A systematic review of the role of C4d in the diagnosis of acute antibody-mediated rejection

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In this study, we conducted a systematic review of the literature to re-evaluate the role of C4d in the diagnosis of acute antibody-mediated rejection of kidney allografts. Electronic databases were searched until September 2013. Eligible studies allowed derivation of diagnostic tables for the performance of C4d by immunofluorescence or immunohistochemistry with comparison to histopathological features of acute antibody-mediated rejection and/or donor-specific antibody (DSA) assays. Of 3492 unique abstracts, 29 studies encompassing 3485 indication and 868 surveillance biopsies were identified. Assessment of C4d by immunofluorescence and immunohistochemistry exhibited slight to moderate agreement with glomerulitis, peritubular capillaritis, solid-phase DSA assays, DSA with glomerulitis, and DSA with peritubular capillaritis. The sensitivity and specificity of C4d varied as a function of C4d and comparator test thresholds. Prognostically, the presence of C4d was associated with inferior allograft survival compared with DSA or histopathology alone. Thus, our findings support the presence of complement-dependent and -independent phenotypes of acute antibody-mediated rejection. Whether the presence of C4d in combination with histopathology or DSA should be considered for the diagnosis of acute antibody-mediated rejection warrants further study.

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Acute antibody-mediated rejection (AAMR) is a major risk factor for kidney allograft failure.[1–3] Until recently, when attributing acute allograft dysfunction to AAMR, the Banff classification[4] warranted the simultaneous presence of three diagnostic criteria: (i) serologic evidence of donor-specific antibodies (DSA), (ii) histopathological evidence of tissue injury, and (iii) presence of peritubular capillary C4d staining (C4d). While a construct reference standard, requiring that all three tests be positive when conducted simultaneously or in sequence, ensures greater specificity of AAMR diagnosis, it also inevitably compromises the sensitivity.[5,6] Consequently, while relying on three criteria to diagnose AAMR may minimize toxicity from unnecessary immunosuppression in vulnerable kidney transplant recipients, it might also result in withholding therapy from patients who could benefit from timely interventions.

Of the three aforementioned criteria, C4d became the cornerstone of AAMR diagnosis in clinical practice,[7–13] as it linked DSA with histopathology, was predictive of allograft failure,[14] and C4d by immunofluorescence (IF) in frozen sections (IF-frozen) demonstrated high specificity when compared with DSA by cytotoxicity assays.[12] Over the past decade, significant developments have been made in the diagnostic methodologies for AAMR. For example, C4d staining by IF and immunohistochemistry (IHC) in frozen and paraffin sections have been evaluated[15–20] and various distribution thresholds in kidney transplant biopsies have been considered;[21–24] the sensitivity and specificity of solid-phase DSA assays have improved;[25] and the definitions of microcirculatory inflammation (MI) features have been refined and standardized.[26–28] In light of these developments, the role of each of the diagnostic criteria of AAMR, and in particular C4d, has been reconsidered in several recent important reviews.[29–31] To date, however, none have reviewed the literature systematically while considering the

heterogeneity between studies in patient selection, conduct and the thresholds defining positive C4d, DSA assays or histopathological features of AAMR. To inform on the role of C4d in the evolving diagnostic schema of AAMR, we conducted a systematic review of the literature assessing the diagnostic performance of C4d compared with histopathological features of AAMR and DSA assays. The prognostic implications of C4d to allograft outcomes in studies reporting on diagnostic accuracy were also reviewed.

RESULTS

Our search yielded 3492 unique citations. Of 143 potentially relevant full text papers, 19 primary studies discussing indication biopsies, four studies discussing surveillance biopsies, and one study discussing indication and surveillance biopsies were included in the review. Five companion reports were used for supplementary data.[9,10,32–34] Figure 1 presents the flow diagram of search results. A total of 3485 indication and 868 surveillance biopsies were included.

Clinical and methodological heterogeneity among studies

Study characteristics are presented in Supplementary Material 1 online. A single study involved pediatric patients.[35] Clinical heterogeneity was noted across studies in patients' immune risk reflected by history of transplantation, presence of DSA, and cross-match status. Importantly, marked methodological heterogeneity was recognized in the conduct and thresholds of C4d and DSA (Tables 1 and 2). Thresholds for histopathological changes of AAMR were for the most part based on the Banff classification.[4,26,36–43]

Diagnostic characteristics and kappa statistics of C4d in indication biopsies against comparator tests

Histopathology. Diagnostic characteristics and kappa statistics of C4d, when compared with histopathology, are presented in Figure 2. Focally distributed (>10%) IF-frozen C4d[13,28,44] exhibited slight to fair agreement (mean kappa: 0.19–0.31 and 0.12–0.35), moderate to high specificity (mean specificity: 0.31–0.88 and 0.81–0.93), low sensitivity (mean sensitivity: 0.30–0.57 and 0.38–0.50), and modest diagnostic odds ratios (DOR) (3.18–3.72 and 4.12–7.43) versus glomerulitis and peritubular capillaritis.[28,44,45] While Verghese *et al.*[45] reported a similar performance of diffusely distributed IF-frozen C4d when compared with glomerulitis, peritubular capillaritis, and MI,[28] Mauiyyedi *et al.*[12] reported substantial agreement, high sensitivity, and high specificity of diffusely distributed IF-frozen C4d in a population with severe rejection when glomerulitis and peritubular capillaritis were defined as the presence of polymorphonuclear cells.

Focally distributed IHC-paraffin C4d compared with glomerulitis, peritubular capillaritis,[2,46–50] and MI[47] demonstrated slight to fair agreement (mean kappa vs. glomerulitis, peritubular capillaritis, and MI: 0.10–0.43, 0.17–0.35, and 0.17, respectively), low to high specificity (mean specificity

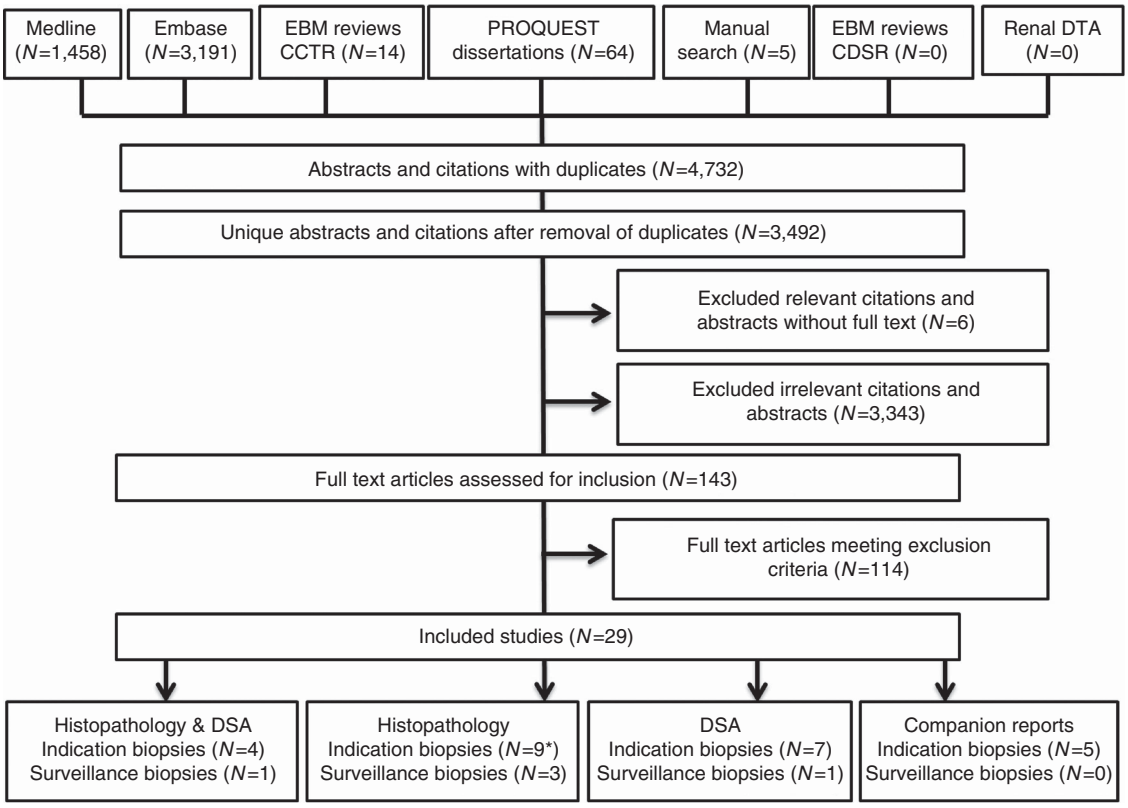

Figure 1 | Summary of study inclusion and exclusion process. *Mengel *et al.*[49] included both indications and surveillance biopsies. CCTR, Cochrane Central Register of Controlled Trials; CDSR, Cochrane Database of Systematic Reviews; DSA, donor-specific antibody; Renal DTA, Cochrane Renal Group Reviews of Diagnostic Test Accuracy.