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Original contribution

Global quality assessment of liver allograft C4d staining during acute antibody-mediated rejection in formalin-fixed, paraffin-embedded tissue ☆

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Summary Discussion of liver antibody-mediated rejection during the 2011, 2013, and 2015 Banff liver sessions raised concerns over reliability of complement fragment 4d (C4d) staining, precipitating a global survey followed by a tissue microarray staining quality assessment study among centers on formalin-fixed, paraffin-embedded tissue. Tissue microarray sections containing tissue plugs of resected native and allograft (with acute antibody-mediated rejection) liver, heart, and kidney (n = 33 total cores) were sent to 31 centers for C4d staining using local method(s) and pathologist scoring. Digital whole-slide images (n = 40) were then semiquantitatively scored by 7 experts for background, distribution, and intensity of portal vein and capillary, hepatic artery, sinusoidal, and central vein endothelia and portal and central stromal staining. Results showed that strong and diffuse portal vein and capillary C4d staining, as determined by both local and central pathologists, clearly distinguished allografts showing acute antibody-mediated rejection from native livers and from those with evidence of weaker donor-specific antibody. Downstream vascular endothelial

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cell C4d staining and assessment were more variable and difficult to identify. C4d staining in the majority of laboratories reliably detects acute liver allograft antibody-mediated rejection in formalin-fixed, paraffin-embedded tissues. Assessment should focus on portal veins and capillaries, sinusoids, and central veins present in peripheral core needle biopsies. C4d staining in one organ does not always translate to staining in another.

1. Introduction

Despite Western world abandonment of ABO-incompatible liver transplantation because of acute antibody-mediated rejection (AMR) in untreated recipients, AMR occurrence in ABO-compatible orthotopic liver transplant (OLTx) is still debated because of its low incidence in OLTx [1-7]. However, accumulating case reports [8-13], cohort studies [14-17], and reviews [7,18] show that AMR can cause liver allograft dysfunction and, rarely, failure. Based on published studies and intense discussion over a 6-year period, AMR has also been incorporated into the Banff scheme for liver allograft pathology [19].

C4d staining is an important, although limited [20], tool used to assist in establishing an AMR diagnosis in all solid organ allografts. Optimal methods of tissue preservation, staining, and compartment scoring of C4d deposition have yet to be established for liver allografts, but in general, immunofluorescence (IF) on frozen tissue is considered the gold standard. Unfortunately, most liver centers obtain only formalin-fixed, paraffin-embedded (FFPE) tissues for C4d immunohistochemistry (IHC). Kozlowski et al [16] suggested that IHC antigen retrieval pH is an important determinant of C4d staining sensitivity and that staining patterns qualitatively differ between IF and IHC, and only the former might be reliable.

AMR discussions during the last 3 Banff meetings (Paris, France [2011]; Comandatuba, Brazil [2013]; and Vancouver, Canada [2015]) prompted a survey to gauge practices and attitudes as to the relevance of AMR in OLTx. C4d usefulness in liver allograft AMR diagnosis. and frozen versus FFPE sections. Based on survey results, this tissue microarray (TMA) reliability was carried out using native and AMR-positive kidney, heart plugs, and liver plugs.

The aims of the study were to (1) elicit current views on AMR in OLTx, (2) determine if C4d staining on FFPE sections is able to identify acute AMR in "gold standard" cases, and (3) identify C4d staining methods and structures that might be used to standardize an acute liver allograft AMR diagnosis.

2. Materials and methods

2.1. Surveys

Two Internet surveys were initially conducted (see Supplementary material for details), both in 2013: The first focused on current understanding and attitudes of hepatologists, surgeons, immunologists, and pathologists toward liver allograft AMR; the second queried centers regarding C4d staining methods, reliability, and interpretation for liver allografts on FFPE sections. Banff-participating and other larger international OLTx centers were targeted as key opinion leaders, but participants were encouraged to disseminate the survey.

2.2. TMA production

Failed allografts are required to obtain enough tissue to create a TMA for multicenter analysis; diagnostic biopsies after clinical assessment do not provide enough tissue. Unfortunately, liver allografts that failed from AMR are sparse, often historical, and lacking complete solid-phase HLA donor-specific antibody (DSA) testing and have no matched fresh frozen tissue for comparison to "gold standard" IF. A 33-plug TMA using FFPE tissues was developed and included perihilar and peripheral plugs of 5 liver allografts from sensitized recipients which failed within 1 month posttransplant, with a strong suspicion of acute AMR as the cause of graft failure. Details of the OLTx histological, immunology, and clinical features are shown in Table 1. As controls, the TMAs included 5 nontransplant (native) hearts, 5 native kidneys, 5 native livers (with a perihilar and peripheral plug from each), 2 cardiac AMR cases, and 6 kidney AMR cases. Native organs with an immunological disease process and allografts without AMR were avoided because (*a*) simultaneous DSA was felt to be necessary and (*b*) a complement deposition role in other diseases has not been thoroughly investigated, especially for livers. Two kidney AMR cases were excluded because of extensive necrosis of the plug, which made comparisons unreliable. Chronic AMR was not considered.

2.3. TMA staining and scoring

Sixty-eight TMA unstained sections were mailed to 31 centers: 2 sections were sent to 25 centers and 3 sections to 6 centers that used 2 methods for C4d staining. TMAs were then stained using local C4d method(s) scored locally using a centrally devised scoring template with instructions; between 33 and 38 local scores were received for each plug. Slides were returned to the University of Pittsburgh Medical Center for creation of digital whole-slide images (WSIs). Forty stained slides from 31 centers (13 North America, 13 Europe, 2 Japan,