A Porcine Vascularized Composite Allotransplantation Model for Assessing Early Prognostic Biomarkers of Rejection

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Introduction: Vascularized composite allotransplantation (VCA) involves transplanting a functional and anatomically complete tissue graft, such as a hand or face from a deceased donor to a recipient. Either acute or chronic, rejection occurs when the recipient's immune system recognizes the transplanted tissue as foreign and attacks it. Vasculopathy appears to be a major feature of chronic rejection in VCA recipients, often leading to ischemic graft loss. Although no definitive treatment currently exists for VCA vasculopathy, early diagnosis may potentially halt or slow the progression of this process. We here describe a consistent VCA rejection model in swine, exploring its potential for testing minimally invasive rejection biomarkers including galectin-3 and transcutaneous tissue oxygenation. Non-invasive approaches that accurately detect early signs of rejection in the skin and deeper tissues could lead to effective management strategies that prevent the progression to acute and chronic VCA rejection clinically.

Methods/Technical Approach: Six allogeneic vertical rectus abdominis myocutaneous VCA grafts were performed using Sinclair as donor pigs into Yucatan as recipients. The recipient animals underwent central line placement for daily blood draws from the day of surgery until euthanasia. Immunosuppression therapy was not used in this cohort. Punch biopsies of the donor graft were performed daily for histological assessment and the results were correlated with physical exam findings and CBC data. Daily serum was collected for future testing of circulating biomarker levels. Using an ultrabright porphyrin molecular oxygen sensor known as a Clickaphor, we measured skin temperature and tissue oxygen concentration transcutaneously in the transplanted VCA compared with healthy recipient skin on the day of surgery and every 24 hours until euthanasia.

Results: The six pigs underwent successful VCA transplantation and were euthanized following complete rejection. Using this model, we consistently observed early signs of inflammation and rejection with erythema and edema visible between POD2 and 4. In general, histological evaluation between POD2-4 revealed classic features of VCA rejection, including increased inflammation surrounding the superficial vascular plexus in the dermis (grades 1-2), that progressed to junctional inflammation, intraepidermal inflammation, and necrosis (grades 3-4). By POD4-5, we observed acute vascular compromise in the context of severe vascular rejection, with vascular congestion, capillary necrosis, and progression to diffuse dermal and partial epidermal necrosis. Daily CBC analysis revealed a spike in both WBC and granulocyte counts for each pig between POD4 and 5, which correlated with the histological data and visual signs of rejection. The majority of the grafts were completely rejected and no longer viable by POD7.

Conclusion: We have successfully developed an allogeneic VCA porcine model. In the setting of no immunosuppression therapy, early signs of rejection were accurately detected, with visual skin rejection occurring by POD2, followed by a progressive vascular rejection that becomes severe between POD3 and 4. These results demonstrate the development of a reproducible model of rejection that can be used for assessment of the effectiveness of minimally invasive methods of detecting early signs of graft rejection. Galectin-3 levels and transcutaneous tissue oxygen levels are currently being analyzed.