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Mixed cellular and antibody-mediated rejection in heart transplantation: In-depth pathologic and clinical observations

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BACKGROUND: Little is known about mixed cellular and antibody-mediated rejection (MR) in heart transplantation. It remains unclear whether cardiac MR has distinctive pathologic and clinical features beyond those of simultaneous cellular rejection (CR) and antibody-mediated rejection (AMR). In this study we systematically explore the pathologic and clinical characteristics of MR in heart transplantation.
METHODS: The UTAH Cardiac Transplant Program database was queried for transplant recipients who survived long enough to have at least one endomyocardial biopsy (EMB) between 1985 and 2014. Only EMBs with both CR and AMR scores documented were included. In addition to detailed pathologic analyses, we also examined the incidence and prevalence of MR, the likelihood to transition *from* and *to* MR, and mortality associated with MR.
RESULTS: Patients ($n = 1,207$) with a total of 28,484 EMBs met the study inclusion criteria. The overall prevalence of MR was 7.8% and it was nearly twice as frequent within the first year post-transplant. *Mild* MR was by far the most common occurrence and was typically preceded by an immune active state. When CR increased in severity, AMR tended to follow, but the reverse was not true. On pathology, individual features of CR and AMR were more easily separated in cases of *mild* MR, whereas they substantially overlapped in more severe cases. MR was associated with a significant cardiovascular death risk that was incremental with severity.
CONCLUSIONS: MR is not common, usually occurs early after transplant, and is associated with worse outcomes. MR reflects a complex interplay between cellular and humoral processes, which varies with rejection severity.
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Experience with cardiac mixed cellular and antibody-mediated rejection (MR) is limited to rare case or small series reports[1,2] and a brief acknowledgment in the updated

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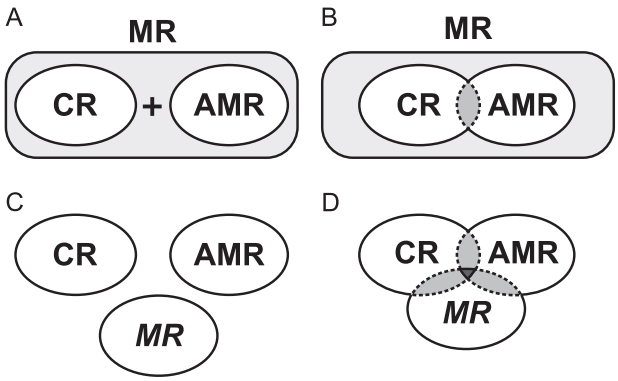

Figure 1 What is MR? (A, B) The *mixed* label requires the presence of pathologic features of both CR and AMR in the diagnosis of MR. They can occur side by side but independently (A), or with a varying degree of overlap (B). (C, D) Cellular and humoral processes result in *MR*, a separate type of rejection with unique pathologic features—thus, MR may in this case be a misnomer. CR, AMR and MR may (D) or may not (C) share some of their individual characteristics. CR, cellular rejection; AMR, antibody-mediated rejection; MR, mixed rejection.

2013 International Society for Heart and Lung Transplantation (ISHLT) working formulation for the standardization of antibody-mediated rejection nomenclature.[3] Despite what the *mixed* designation would, by default, infer, a fundamental question remains whether MR is a separate entity with unique pathologic and clinical characteristics or merely cellular rejection (CR) and antibody-mediated rejection (AMR) occurring concomitantly (**Figure 1**). Central to this dilemma is whether there are independent or related mechanisms driving these entities. It would be a daunting puzzle to elucidate the nature and extent of interplay, if present, between cellular and humoral mechanisms in MR. Another conundrum is whether MR can be the first manifestation of rejection or always a downstream event stemming from either CR or AMR first, invoking the possibility of cross-activation between T-cell and B-cell arms of immunity.

AMR and CR are not mutually exclusive processes immunologically. It is not unusual to see features of both on the same endomyocardial biopsy (EMB).[4,5] Clinically, it is unclear whether the effects of MR on graft function and longevity in cardiac transplant patients are cumulative outcomes from CR and AMR or more closely resemble the natural history of one type of rejection or the other. A better understanding of what truly constitutes MR and its associated outcomes may help guide management of these patients with biopsies showing MR.

Methods

Study population

Eligible patients comprised all pediatric and adult heart recipients within the Utah Transplantation Affiliated Hospitals (U.T.A.H.) Cardiac Transplant Program (UCTP) between 1985 and 2014. Included were patients who survived long enough to have at least 1 EMB post-transplant based on a routine surveillance protocol. Endomyocardial biopsies are done on a weekly basis in the first month after transplant, then bi-weekly in the second month, then every 3 weeks twice. Beyond that, EMBs are done monthly until Month 7 post-transplant. Between Months 7 and 10, 2 EMBs are done with a 6-week interval. The last EMB within the first year is done 2 months later at the transplant 1-year anniversary. In the second, third and fourth years post-transplant, EMBs are done every 3, 4 and 6 months, respectively. Beyond that, EMBs are done on a yearly basis unless otherwise clinically indicated. Excluded were patients with incomplete follow-up records pertinent to the study. To focus on MR, only EMBs with both CR and AMR scores reported were included. Biopsies entered in the registry with mismatched *new* versus *old* CR nomenclatures (e.g., Grades 1A and 3R) and those missing relevant information (e.g., date of EMB) were also excluded from analyses.

Induction and maintenance immunosuppression as well as management of acute rejection were standardized across the UCTP institutions.

Data source

Our pathology database of all recorded EMBs performed in the study group was queried. This large registry, created in 1985, has kept detailed information on biopsies using semi-quantitative scales to independently grade histologic and immunofluorescence findings. The entry document used to populate the database and the biopsy grading scales have been detailed elsewhere.[6] Cellular rejection and AMR were also graded according to the International Society for Heart and Lung Transplantation Working Formulation (ISHLT WF).[3,7] Since this has evolved over the past decade, retrospective conversion to the most current working formulation was performed using the semi-quantitative parameters relevant to the new ISHLT (e.g., number of inflammatory foci and foci of myocyte damage, endothelial activation, intravascular mononuclear cells, C4d and C3d). Whenever possible, biopsies with ambiguous or equivocal findings were re-examined and assigned the appropriate ISHLT score. Most of the ambiguity related to biopsies with *borderline* or *suspicious* histologic features for AMR or else weak immunofluorescence staining for complement. Examination of tissue samples was typically blinded to the clinical history.

The pathology registry also includes patient demographics, the presence and extent of cardiac allograft vasculopathy and date and cause of death.

Patients consented to data collection and the study was approved by our institutional review board.

Statistical analysis

Standard descriptive statistics were used to describe prevalence and incidence rates as well as the rates at which patients transitioned between specific rejection states. For these analyses, the rejection states (measured at each biopsy) were: no rejection; CR only; AMR only; and MR. Cellular rejection was defined as any ISHLT Grade $\geq$1R, and AMR was defined as any pathologic AMR (pAMR) value of $\geq$1. Mixed rejection required histologic evidence of both conditions. Other outcomes of interest were mortality [cardiovascular (CV) and all-cause] of patients based on rejection status and severity of rejection. Cardiovascular mortality was defined as death resulting from acute rejection, acute myocardial infarction, advanced cardiac allograft vasculopathy, sudden cardiac death, heart failure and cardiogenic shock, primary allograft failure, cardiac arrhythmias and pulmonary embolism. Re-do heart transplantation was also considered a CV mortality end-point.

For the survival analysis, Cox proportional hazards models with time-varying covariates were used. The rejection status on the most