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Guidelines for the Diagnosis of Antibody-Mediated Rejection in Pancreas Allografts—Updated Banff Grading Schema

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The first Banff proposal for the diagnosis of pancreas rejection (Am J Transplant 2008; 8: 237) dealt primarily with the diagnosis of acute T-cell-mediated rejection (ACMR), while only tentatively addressing issues pertaining to antibody-mediated rejection (AMR). This document presents comprehensive guidelines for the diagnosis of AMR, first proposed at the 10th Banff Conference on Allograft Pathology and refined by a broad-based multidisciplinary panel. Pancreatic AMR is best identified by a combination of serological and immunohistopathological findings consisting of (i) identification of circulating donor-specific antibodies, and histopathological data including (ii) morphological evidence of microvascular tissue injury and (iii) C4d staining in interacinar capillaries. *Acute AMR* is diagnosed conclusively if these three elements are present,

whereas a diagnosis of *suspicious for AMR* is rendered if only two elements are identified. The identification of only one diagnostic element is not sufficient for the diagnosis of AMR but should prompt heightened clinical vigilance. AMR and ACMR may coexist, and should be recognized and graded independently. This proposal is based on our current knowledge of the pathogenesis of pancreas rejection and currently available tools for diagnosis. A systematized clinicopathological approach to AMR is essential for the development and assessment of much needed therapeutic interventions.

Key words: Acinar cell injury, active chronic antibody-mediated rejection, amylin, amyloid, C4d, cell-mediated rejection, donor-specific antibody, interacinar capillaries, pancreas biopsy, transplant arteriopathy

Abbreviations: AMR, antibody-mediated rejection; ACMR, acute T-cell-mediated allograft rejection; DSA, donor-specific antibody; MHC, major histocompatibility complex; MICA, MHC class I–related chain A; IAC, interacinar capillaries in pancreatic exocrine lobules; IAPP, islet amyloid polypeptide; IVIG, intravenous immune globulin; PRA, panel-reactive antibody; SMA, smooth-muscle antibody.

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Introduction

Over the past three decades, refinements in surgical techniques and greater understanding of the histopathological features of allograft rejection have been achieved in vascularized pancreas transplantation (1–9). Moreover, major pharmacological advances have been made in the prevention and treatment of classical acute T-cell-mediated allograft rejection (ACMR) leading to higher expectations for improved short- and long-term outcomes. Unfortunately, in spite of a reduction in the rates of early acute rejection, long-term outcomes have not improved markedly, because some subsets of patients continued to lose their grafts to intractable rejection or to slow but inexorable progression to graft fibrosis (10,11).

In recent years an increasing number of cases of pancreas allograft dysfunction and loss attributed to antibody-mediated allograft rejection (AMR) have been reported in the literature (6,11–15). AMR which does not respond to standard treatments for ACMR is caused by antibodies directed against donor-specific human leukocyte antigen (HLA) molecules or other cellular antigens (12,16,17). AMR can result from a strong anamnestic antibody response to previous antigenic exposure (i.e. retransplantation and pregnancy) or from *de novo* development of donor-specific antibody (DSA; Ref. 11). The development of AMR was documented in a pancreas-transplant recipient who was presensitized after two previous islet transplantations (18) and has also been reported in association with viral infections (19,20).

AMR causes graft failure through acute and/or chronic immunoglobulin and complement induced microvascular injury and remodeling that eventually leads to graft fibrosis (21,22). It has been postulated that chronic AMR is the single most important factor limiting long-term graft survival in solid organ transplantation (21,23,24). The interplay between AMR and autoimmunity is currently unknown (25), but anti-HLA DSA and AMR have also been reported in association with recurrence of autoimmune diabetes mellitus (14).

Distribution of HLA Class I and Class II in pancreas tissue

Major histocompatibility complex (MHC) disparities have been associated with an increased risk of humoral rejection and graft loss (26), but in practice minimal emphasis is placed on HLA matching in simultaneous kidney-pancreas transplantation. HLA matching may have a greater role in solitary pancreas transplantation, in which the incidences of ACMR and immunological graft loss are inherently higher (1).

The normal pancreas expresses MHC Classes I and II differently in the exocrine and endocrine components (27–29). Expression is altered in inflammatory conditions, including ACMR, which is typically associated with aberrant expression of Class I and Class II antigens (30–33). Similarly, with the development of diabetes mellitus there is hyperexpression of Class I antigens and aberrant expression of Class II antigens in the endocrine islets (34; Table 1).

Considerations on DSA testing

Prevention, diagnosis and treatment of AMR require monitoring for the presence of circulating DSA (35). It is recommended that routine antibody monitoring be performed at regular intervals after transplantation, as well as at the time of biopsy, and whenever rejection is suspected (36). Specific clinical settings may warrant development and implementation of protocols tailored to individual patients (i.e. desensitization protocols, weaning of immunosuppression, etc.) (37).

In recent years, marked improvements in the sensitivity and specificity for detecting alloantibodies have led to ongoing assessment of the clinical relevance of anti-HLA antibody levels, specificities and the significance of antibodies to non-HLA antigens (e.g. MHC class I–related chain A [MICA], auto antigens) and to non-AB-DR HLA antigens (37–39). Although an earlier study found a strong association between DSA to MHC Class II and chronic allograft rejection/graft loss (26), subsequent studies have not found

Table 1: Class I and II HLA expression in normal and abnormal pancreas tissue*

	Normal pancreas histology sections		Tissue culture–inflammatory milieu (ß IFN, γ IFN and IL2)		Diabetes mellitus (DM)	
Cell type	Class I	Class II	Class I	Class II	Class I	Class II
Acinar cells	–	–	+ Aberrant expression	+ Aberrant expression	n/a	n/a
Ductal cells	++	–	++	+ Aberrant expression	n/a	n/a
Islet cells	+/– (Weak)	–	++ Hyperexpression	++ Aberrant expression	++ all islet cells hyperexpression (with insulitis -early DM)	+ ß cells aberrant expression, +/– insulitis
Capillary endothelium	++	++	n/a	n/a	n/a	n/a
Large vessel endothelium	++	Variable	n/a	n/a	n/a	n/a

*Based on Refs. 10–17.
n/a = data not available.

significant clinicopathological differences between DSA to Class I and Class II antigens (6,12,15). Antibodies to MICA were associated with histopathological features of AMR in the pancreas in one series (15)

Although there has been remarkable progress to date, much work remains to be done in the area of histocompatibility and immunogenetics in order to better understand and treat AMR (see section later on future directions).

C4d Staining

Background

Circulating DSA directed against endothelial cells leads to widespread activation of the complement and coagulation cascades in the vascular walls with consequent mobilization of a variety of inflammatory mediators. Demonstration of immunoglobulins and active complement components in the microvasculature has proven difficult due to the rapid turnover and degradation of the various products (40). In contrast, the complement fragment C4d which is generated through the classical (antibody-induced) activation pathway is resistant to shedding and degradation and remains detectable in the vessel walls for at least several days following the initial immunological event (17,40,41).

Multiple studies have demonstrated that C4d staining in renal biopsies performed for allograft dysfunction is predictive of poorer graft outcomes and helps identify patients with AMR (16). In pancreas allograft biopsies, C4d staining is typically absent in cases of pure ACMR or in protocol biopsies from well-functioning grafts (6,12).

More recently, it has been emphasized that C4d staining alone lacks enough sensitivity and specificity to be used as an unequivocal marker for the presence or absence of renal AMR, especially late posttransplant (42,43). However, microvascular inflammation/injury with concurrent detection of circulating DSA can identify AMR independently of positive C4d staining (22,44). For practical purposes, it is generally agreed that the clinical diagnosis of AMR is best achieved with a combination of careful histological evaluation, including C4d staining and correlation with concurrent DSA evaluation (9,45).

Technical aspects and interpretation

There is general consensus that C4d staining should be performed in pancreas allograft biopsies in addition to the standard set of slides and stains (Hematoxylin and Eosin [H&E] ×3 and Masson's trichrome stain; Ref. 9). Both immunohistochemical and immunofluorescence C4d stains are adequate for diagnosis and yield a similar staining pattern in interacinar capillaries (IAC; Figure 1). In renal and cardiac allograft biopsies, the immunofluorescence technique has been reported to yield stronger staining compared to the immunohistochemical method but the difference was not considered significant for clinical purposes (45,46). Comparison of the two methods in pancreas allograft biopsies, showed that with the immunofluorescence technique the estimated areas of lobular IAC staining were 10–50% larger than with the immunohistochemical method (E. Rangel, D. U. Kim and P. Revelo, ongoing data collection). Based on these preliminary data and the earlier work by Torrealba et al. (6), it is recommended that the threshold for C4d positivity in pancreas-allograft biopsies should remain at ≥5% (9), until additional data become available. When immunohistochemical staining is used, a low threshold is preferable, considering that several studies in the kidney

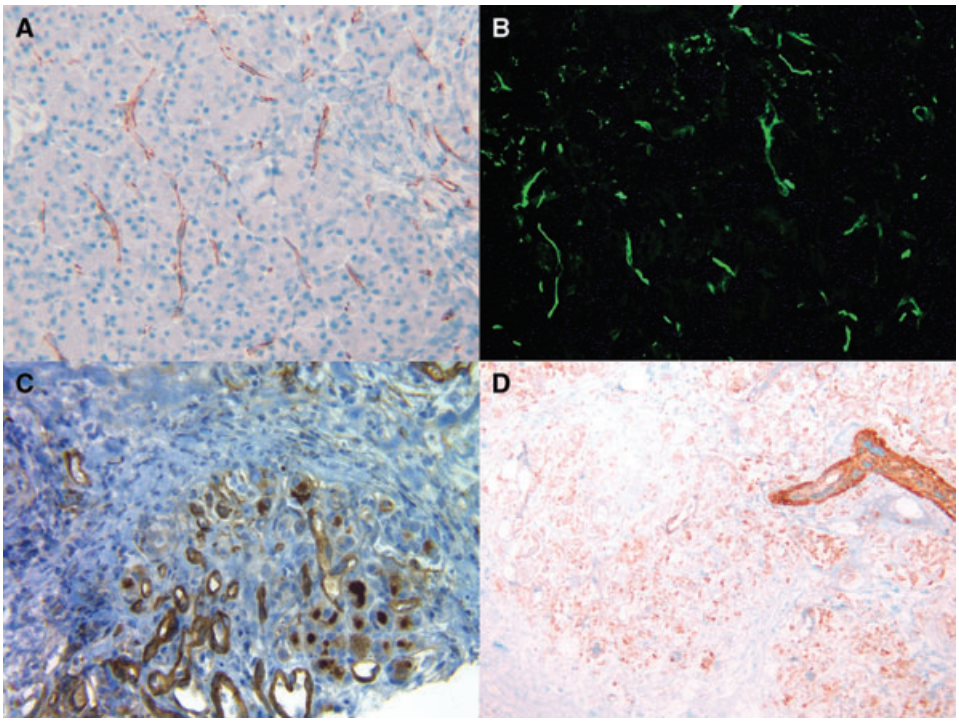

Figure 1: C4d staining in pancreas allografts. (A and B) Immunohistochemical and immunofluorescence C4d staining demonstrates comparable interacinar capillary staining (Courtesy: Dr. Revelo). (C) Atrophic lobule in chronic active AMR shows strong C4d positivity in residual interacinar capillaries. The acinar component is atrophic. Note "lobular" arrangement of the staining capillaries. (D) C4d staining in severe acute AMR. Due to extensive parenchymal necrosis there is nonspecific background staining with very rare recognizable positive interacinar capillaries. A thrombosed necrotic artery shows positive staining in its wall and contents.

have shown that both focal and diffuse C4d staining were associated with poorer graft outcomes (17,47). In a patient with pancreatic AMR, semiquantitive evaluation of intensity and extent of C4d staining in serial biopsies was found to correlate with DSA levels (S. Seshan, personal observation).

C4d staining in parenchymal-IAC is to be reported semiquantitatively based on the extent of exocrine lobular biopsy surface staining, as follows: Negative <5%, Focal 5–50% and Diffuse >50%. Only linear or granular staining along the IAC correlates with the presence of circulating DSA (6,12). In contrast, staining in other tissue components such as the endothelium of larger vessels including veins and arteries, the interstitial or septal connective tissue or the peripancreatic soft tissues is considered nonspecific (6). In biopsies with chronic active AMR (see later), the lobular architecture is expected to be disrupted by interstitial fibrosis and acinar atrophy and it may be more difficult to identify the IAC. On the other hand, despite the sclerosing architectural changes, C4d positivity typically remains in residual capillary vessels often with partial preservation of the lobular arrangement (Figure 1C).

In severe AMR with extensive parenchymal necrosis, most of the IAC staining could be lost. In contrast, strong C4d staining is typically found in the necrotic vascular walls (Figure 1D). Correlation with DSA studies is strongly recommended in this setting.

Clinicopathological Spectrum of AMR in Pancreas Allografts

Hyperacute rejection

The inescapable effects of preformed antidonor antibodies leading to "hyperacute rejection" and immediate graft destruction were identified early in the history of solid organ transplantation as a strong immunological barrier to successful engraftment. With respect to pancreas transplantation, the recognition of *hyperacute rejection* has been obscured by the high propensity of this organ for early graft thrombosis that may or may not be related to rejection (2,48). Sibley (49) first described a case of hyperacute rejection, in a patient with a negative pretransplant cross match but high-level panel-reactive antibody (PRA) when retested after the removal of the thrombosed organ. Similar cases were described later, with graft loss occurring either immediately (*hyperacute rejection*) or within hours posttransplantation. As in the case reported by Sibley (49), circulating DSA were identified retrospectively, in the setting of an initially negative cytotoxic cross match (2).

Pathological findings in biopsies with severe, irreversible AMR correspond to those observed in experimental models of hyperacute rejection (50). The earliest changes occur within minutes of revascularization and consist of edema, congestion, spotty acinar cell injury (i.e. vacuolization, degranulation and necrosis) and capillary and venular neutrophilic margination. Progressive graft destruction occurs within a few hours and is characterized by confluent foci of

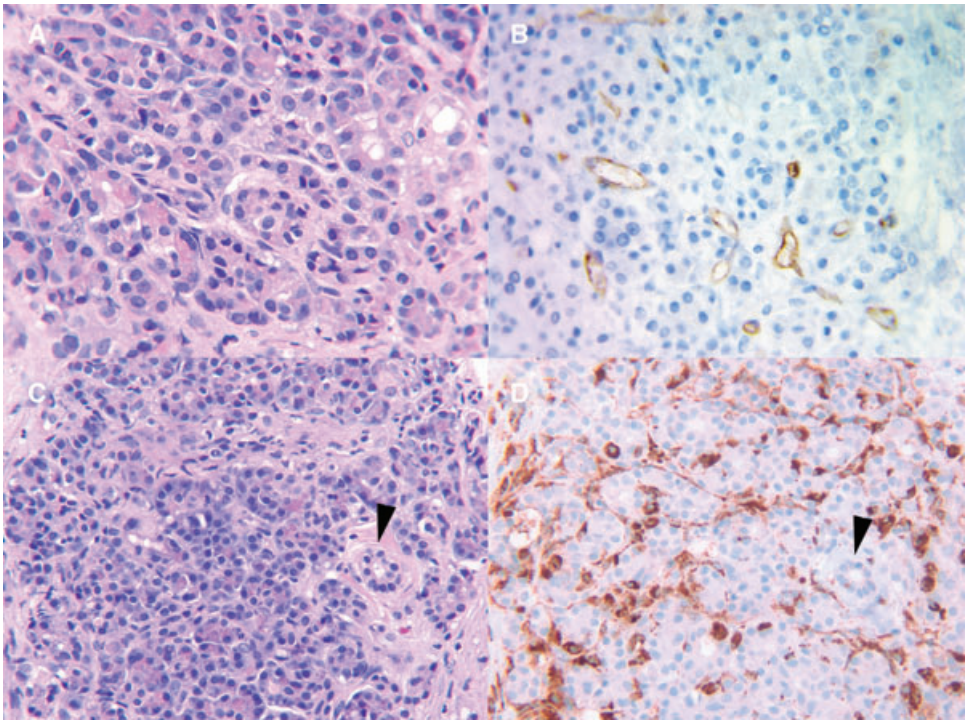

Figure 2: Mild acute AMR. (A) Exocrine area with very subtle interstitial, interacinar inflammation and (B) scattered IAC outlined with the C4d stain. (C) Preserved lobular/acinar architecture and mild mononuclear infiltrates that are underestimated on the H&E stain. (D) CD68 stain for macrophages in the same area of the biopsy as (C), demonstrates the extent of the infiltrates (arrowheads in C and D mark a small duct for orientation).

hemorrhagic necrosis in acini, islets and ducts, with prominent neutrophilic infiltrates and widespread fibrinoid vascular necrosis and thrombosis (50). Immunoglobulin (i.e. IgG) and complement deposition including C4d staining are found throughout the graft vasculature (2,9).

Acute AMR

Awareness of acute AMR in pancreas allografts was heightened by the characterization of this entity in kidney transplants (16) and recognition of the negative impact of circulating DSA on both short- and long-term pancreas graft survival rates (6,11,12,51–54).

In recent years, a considerable number of studies have reported the clinical and pathological findings of acute AMR in pancreas allografts, which typically presents with graft dysfunction. In the studies of de Kort (12), Rangel et al. (15) and Torrealba et al. (6) exocrine abnormalities (increase in serum amylase/lipase or decrease in urine amylase levels) represented the most common indication for allograft biopsy (55–70%) followed by combined exocrine and endocrine abnormalities (15–20%). Isolated endocrine dysfunction (hyperglycemia) was a relatively rare indication for allograft biopsy (6–8%). The unusual association between AMR and pancreatic panniculitis was recently reported (55).

Although approximately 75% of cases of acute AMR were diagnosed in the first 6 months posttransplantation, late occurring cases were not unusual (average 248 days, median 79 days, range 1–3331 days; Refs. 6,11–14,52), clearly paralleling the clinico-pathological spectrum described with AMR in renal allografts (17). ACMR and acute AMR could not be distinguished from each other on clinical grounds, stressing the importance of DSA monitoring and biopsy evaluation (15)

The morphological findings in acute AMR may consist of various degrees of inflammation and tissue injury as detailed below:

- Acinar/interacinar inflammation
  In its earlier, milder forms acute AMR presents with overall preservation of the architecture and mild interacinar monocytic and/or neutrophilic infiltrates associated with subtle spotty acinar cell dropout/apoptosis (Figures 2A and B). In cases with predominantly monocytic inflammation (i.e. few or no neutrophils), the interacinar inflammation may be inconspicuous on routine H&E stains, whereas the monocyte/macrophage infiltrates can be highlighted with the performance of a CD68 immunostain (Figures 2C and D; Refs. 6,14).
- Interacinar capillaritis
  In more severe or advanced forms of acute AMR there is dilatation and congestion of the IAC which allows for easier identification of marginating and intraluminal inflammatory infiltrates (interacinar capillaritis). The latter is similar morphologically and presumably pathogenetically to peritubular capillaritis in renal allografts (56), but in comparison to the renal peritubular capillaries, the pancreatic IAC have a less predictable distribution

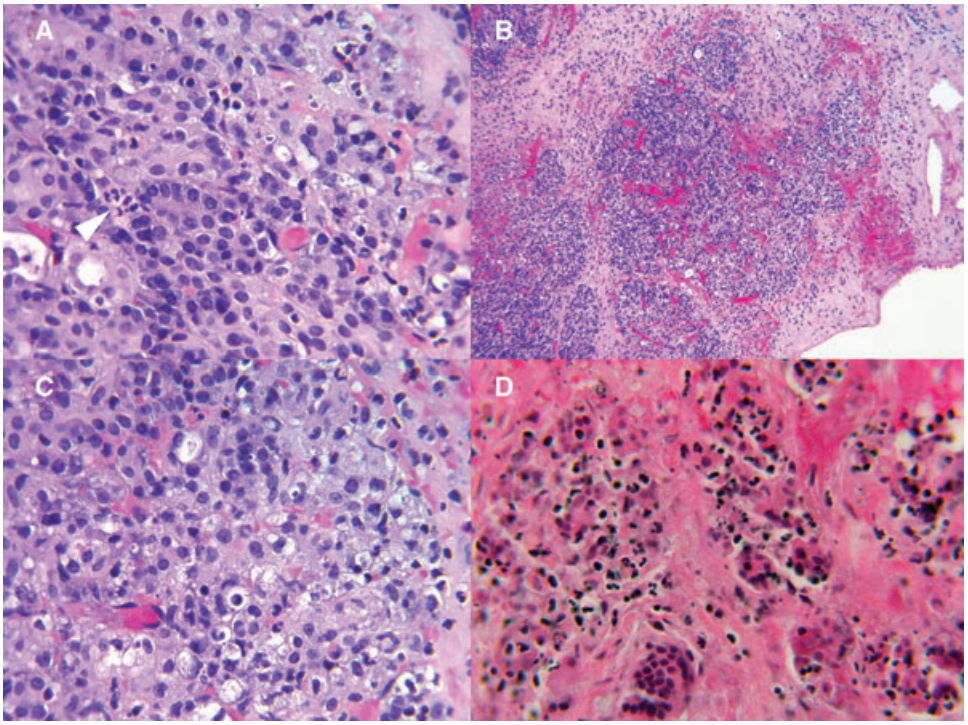

Figure 3: (A and C) Moderate acute AMR characterized by acinar/interacinar inflammation and capillaritis. The acinar cell injury (swelling, vacuolization and cell dropout) appears disproportionate to the extent of the inflammation. Arrowhead (A) marks neutrophilic capillaritis. (B and D) Severe acute AMR represented at low magnification to show marked vascular congestion and confluent areas of hemorrhagic necrosis (B). Exocrine area with multicellular necrosis and mixed interacinar/acinar inflammation (D).

and are relatively sparse. Furthermore in fully developed acute AMR in pancreas allografts, extensive microvascular injury leads to prominent interstitial hemorrhage, edema and multicellular necrosis of interstitial and acinar cells making the identification of interacinar capillaritis more difficult (see later; Figure 3; Ref. 57). Identification of interacinar infiltrates with associated interacinar capillaritis has been found to be strongly associated with C4d positivity and detection of DSA (6,7,12,14). In one study capillaritis was found in >80% of biopsies with focal and diffuse C4d positivity (6).

- Acinar cell and overall tissue injury
  In pancreatic acute AMR, there is increased acinar cell injury manifested with cytoplasmic swelling and vacuolization as well as apoptotic or necrotic cell dropout. The identification of acinar cell injury in an otherwise bland appearing biopsy (Figures 2 and 3) should alert the pathologist to the possibility of subtle interacinar inflammation or capillaritis, and warrants correlation with the C4d staining and DSA studies (6,9,12,14).

In addition to the features described earlier, very severe or advanced forms of acute AMR have morphological features approaching those found in hyperacute rejection. These findings consist of widespread vascular necrosis and thrombosis in small or larger vessels and small or confluent foci of parenchymal necrosis (Figures 1D, 3B and D; Ref. 2).

Acute AMR is graded histologically (mild, moderate or severe) based on the extent of the interacinar infiltrates/capillaritis and tissue damage, as presented in Table 2.

Chronic active AMR

Chronic exposure to circulating DSA is associated with development of graft fibrosis and graft failure (58). The histological diagnosis of *chronic active AMR* is based on the following triad: (i) features of acute AMR as described in the previous section (also see Table 4; AMR diagnostic components), (ii) absence of features of ACMR and (iii) underlying graft fibrosis (Banff diagnostic category 6). The utilization of this diagnostic category presupposes that the main cause of graft fibrosis is ongoing AMR, and therefore requires that other causes of graft fibrosis/sclerosis are ruled out, such as previous episodes of ACMR. In clinical practice this conclusion would be most accurate when serial biopsies are available for evaluation.

Mixed ACMR and AMR

A generalized increase in interstitial inflammation, both in septa and acini, as well as edema were found to be more common in biopsies with C4d positivity and concurrent circulating DSA (12), which raises the possibility of cases having mixed ACMR and AMR.

Stereotypical cases of isolated AMR or ACMR can be classified by a systematic evaluation of the various features described in Table 3, but it is not unusual for the two processes to coexist in the same biopsy (mixed rejection) and appear with overlapping features. The pathology report should clearly indicate the type of rejection present

Table 2: Histological grading of acute antibody-mediated rejection (See Table 4*^ for other diagnostic components)

Grade I/Mild acute AMR
Well-preserved architecture, mild monocytic-macrophagic or mixed (monocytic-macrophagic/ neutrophilic) infiltrates with rare acinar cell damage
Grade II/Moderate acute AMR
Overall preservation of the architecture with interacinar monocytic-macrophagic or mixed (monocytic-macrophagic/neutrophilic) infiltrates, capillary dilatation, capillaritis, congestion, multicellular acinar cell dropout and extravasation of red blood cells
Grade III/Severe acute AMR
Architectural disarray, scattered inflammatory infiltrates in a background of interstitial hemorrhage, multifocal and confluent parenchymal necrosis, arterial and venous wall necrosis and thrombosis

(AMR, ACMR or mixed), estimate the degree of activity (mild, moderate or severe) of each process and indicate the extent of chronicity/graft fibrosis (stage).

Specific Considerations on the Updated Banff Schema for Grading Pancreas Allograft Rejection

1. It is now recognized that one of the main features of acute AMR in the pancreas is the presence of prominent acinar cell injury ranging from spotty cell necrosis/apoptosis to confluent necrosis. Accordingly it is now stated in the schema that Grade II and III/Moderate and Severe ACMR, which can also present with prominent acinar cell injury/necrosis, require differentiation from acute AMR (Table 4).
2. Diagnosis of acute AMR is based on a combination of diagnostic components as listed in Table 4. Criteria for the histological grading of acute AMR (mild, moderate or severe) are provided in a separate table (Table 2).
3. Chronic active AMR is based on the combination of acute AMR and graft sclerosis-fibrosis (Categories 4 and 6), in the absence of ACMR (Category 3). A conclusive diagnosis of *chronic active AMR* requires C4d positivity, interacinar inflammation/capillaritis/acinar damage and circulating DSA in addition to graft sclerosis-fibrosis. If only two of the AMR diagnostic elements are present, a diagnosis of *suspicious for chronic active AMR* can be rendered.
4. Chronic allograft arteriopathy was initially considered to be an expression of T-cell-mediated allograft rejection (45), but recent studies have shown that acute and chronic arterial lesions can be also associated with DSA and AMR (59–61). Accordingly, this lesion is now listed as a separate morphological category (independent from ACMR and AMR). Recognition of chronic allograft arteriopathy in biopsy samples is clinically important because it indicates ongoing (chronic) alloimmune injury and for its association with late graft thrombosis (2).
5. A separate category has been created for lesions specifically involving the endocrine islets. The main purpose of this category is the recognition of *recurrent autoimmune diabetes mellitus*, characterized by insulitis and/or selective ß cell loss (62,63). In addition, islet deposition of Amylin (also known as islet amyloid polypeptide (IAPP)) appearing as amorphous Congo red positive material is placed in this category. Amylin, a protein normally cosecreted with insulin by ß cells, accumulates in the pancreatic islets under abnormal circumstances in particular hyperglycemic states (i.e. Type 2 diabetes mellitus), pancreatitis and possible allograft rejection (64,65). Deposition of amylin in otherwise normal islets of pancreas allografts is usually associated with loss of glycemic control (hyperglycemia). Acute calcineurin inhibitor toxicity (9) is also included in this category.

   The impact of AMR in pancreatic islets remains unclear. Whereas hyperglycemia was documented in early reports of AMR (13,58), this was a rare indication for biopsy in subsequent larger studies (6,12,15). C4d staining in islet capillary endothelium was found in

Table 3: Predominance of histological features in stereotypical ACMR and AMR

	ACMR	AMR
Septal infiltrates	+++	− to +
Eosinophils	+ to +++	− to +
Neutrophils	− to ++	+/− to +++
T-Lymphocytes	++ to +++	+/− to +
Macrophages	++	++++
Venulitis	++	−
Ductitis	++	−
Acinar cell injury	+/− to ++	+++
Acinar inflammation	− to +++	+ to +++
Acinitis (mononuclear infiltrates within the basement membrane of individual acini)	+ to +++	− to+/−
Interacinar capillaritis	− to +/−	+ to +++
Intimal arteritis	+	+
Necrotizing vasculitis/thrombosis	− to +	+++
Confluent hemorrhagic necrosis	− to ++	− to ++++
Active transplant arteriopathy	+	+

Table 4: Banff pancreas allograft rejection grading schema—update diagnostic categories#

1. Normal. Absent inflammation *or* inactive septal, mononuclear inflammation not involving ducts, veins, arteries or acini. There is no graft sclerosis. The fibrous component is limited to normal septa and its amount is proportional to the size of the enclosed structures (ducts and vessels). The acinar parenchyma shows no signs of atrophy or injury.

2 Indeterminate. Septal inflammation that appears active but the overall features do not fulfill the criteria for mild cell-mediated acute rejection

3. Acute T-cell-mediated rejection+

- Grade I/Mild acute T-cell-mediated rejection

Active septal inflammation (activated, blastic lymphocytes and ±eosinophils) involving septal structures: Venulitis (subendothelial accumulation of inflammatory cells and endothelial damage in septal veins, ductitis (epithelial inflammation and damage of ducts)

and/or

Focal acinar inflammation. No more than two inflammatory foci^per lobule with absent or minimal acinar cell injury

- Grade II / Moderate acute T-cell-mediated rejection (requires differentiation from AMR)

Multifocal (but not confluent or diffuse) acinar inflammation (≥3 foci^per lobule) with spotty (individual) acinar cell injury and dropout

and/or

Mild intimal arteritis (with minimal, <25% luminal compromise)

- Grade III / Severe acute T-cell-mediated rejection (requires differentiation from AMR)

Diffuse (widespread, extensive) acinar inflammation with focal or diffuse multicellular/confluent acinar cell necrosis

and/or

Moderate or severe intimal arteritis, >25% luminal compromise.

and/or

Transmural inflammation—Necrotizing arteritis.

4. Antibody-mediated rejection (AMR*, See diagnostic components below**)

*Confirmed circulating donor-specific antibody (DSA)

*Morphological evidence of tissue injury (interacinar inflammation/capillaritis, acinar cell damage swelling/necrosis/apoptosis/dropout, vasculitis, thrombosis)

*C4d positivity in interacinar capillaries (IAC, ≥5% of acinar lobular surface)

Acute AMR 3 of 3 diagnostic components*^

Consistent with acute AMR 2 of 3 diagnostic components*^

Requires exclusion of AMR 1 of 3 diagnostic components*

See separate table for histological grading of acute AMR^

Chronic active antibody-mediated rejection: Combined features of categories 4* and 6 in the absence of features of category 3

5. Chronic allograft arteriopathy. Arterial intimal fibrosis with mononuclear cell infiltration in fibrosis.

6. Chronic allograft rejection/graft fibrosis

- Stage I (mild graft fibrosis)

Expansion of fibrous septa; the fibrosis occupies less than 30% of the core surface but the acinar lobules have eroded, irregular contours. The central lobular areas are normal.

- Stage II (moderate graft fibrosis)

The fibrosis occupies 30–60% of the core surface. The exocrine atrophy affects the majority of the lobules in their periphery (irregular contours) and in their central areas (thin fibrous strands criss-cross between individual acini).

- Stage III (severe graft fibrosis)

The fibrotic areas predominate and occupy more than 60% of the core surface with only isolated areas of residual acinar tissue and/or islets present.

7. Islet pathology

Recurrence of autoimmune DM (insulitis and/or selective ß cell loss)

Islet amyloid (amylin) deposition

8. Other histologic diagnosis. Pathologic changes not considered to be due acute and/or chronic rejection. For example, CMV pancreatitis, PTLD, etc.

#Categories 2 to 8 may be diagnosed concurrently and should be listed in the diagnosis in the order of their clinicopathological significance.
+Histological features of stereotypical ACMR and AMR, see Table 3.
^Histological grading of acute AMR, see Table 2.
See Ref. 9 for morphological definition of lesions of cell-mediated rejection and for list of other histological diagnosis.

approximately 20% of samples from patients with DSA but this finding did not correlate with hyperglycemia (6). In severe necrotizing AMR (as well as severe ACMR) hyperglycemia typically develops, correlating with the extent of parenchymal necrosis (2,9).

6. The proposed schema for diagnosis of AMR in pancreas allografts follows the same approach as the Banff 09 update for diagnosis of acute AMR in kidney allografts (66). Both schemas rely on the combination of C4d positivity, presence of circulating DSA and

evidence of associated tissue injury for a diagnosis of *acute AMR*. In both schemas, a diagnosis of *suspicious for AMR* is rendered in the absence of any one of these diagnostic components (66).

Discussion

AMR is a complex, dynamic process with protean clinicopathological manifestations that range from cataclysmic graft loss to various forms of allograft inflammation associated with protracted graft sclerosis-fibrosis (67). From a practical point of view, severe untreatable acute AMR can be easily diagnosed on the basis of morphological criteria alone, but a systematic approach and high degree of suspicion are necessary in order to recognize the milder or more indolent and potentially treatable forms of the disease (9,17,45). Biopsy evaluation remains the gold standard for the diagnosis of allograft rejection, as clinical parameters lack specificity and cannot discriminate between ACMR and AMR which require different therapeutic approaches (15).

Detailed histological examination, immunostaining for C4d and serological search for circulating DSA currently represent the cornerstones for the diagnosis of AMR, although significant questions remain with respect to determining the most adequate tools and thresholds for this diagnosis (16,44,67). In particular, fluctuating levels of DSA (and C4d staining) as well as technical limitations for the identification of DSA have been problematic (36–38). However, other promising tools are not yet available for routine diagnosis (3,16,68).

The schema proposed here relies on the combination of the currently available tools (DSA, C4d and histological findings), based on their perceived strengths and limitations, and also their complementary value (12,45; see Table 4). A diagnosis of "suspicious for AMR" increases the sensitivity of the schema by addressing the not too unlikely clinical situation in which a complete constellation of elements is not identified (i.e. only two of the three elements are present). On the other hand, the presence of C4d positivity, DSA or tissue injury in isolation from the other elements is not considered sufficient to warrant clinical intervention for AMR but should prompt thorough clinicopathological correlation and close follow-up.

Currently there is no adequate treatment for either acute or chronic AMR. This process is currently one of the most challenging problems in solid organ transplantation (10,16,21). Interventions in pancreas AMR have followed the approach used in other organs, mainly consisting of rabbit antithymocyte globulin, intravenous immune globulin (IVIG) and plasmapheresis with or without rituximab. Less well-established treatments include the addition of bortezomib and/or eculizumab, but these therapies need to be evaluated in formal clinical trials (69).

The goal of this Banff working proposal is to provide uniform diagnostic criteria that can be applied both for diagnostic and investigational purposes in pancreas transplantation. Morphological classifications such as this one have inherent limitations related to intra- and interobserver reproducibility (70). Studies are being currently undertaken to evaluate reproducibility issues in this specific context.

Future Direction

In the field of pancreas transplantation there are multiple areas of investigation that require attention, some of which are listed below.

Clinical studies

- Need for better understanding of the pathogenetic mechanisms in AMR including better characterization of the role of presensitization, role of surveillance DSA/PRA, determination of meaningful clinical cut-offs for DSA levels (e.g. highest levels vs. broader sensitization), potential role of circulating non-HLA antidonor antibodies and autoantibodies (including antiislet antibodies and other autoantibodies to SMA or collagen), impact of AMR on the exocrine versus endocrine components and relationship between alloimmunity and autoimmune recurrence.
- Single-center and multicenter studies to determine the utility of protocol biopsies enabling identification and determination of the significance of subclinical rejections, early identification of AMR versus ACMR and refinement of clinicopathological correlations.

Pathological studies

- Histopathological characterization of the inflammatory infiltrates in AMR and ACMR (e.g. immunohistochemical application of lymphoid markers) to improve diagnostic yield and increase data accumulation of pathophysiologic significance.
- Further refinement of the morphological characterization of microvascular and endothelial injury and study of the pathogenesis of chronic rejection/graft sclerosis.

Molecular studies

- Application to pancreas transplantation of the currently available tools in gene profiling, gene transcription and proteomics to improve understanding of the pathogenetic mechanisms of allograft rejection (ACMR, AMR and mixed rejection) and other processes leading to graft failure.
- Exploration of the potential use of limited microarray analysis or multiplex polymerase chain reaction (PCR) for obtaining diagnostic and pathophysiologic data (e.g. PCR for Th1, Th2, Th17 cytokines or cells).

Individual efforts in these areas will advance understanding of basic mechanisms and enhance clinical management of pancreas allografts. This review and update of the Banff grading schema for AMR will hopefully provide standardization, improve diagnosis and understanding and help elucidate mechanisms of graft failure as well as target interventions for improving long-term outcomes.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose, as described by the *American Journal of Transplantation*.

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