MRI-based measurement of the pancreatic extracellular volume detects immune cell infiltration in a mouse model of type 1 diabetes

Patrick Antkowiak[1], Brian Stevens[1], Marcia McDuffie[1], and Frederick Epstein[1]

[1]University of Virginia, Charlottesville, Virginia, United States

Introduction: Type 1 diabetes mellitus (T1DM) is an autoimmune metabolic disorder that affects approximately 3 million Americans. In T1DM, immune cells infiltrate and destroy the insulin-producing pancreatic β cells, which are critical for maintaining blood glucose homeostasis. Early detection of insulitis (cellular infiltration of the pancreas) may identify a key therapeutic window, as it occurs before overt β cell destruction. Noninvasive imaging is a potential method for early detection of insulitis, although no satisfactory method yet exists. Current methods use specialized contrast agents that lack FDA approval[1,2]. Recently, techniques have been developed to measure the extracellular volume (ECV) using the common clinical contrast agent Gd-DTPA[3]. These methods have detected increases in ECV in the setting of cardiac diffuse fibrosis[3]. In the pre-diabetic pancreas, we hypothesized that infiltration of immune cells decreases the ECV. Accordingly, we investigated whether MR measurements of the ECV with Gd-DTPA could detect immune cell infiltration in a mouse model of T1DM. This method could be clinically applicable and readily translatable to humans.

Methods: *Mouse model of T1DM:* All methods were approved by our institutional animal care and use committee. To investigate the ability of MRI to detect immune cell infiltration, an adoptive splenocyte transfer mouse model of T1DM was used[4]. Specifically, 20×10^6 diabetogenic splenocytes from NOD-BDC2.5 Tcr-positive mice were transferred into 6-week-old immunocompromised NOD.scid mice (N=5). In this model, insulitis develops over a period of 10-12 days after cell transfer and persists for a few weeks[4]. Age-matched NOD.scid mice (N=2-3) injected with vehicle were used as a control. Urine glucose was monitored as an indicator of T1DM during the study, and blood glucose was measured immediately before animals were sacrificed. Histology (aldehyde fuchsin to stain insulin-positive β cells, hematoxylin & eosin to stain immune cells) was used as a gold standard measurement of cellular infiltration.

MRI measurement of the pancreatic ECV: All MRI was performed on a 7.0T Clinscan small animal imaging system. Imaging was performed on days 7, 14, 21, and 28 after injection of diabetogenic cells/vehicle. Mice were anesthetized with 1.25% isoflurane. An indwelling catheter was established in the tail vain for intravenous administration of Gd-DTPA. The pancreas was localized using axial images. T1 mapping images were acquired in the pancreas before Gd-DTPA infusion, and images were again acquired in the pancreas and in the heart (for measurement of blood pool T1) after Gd-DTPA equilibrium had been established. T1 mapping images had an in-plane spatial resolution of 250μm, and image acquisition took approximately 10-20 minutes per set of T1 mapping images. Equilibrium-phase Gd-DTPA was established by injecting a bolus of 0.1mmol/kg Gd-DTPA, followed by an infusion of Gd-DTPA at a rate of 0.075 mmol/kg/hour with a typical infusion duration of approximately 40 minutes to establish equilibrium, for a dosage of 0.15 mmol Gd-DTPA/kg. Regions of interest were drawn around the pancreas and in the left ventricular cavity to generate signal intensity vs. time curves for the pancreas and blood, respectively. These curves were modeled as monoexponential recovery curves of the form $SI(t) = 1 - e{\text{-}t/T1}$ and were fit for T1 using a least-squares optimization algorithm implemented in MATLAB. ECV was calculated from the following equation[3], assuming a hematocrit of 0.45[5] and a pre-contrast blood T1 of 1.6s[6]: $ECV = (1-Hct) * (T1_{pancreas, equilibrium} - T1_{pancreas, pre}) / (T1_{heart, equilibrium} - T1_{heart, pre})$

Results: Example ECV maps of the pancreas are shown in Figure 1 for the same mouse on day 7 and day 21 after cell transfer, demonstrating an ECV reduction at day 21. Figure 2 compares pancreatic ECV vs. time in mice who underwent adoptive cell transfer and control mice. Pancreatic ECV remained unchanged in control mice during the course of the study, while it decreased significantly after day 14 in mice who underwent cell transfer. Two-way ANOVA revealed a significant difference between cell transfer and control mice (p = .003), and Tukey tests revealed significant differences in cell transfer mice at days 7 and 28 (*p < .05) and between cell transfer mice and control mice at day 28 (#p = .018). Histology confirmed the presence of cellular infiltrate after day 14. Blood glucose was 231 ± 49 in cell transfer mice after day 21.

Conclusions: Using a standard approved contrast agent, ECV measurements detected pancreatic cellular infiltrate in a mouse model of diabetes. This clinically-translatable method might allow the early detection of insulitis prior to loss of β cell mass, and could potentially identify a therapeutic window wherein treatments that protect against invading inflammatory cells could be applied. It may additionally be useful as an early predictor of T1DM development in high-risk patients, as current biomarkers are not highly predictive of T1DM development. Furthermore, this technique may be investigated in other diseases, such as acute organ rejection, where cellular infiltrate plays key roles in the pathophysiology and progression of the disease.

References: 1). Gaglia JL et al, *J Clin Invest* 2011. 2). Fu W et al. *Nat Immunol* 2012. 3). Flett AJ et al, *Circulation* 2010. 4). Rohane PW et al, *Diabetes* 1995. 5). Baxter AG and Mendel TE, *Eur J Immunol* 1991. 6). Vandsburger MH et al, *Magn Reson Med* 2010.
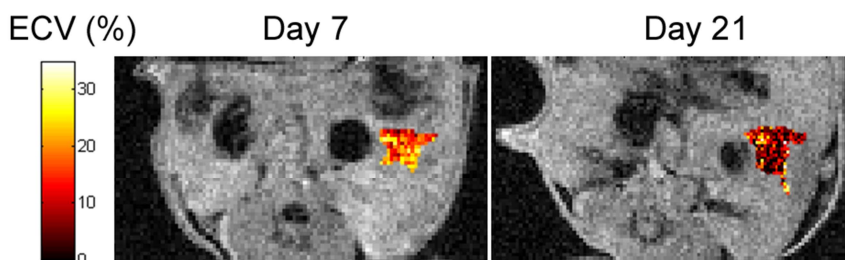
Figure 1: Example extracellular volume maps of the mouse pancreas on *(left)* day 7 after adoptive cell transfer, prior to pancreatic infiltration and *(right)* on day 21 after cell transfer, by which time immune cells have invaded the pancreas and decreased the extracellular volume.
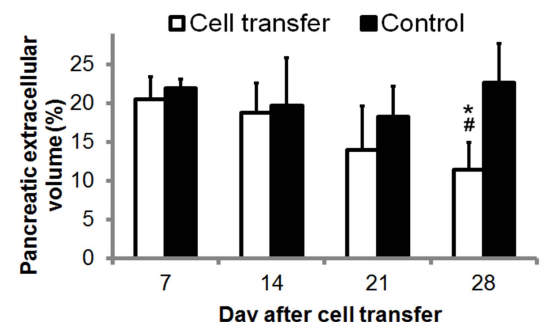

Figure 2: Comparison of pancreatic extracellular volume in NOD.scid mice who underwent adoptive transfer of diabetogenic splenocytes (*white*) and control mice injected with vehicle *(black)*. *p<0.05 vs. days 7 cell transfer, #p<0.05 vs same-day control.