than answers: when and how to use them, including which gene sets, platforms, thresholds and validations • Until the clinical utility has been demonstrated, the use of molecular tools will remain uncertain	• Provide robust scientific evidence supporting the right context of use (eg, diagnostic, prognostic, and guiding therapy) for a biopsy-based molecular diagnostic in the real-world clinical setting • Develop clinical trials comparing graft survival in 2 arms using or not molecular diagnostics as a companion tool to the histomorphologic findings of biopsy • Clarify the gene sets to use according to the platform
Minimally invasive tools	• Little mentioned in the Banff 2022 meeting and manuscripts	• Provide more data on minimally invasive tools and their role in the management of patients • Studies need to be conducted to establish whether they can be diagnostic for rejection or should remain as screening tests
Situations not captured by the Banff classification	• Some situations are not captured by the actual Banff classification, for example, glomerulitis in the context of AMR and recurrent GN, ptc with no glomerulitis in the context of BL or TCMR	• Provide guidance in these situations • Further research is needed in these situations

AMR, antibody-mediated rejection; BL, borderline; DSA, donor-specific antibodies; GN, glomerulonephritis; MVI, microvascular inflammation/injury; ptc, peritubular capillaritis; TCMR, T cell–mediated rejection.

Alterations to Category 2 "Antibody-mediated Changes and Microvascular Inflammation"

The changes in category 2 "antibody-mediated lesions and microvascular inflammation" were unanimously congratulated, particularly since the elimination of the "suspicious for antibody-mediated rejection (AMR)" category in Banff 2017[3] excluded many patients from this diagnostic category,[4] a patient population that no longer fit into any of the Banff diagnosis despite displaying microvascular inflammation. These patients now fall into the newly recognized entity of microvascular inflammation of unknown origin: "microvascular inflammation/injury (MVI), C4d negative, donor-specific antibodies (DSA) negative" and this was recognized as a positive move. It was also acknowledged that stepping back to a more descriptive phenotype (rather than assigning cause, ie, suspicious for AMR) would also likely have a positive effect by encouraging studies aiming at understanding the pathophysiology responsible for MVI lesions in these patients. Removing a direct link to causality in the title prevents inappropriate treatment of patients with tedious and costly therapies while at the same time allowing us to consider this previously excluded phenotype more widely for potential treatments. Even if it could be perceived as being less directive, potentially making it more difficult for physicians to direct treatment, it is also vital as it remains to be determined whether MVI is antibody-mediated (because of non-HLA DSA) or antibody-independent. Accumulating data indeed points to the possibility that missing self-induced natural killer (NK) cell activation could explain a significant fraction of MVI lesions in the absence of DSA.[5] Furthermore, monocyte/macrophage allorecognition[6] or even T cell responses[7] might be involved. Importantly, the field of DSA testing remains challenging. The definition of positive versus negative DSA continues to be debated because of the semiquantitative nature of mean fluorescence intensity and the limitations of solid phase assays.[8] There are even more gaps in our understanding of non-HLA DSA.[9] These 2 fields are important topics discussed by the "Sensitization in Transplantation: Assessment of Risk" Working Group.

The second main change was the introduction of the "probable AMR" entity, including biopsies not reaching the MVI cutoff in patients with DSA, but no C4d, and this was also acknowledged because this is commonly seen in clinical practice. The term "probable" was chosen to avoid confusion with the previous "suspicious" category because this does not correspond to the same patient group. Interestingly, this phenotype can trigger AMR treatment depending on the clinical context (eg, high-risk crossmatch positive patients, rapidly declining function) or surveillance only in other cases.

Finally, pathologists and transplant nephrologists acknowledged that in manuscript no. 1, Figure 2 (Figure 1) together with Table 3 and Supplemental Table S1 clarified the framework of the diagnosis of category 2 with first, the determination of the MVI threshold and biopsy-based transcripts, then the presence or absence of DSA and C4d. This is in contrast to the previous Banff Reports in which category 2 was made up of 3 criteria, namely (1) histology, (2) evidence of current or recent antibody interaction with vascular endothelium, and (3) serologic evidence of circulating DSA, among which some elements belonged to several criteria at the same time (eg, MVI in [1] and [2], C4d in [2] and [3]) which led to a nonintuitive flowchart. Particularly noteworthy is that the names given to criteria 2 and 3 are now omitted since they were not wholly accurate (g+ptc is not evidence of interaction between antibody and endothelium since MVI lesions can be seen in the absence of DSA (see above)[5] and positive C4d staining, which reflects local activation of the classical complement pathway, is not a good marker for the presence of low titer circulating DSA).

Additional minor changes were also made to this category including the elimination of acute tubular injury (ATI), arterial intimal fibrosis of new onset as histologic criteria, and the omission of the term inactive for the definition of chronic (inactive) AMR. The reason these changes were also interpreted as positive is twofold. First, the aforementioned categories were introduced based on insufficient data directly proving causality and second, in the clinical setting these lesions can have multiple potential causes making it frequently impossible to assign a definite cause. Although in itself removing ATI is a relatively minor change to the classification, the impact of this change could be significant. Given the finding of ATI is so prevalent and its direct link to rejection is not established, removing these cases will refine and strengthen the AMR category, again facilitating future research.

Finally, because the DSA status is often not available to pathologists at the time of biopsy interpretation, it is often impossible for pathologists to formulate a final causal diagnosis. As a result, it is most welcome that clear directives are now given for reporting and clinical interpretation in this context. Indeed, it is now clearly stated in manuscript no. 1 that a differential diagnosis needs to be formulated in the absence of information on DSA.

Complexity of the Banff Classification

Addressing the complexity of the Banff classification must be a crucial focus for the Banff community. The main challenge arises from finding a way to integrate changes as the Banff classification evolves over time. As highlighted in the Supplementary Table S3 of manuscript no. 1, the comprehensive alterations made to category 2, include over 15 changes, encompassing 5 additions, 5 deletions, and 5 rewordings. The constant evolution and subsequent changes can lead to difficulties using the classification both in daily practice and research, but it can also be viewed as beneficial, providing a classification and diagnostic system aligned and reflective of the latest breakthroughs in transplant immunology research.[5-7]

To tackle this challenge, the authors of the latest Banff report have made a concerted effort to present these changes in an accessible format. For instance, Figure 2 in manuscript no. 1 attempts to simplify category 2 through visual representation. Despite this, challenges persist as indicated by the inclusion of 9 footnotes and various modifications within the Banff report, undermining aforementioned efforts required for clarity and accessibility.

The group was also concerned that the overall complexity of the Banff classification system could hinder worldwide adoption of the Banff classification. It could be particularly challenging to those clinicians or pathologists for whom transplantation is not their main domain of expertise. On this basis, the group proposed it would be worthwhile extending the invite for pathologists and transplant nephrologists from nonexpert centers and low- or middle-income countries to also provide feedback on Banff classification and changes.

An overly complex system, which is hard to navigate may also prevent the reproducibility of the defining histopathologic Banff lesions and therefore, ultimately, the diagnosis. To improve clarity and ease of use for all, the most up-to-date, entire classification, is now clearly accessible online on the Banff website https://banfffoundation.org/central-repository-for-banff-classification-resources-3/. In that regard, the group also unanimously encouraged the development of an image repository on the Banff website and of automatic algorithms[10,11] to aid the interpretation of Banff lesions, with some caveats. Any algorithms should correctly and accurately reflect the Banff classification and must be up to date reflecting the most recent Banff iterative changes. If implemented, users must be given the opportunity to give feedback and it should be made clear that such algorithms do not take into account the clinical context which has a significant impact on interpretation and subsequent diagnoses in a real-life setting. We acknowledge that the development of these tools is already the main goal of the Digital Pathology Working Group (Table S10 of manuscript no. 2) and we welcome updates on these developments to be presented during the next Banff meeting in September 2024 in Paris, France.

Chronic Active TCMR

During the 2 discussions of the group, a significant amount of time was spent discussing CA TCMR. It is the view of this group of experts that there is insufficient evidence underpinning the CA TCMR category. Although it is known that inflammation within areas of fibrosis is a poor prognostic indicator, assigning a definite diagnosis of CA TCMR raises questions, notably the lack of specificity of the required histologic features (ie, inflammation in areas of interstitial fibrosis and tubular atrophy [i-IFTA] and total inflammation), which in themselves may not always reflect an alloimmune process. Molecular tools such as the MMDx platform show that

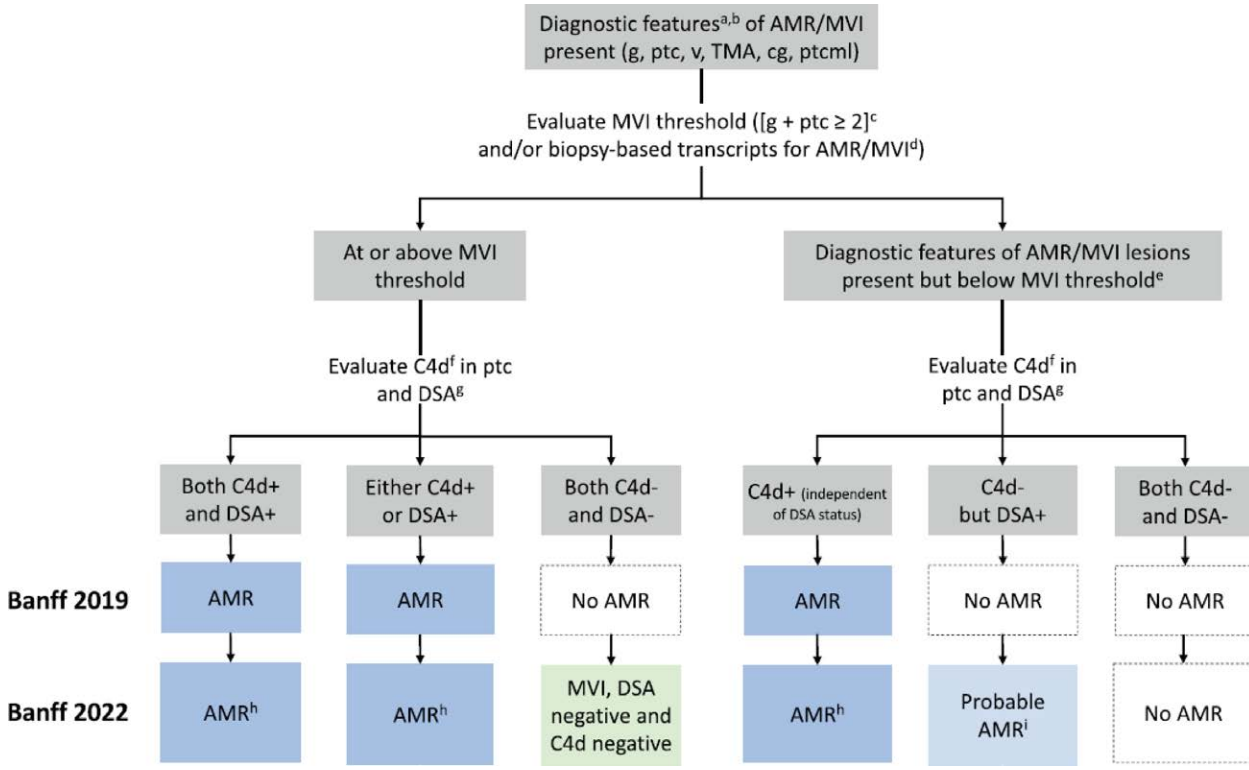

FIGURE 1. Figure 2 from the manuscript no. 1 (Naesens M, Roufosse C, Haas M, et al. The Banff 2022 Kidney Meeting Report: reappraisal of microvascular inflammation and the role of biopsy-based transcript diagnostics. *Am J Transplant*. 2024;24:338–349) with new framework in the diagnosis of Banff category 2 ("Antibody-mediated rejection and microvascular inflammation/injury [AMR/MVI]") with first, the determination of the microvascular inflammation/injury (MVI) threshold and biopsy-based transcripts, then the presence or absence of donor-specific antibodies (DSA) and C4d, and lastly differentiation of disease stage. A, Other lesions can be observed in antibody-mediated rejection (AMR) and strengthen the diagnosis but are not diagnostic by themselves: arterial intimal fibrosis (cv) of new onset, excluding other causes; leukocytes within the sclerotic intima favor chronic AMR if there is no prior history of T cell–mediated rejection (TCMR); acute tubular injury, in the absence of any other apparent cause. B, Definitions of "diagnostic features of AMR/MVI": glomerulitis (g) > 0 in the absence of glomerulonephritis; peritubular capillaritis (ptc) > 0 in the absence of acute TCMR or borderline (suspicious) for acute TCMR; v > 0; acute thrombotic microangiopathy (TMA) in the absence of any other cause; double contours (cg) > 0 by light microscopy, or electron microscopy (EM) if available, if no evidence of chronic TMA and if absence of recurrent or de novo glomerulonephritis; peritubular capillary multilamellation (ptcml) = 7 or more layers in 1 cortical peritubular capillary and 5 or more layers in 2 additional capillaries, avoiding portions cut tangentially by EM, if available. C, [g + ptc ≥ 2] in the absence of recurrent or de novo glomerulonephritis. If borderline (suspicious) for or acute TCMR, or infection are present, [g + ptc ≥ 2] is not sufficient and Banff lesion score g ≥ 1 is required. D, Biopsy-based transcript diagnostics for AMR/MVI above a defined threshold, if thoroughly validated for use as a substitute for AMR/MVI and available. E, In cases of MVI below the threshold, biopsy-based transcript diagnostics can be applied, if thoroughly validated for use as a substitute for AMR/MVI and available. F, C4d deposition should be evaluated in peritubular capillaries and vasa recta (C4d positive = C4d2 or C4d3 by IF on frozen sections, C4d > 0 by immunohistochemistry on paraffin sections). G, If thorough testing for DSA (anti-HLA or other specificity) has not yet been performed, this should be done, following the STAR guidelines. Detection of non-HLA antibodies (including ABO antibodies in ABO-incompatible transplantation) can be used as a serologic Banff criterion for diagnosis of AMR, if the testing protocols are sufficiently standardized and clinically validated for the appropriate clinical context. At present, no non-HLA antibodies (apart from ABO antibodies) have been validated sufficiently for inclusion into the routine clinical classification of kidney transplant biopsies. H, Upon diagnosis of AMR, further differentiation of disease stage is as follows. Active AMR: presence of only active features (including C4d positivity) (cg = 0; ptcml = 0); Chronic active AMR: presence of both active (including C4d positivity) and chronic (cg > 0 and severe ptcml) features. I, Cases with "Probable AMR" and histologic chronic lesions (cg or ptcml) can be labeled as "chronic AMR." For these cases, prior documented diagnosis of active or chronic active AMR, or documented prior evidence of DSA, also count as DSA positivity.

inflammation in areas of fibrosis has a heterogenous phenotype with only a subset showing typical TCMR signature.[12] As immune cell infiltrates could also be composed of regulatory immune cells,[13] further molecular characterization may allow differentiation of pathogenic processes compared with others that may reflect resolution or immune regulation. Furthermore, there is no study clearly proving that therapeutic intervention could prevent graft loss or renal function decline. Only 2 studies have evaluated the efficacy of steroids in the setting of CA TCMR.[14-17] In the study by Kung et al,[14] 48 cases of isolated CA TCMR were identified, of which 44 were treated with pulse steroids and antithymocyte globulin. Response, defined as an at least 50% estimated glomerular filtration rate recovery, was achieved in 20% of cases (n = 9) at 4 wk. Treatment responsiveness did not reflect the presence of concomitant acute TCMR and was not associated with the degree of interstitial fibrosis or tubular atrophy. Noguchi et al[15] retrospectively analyzed 37 cases of CA TCMR of which 32 were treated by either increasing immunosuppression, bolus steroids, and antithymocyte globulin. Of the 32 treated cases, 23 were re-biopsied. Thirteen cases responded, with response being defined as no evidence of acute rejection or only borderline changes. No Banff lesion scores on initial biopsy or treatment type were significant in predicting histologic response, even by

univariate analysis. Applying the clinical response outlined by Kung et al[14] above, 6 of 32 (19%) responded, nearly identical to the 20% of Kung. Hence, the group has concerns that attributing a definite pathophysiology (ie, an alloimmune process) to a nonspecific lesion may trigger the induction of harmful treatments that are not proven to be effective in the majority of patients.

Finally, questions arose during the group discussions as to why Banff appears to take 2 different approaches about 2 different entities (AMR and CA TCMR) on 1 hand it chose to be "diagnosis-agnostic" in the MVI DSA-negative C4d-negative category but not for the CA TCMR category. If the use of "diagnosis-agnostic" better reflects its uncertain pathogenesis, should Banff take the same principle and apply this to i-IFTA and CA TCMR? This could be beneficial by stimulating research aimed at shedding light on the different pathophysiologic processes leading to i-IFTA. Also, as previously mentioned, one of the downfalls of the Banff classification is the piecemeal way in which changes are introduced. It could be worthwhile, therefore, to simultaneously assess other areas where applying the same terminology may also be appropriate (eg, CA TCMR category).

Molecular Diagnostics

Molecular diagnostics was also a major area of discussion in the 2 group meetings. A more cautious approach was taken following the 2022 Banff meeting and the survey that followed, with the change in the wording for the transcript-based diagnosis from "if thoroughly validated" to "if thoroughly validated for this context of use, and available." This feasibility of the use of molecular diagnostics in the diagnosis of kidney transplant disease seems to raise far more questions than answers. These questions are nicely summarized in manuscript no. 2, Table 3.

First of all, it was acknowledged that, around the world, the utilization, availability, and reimbursement for molecular diagnostics are heterogeneous and that molecular tools are likely to be too expensive and unavailable to the majority. However, it seems that more and more centers have started using molecular studies, mostly as a research tool. A worldwide survey about the use of molecular diagnosis could be interesting in this setting.

Major questions on the feasibility of using molecular platforms in kidney transplant pathology diagnostics still remain unanswered: when and how to use them, including which gene sets, platforms, thresholds. and validations.

Several attempts have already been made to delineate the right context of use of the molecular diagnostics. In the Banff 2017 report,[3] a list of indications for molecular diagnostic use was established in Table 6 (recommended indications for use of molecular diagnostics in renal allograft biopsy diagnosis). However, until the clinical utility has been demonstrated, the use of molecular tools will remain uncertain. Studies must demonstrate the added value of the test for the patients, as well as for the health system, in terms of cost, therapeutic, and prognostic impact. Therefore, as stated in the Editorial from Michael Mengel and Mark Haas, "robust scientific evidence supporting the right context of use (eg, diagnostic, prognostic, and guiding therapy) for a biopsy-based molecular diagnostic in the real-world clinical setting is required."[18] Individual participants of the group routinely using the MMDx platform (Vienna) state that they find it helpful in specific situations (isolated v-lesions, borderline lesion, CA TCMR) but acknowledge that proving its help as stated above is difficult. Other participants have indicated that the evidence on its feasibility and clinical use is still lacking and they therefore do not incorporate it in their routine diagnostics. Clinical trials comparing graft survival in 2 arms using and not using molecular diagnostics as a companion tool to the histomorphologic findings of biopsy could be interesting. For example, conducting an interventional parallel-group trial in the setting of borderline lesions, to evaluate the added value of molecular diagnostics for guiding steroid treatment could be of interest.

Obviously, the situations in which molecular diagnostics seems to be of interest for the clinicians are when the histologic diagnosis of rejection is doubtful: borderline lesions, isolated v-lesions, CA TCMR, MVI DSA negative C4d negative, so on.

However, recent studies have shown that if molecular phenotypes are usually in line with the histology, they do not always reflect the pathophysiology.[19-21] For example, Callemeyn et al[19] studied the transcriptome of 56 biopsies with AMR histology by Affymetrix technology; 26 of these (46.4%) lacked detectable serum HLA-DSAs. HLA-DSA–positive and HLA-DSA–negative biopsy specimens with AMR histology displayed similar upregulation of pathways and enrichment of infiltrating leukocytes. Biopsies with AMR histology and HLA-DSA had higher allograft failure risk than cases without HLA-DSA, despite the absence of transcriptional differences, which may imply different pathophysiology. The same results were found by Halloran et al[20] in 148 DSA-negative versus 248 DSA-positive molecular AMR biopsies, compared with 864 no rejection (excluding TCMR and mixed), where the top AMR-associated transcripts were identical in DSA-negative versus DSA-positive AMR, for example, NK-associated (eg, killer cell Lectin like receptor D1 and granzyme B) and gamma interferon-inducible (eg, phospholipase A1 Member A). These 2 independent studies demonstrate that the molecular signature of genuine AMR (MVI+ DSA+) and MVI DSA negative C4d negative are indistinguishable[19] including when validated MMDx set of genes was used.[20] Therefore, it remains doubtful whether integrating molecular tools as additional information to histology could significantly enhance diagnostic reports in these challenging scenarios.[21] However, one may speculate that in specific situations molecular diagnostics could play a crucial role in guiding therapeutic decisions. One scenario could be the use of specific therapeutic interventions, such as NK cell depletion via targeting CD38, based on the dominance of NK cell transcripts in MVI, independent of the initial trigger (whether it is antibody-mediated, missing-self, or other mechanisms).

The question of which platform and what gene set to use is still ongoing. To date, 2 molecular platforms are endorsed by the Banff consortium (MMDx and NanoString with the B-HOT panel). However, other (less costly) technologies may also be of value (such as the Reverse Transcriptase Multiplex Ligation-dependent Probe Amplification, RT-MLPA). Furthermore, the molecular signatures differ from one center to another and translation of gene sets from one platform to another platform are not proven to be possible. Identifying housekeeping genes that are expressed at constant levels in different conditions or time

points on those biopsies will be important for the normalization across platforms and samples, ensuring that observed differences are because of biological variation rather than technical biases. Several studies have compared gene set signature in different settings, across different platforms and have shown contradictory results.[22-25]

Tissue sampling and preservation can also influence the results. In 2020, Toulza et al[26] investigated the effect of tissue sampling and preservation on candidate genes on the expression of 219 genes in 51 samples, split for formalin-fixation and paraffin-embedding (FFPE) and RNA*later* preservation (RNA*later*) using the NanoString platform. Overall, gene expression significantly correlated between FFPE and RNA*later* samples. However, at the individual gene level, 46 of the 219 genes did not correlate across the 51 matched FFPE and RNA*later* samples. Selection of gene panels for routine diagnosis should therefore also take this information into consideration.[26]

In addition to the mentioned need for accurate and reproducible technological platforms, the application of sophisticated machine learning tools using large reference sets of cases may be crucial, for example, including the generation of probabilistic individual rejection classifiers or unsupervised clustering methods such as archetypal analysis, as established for the MMDx platform. This may be especially relevant considering the potential considerable overlap between entities regarding individual transcripts; "herds of genes" may be affected and analysis of individual genes may be insufficient for dissecting rejection entities (eg, for MMDx this was an extensive development process, which will also be necessary for all the other platforms).[27]

To conclude, although few studies have already shown the interest of restricted signatures in the setting of AMR diagnosis, technical considerations are important and the clinical impact on graft survival needs to be proven.

Minimally Invasive Tools

In parallel with the development of molecular diagnostics, big steps have been made with minimally invasive tools to assess the allograft state. The group noticed that little was mentioned on this topic in both the Banff manuscripts and during the Banff meeting. However, a new working group was formed in 2021 on this topic and has published a white article on this topic.[28] The group suggests that even if data do not currently support the adoption of minimally invasive biomarkers as stand-alone tests into the classification, a framework should be provided for how they might fit for the future. Given the increase of these new tests and the temptation to interpret abnormal results as rejection in clinical practice, studies need to be conducted to establish whether they can be diagnostic for rejection or should remain as screening tests. Although the currently available commercial tests lack specificity for rejection, future studies should address whether these biomarkers can support a diagnosis of rejection within the appropriate context, such as DSA without histologic features of rejection, MVI without DSA or C4d, or other ambiguous states. More needs to be understood about how to integrate biomarker data with clinical and immunologic risk information, such as recently shown by the Manitoba group[29] and one proposal is to move toward probabilistic models of diagnosis, integrating clinical information (estimated glomerular filtration rate, proteinuria, sensitization status), immunologic variables (DSA, chemokines), and molecular and transcriptomic data (donor-derived cell-free DNA, transcriptomics) together with histology to define archetypes that resemble rejection. However, in addition to transcriptomics any minimally invasive biomarkers should also be accessible worldwide. Results should be reproducible, regardless of where the test is performed. This topic should be addressed more at the next Banff meeting, in September 2024.

Situation Not Captured by the Banff Classification

Lastly, the group briefly discussed a couple of situations they felt were still not fully captured and represented by the Banff classification even after the introduction of the 2 new entities in the category 2. An example might include, a biopsy with borderline changes or TCMR, a peritubular capillaritis (score 2 or 3), and a positive C4d (or positive DSA), but no glomerulitis cannot fall into the AMR category, since glomerulitis is required. Another situation not captured by the Banff classification is the simultaneous diagnosis of glomerulonephritis and AMR, because glomerulitis is not taken into account for AMR in case of glomerulonephritis. A third unanswered question is whether the presence of a v-lesion in the context of AMR lesions leads to the diagnosis of associated acute TCMR or mixed rejection. These situations are of potential interest and currently uncaptured in the current Banff classification (Table 3). Banff could consider whether providing the pathologists with an appropriate commentary for such situations would be helpful. This has already been done and received well in the revised AMR category, for example, comments around DSA status.

TABLE 3.

Examples of situations not covered or addressed by the Banff classification

Pathologic condition	Issue
Peritubular capillaritis with positive C4d and no glomerulitis with borderline lesions, TCMR, or infection	"AMR" diagnosis is not possible
Glomerulitis and positive C4d, with concomitant recurrent or de novo glomerulonephritis	"AMR" diagnosis is not possible
C4d staining with no diagnostic features of AMR/MVI present and no acute or chronic active TCMR, or borderline changes, but biopsy-based transcripts not available	"C4d staining without evidence of rejection" diagnosis is not possible because molecular results must be part of the diagnosis
Presence of a "v"-lesion concomitant with AMR features	Is it AMR or mixed rejection?

AMR, antibody-mediated rejection; MVI, microvascular inflammation/injury; TCMR, T cell–mediated rejection.

CONCLUSION

To conclude, the group commends the overarching initiative which facilitates peer prereview and prepublication review of the latest Banff manuscripts, aligning with the collective intention to enhance the overall Banff process. We express our satisfaction with the incorporation of 2 new entities, namely "MVI DSA-negative and C4d negative" and "probable AMR," into category 2. These changes expand the classification, facilitating capture of more biopsies and provide an opportunity to explore the clinical implications of these lesions further.

Despite these positive developments, we find that the Banff classification remains complex, potentially hindering its widespread use. Retaining a degree of complexity may be unavoidable; however, given the intricate pathophysiology of kidney allograft pathology. Elaborating on the recent works, which have demonstrated the existence of innate rejection mimicking the MVI lesions of AMR, our primary focus should center on studying MVI DSA-negative and C4d-negative cases. Addressing the histomorphologic diagnosis of CA TCMR, potentially reconsidering a diagnostic-agnostic approach to i-IFTA and CA TCMR is also an important objective.

At this stage, there is a need for more evidence before molecular diagnostics can be routinely integrated. This also emphasizes the need for clinical and histologic context determination and the substantiation of its clinical impact through rigorous clinical trials.

Table 2 summarizes key take-home messages and future directions.

Our discussions emphasized the ongoing necessity for multidisciplinary decision-making in patient care, involving pathologists, transplant clinicians, immunologists, and experts in molecular and noninvasive tools—a collaborative approach already established in various medical fields, such as oncology.

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