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Links between a biomarker profile, cold ischaemic time and clinical outcome following simultaneous pancreas and kidney transplantation

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ABSTRACT

In sepsis, trauma and major surgery, where an explicit physiological insult leads to a significant systemic inflammatory response, the acute evolution of biomarkers have been delineated. In these settings, Interleukin (IL) -6 and TNF-α are often the first pro-inflammatory markers to rise, stimulating production of acute phase proteins followed by peaks in anti-inflammatory markers. Patients undergoing SPKT as a result of diabetic complications already have an inflammatory phenotype as a result of uraemia and glycaemia. How this inflammatory response is affected further by the trauma of major transplant surgery and how this may impact on graft survival is unknown, despite the recognised pro-inflammatory cytokines' detrimental effects on islet cell function.

The aim of the study was to determine the evolution of biomarkers in omentum and serum in the peri-operative period following SPKT. The biochemical findings were correlated to clinical outcomes.

Two omental biopsies were taken (at the beginning and end of surgery) and measured for CD68+ and CD206+ antibodies (M1 and M2 macrophages respectively). Serum was measured within the first 72 h post-SPKT for pro- and anti-inflammatory cytokines (IL -6, -10 and TNF-α), inflammatory markers (WCC and CRP) and endocrine markers (insulin, C-peptide, glucagon and resistin).

46 patients were recruited to the study. Levels of M1 (CD68+) and M2 (CD206+) macrophages were significantly raised at the end of surgery compared to the beginning ($p = 0.003$ and $p < 0.001$ respectively). Levels of C-peptide, insulin and glucagon were significantly raised 30 min post pancreas perfusion compared to baseline and were also significantly negatively related to prolonged cold ischaemic time (CIT) ($p < 0.05$). CRP levels correlated significantly with the Post-Operative Morbidity Survey ($p < 0.05$).

The temporal inflammatory marker signature after SPKT is comparable to the pattern observed following other physiological insults. Unique to this study, we find that CIT is significantly related to early pancreatic endocrine function. In addition, this study suggests a predictive value of CRP in peri-operative morbidity following SPKT.

1. Introduction

In sepsis, trauma and major surgery, where an explicit physiological insult leads to a significant systemic inflammatory response, the acute temporal biomarker expression patterns of acute phase proteins have been described [1–6]. In these conditions, Interleukin (IL) -6 and TNF-α appear to be the first pro-inflammatory markers to rise, triggering the production of acute phase proteins, leading to peaks in anti-inflammatory markers [5]. Biomarkers are now used to improve specificity for risk stratification and predict outcomes at an early stage of the disease process [7–9], estimate extent of tissue damage [6,10,11] and provide targets for novel pharmacological therapies [12–14]. In chronic diseases such as insulin dependent diabetes mellitus (IDDM) and end-stage renal failure (ESRF), specific pro-inflammatory markers (IL -4, -6, -8, TNF-α) are persistently raised [14,15], leading to higher cardiovascular risk in this cohort [16].

Simultaneous pancreas and kidney transplantation (SPKT) recipients suffer with an exacerbated inflammatory phenotype as a result of uraemia and glycaemia, which has contributed to multi-system morbidity, end-organ failure, while and at transplantation, they undergo high-risk surgery, which further aggravates the inflammatory response. This reaction is clinically akin to sepsis and major trauma,

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and provides the impetus for current guidelines to recommend a more detailed surveillance. There is an urgent and unmet need to identify and validate accurate biomarkers for the detection of complications to improve quality of life for the patients and reduce costs for health care providers, while maintaining or improving current outcomes. Explicit clinical factors, which include a prolonged cold ischaemic time (CIT), recipient BMI greater than 30 kg/m^2 [17], recipient age greater than 45 years [18] and donor age greater than 40–45 [19,20] have all been identified as clinical markers correlating to poor outcomes. However, relevant biological profiling in this clinical context has not been investigated and the temporal evolution and interactions of peri-operative inflammatory markers (IMs) have not been characterised.

In solid-organ transplantation, biomarkers have been investigated in relation to donation after brainstem death (DBD), the impact of ischaemia-reperfusion-injury (IRI) and in rejection [21–23]. High levels of IL-1, -2, -4, -10 and TNF-α in the peri-operative period correlate with reduced long-term graft survival and increased rates of rejection. During islet cell transplantation, β-cells produce pro-inflammatory cytokines leading to β-cell dysfunction, graft toxicity and islet-cell death [24–27]. This has led to the introduction of anti-TNF-α agents being used as part of induction immunosuppression in some islet transplant centres, with evidence suggesting improved rates of graft survival [28–30]. In the setting of pancreatitis, the most widely available stressor model affecting the pancreas, raised levels of inflammatory cytokines (IL-6, IL-8, TNF-α and IL-10) have been observed and aid in prognosis prediction [7]. We propose that a measure of a panel of independent complementary biomarkers, including the markers with classic prognostic properties, together with novel inflammatory and endocrine markers may provide a more accurate prediction of outcome, compared with a few individual markers, thus improving risk stratification and clinical management of patients following SPKT.

Additionally, measuring both tissue and circulating biomarkers add valuable novel information to the study for diagnosing further surgical complications. Human adipose tissue is a metabolically active organ. The function and metabolic activity of the fat varies, depending on the location within the body [31], but higher levels of obesity correlate with higher levels of circulating IMs [32] leading to increased cardiovascular risk in the obese patient.

Within the abdomen, the omentum is an apron of fat, which like other deposits of fat, is a physiologically and metabolically active organ [33]. In surgery, it is known as the “abdominal policeman” as it is often found to encase areas of intra-abdominal inflammation and infection. The omentum is also involved in the formation of peritoneal adhesions and the production of growth factors and cytokines [33,34]. It reacts to a localised inflammatory insult via macrophage production of IMs (TNF-α, Resistin, plasminogen activator inhibitor-1 (PAI-1) and multiple interleukins [35]) resulting in a systemic effect [36]. This role has been outlined in cases of intra-abdominal sepsis, but minimal evidence exists as to the role of the omentum in instigating a systemic inflammatory reaction in response to an elective intra-abdominal procedure [37].

In SPKT, assumptions are also made regarding the production of endocrine markers by the allograft pancreas. Given that serum blood sugar levels tend to fall within the first hour post-pancreatic perfusion, and demonstrable primary pancreatic graft dysfunction is exceptionally rare, it is assumed that endocrine function is instantaneous and uniform, with the distinction between “impaired” or “delayed” graft function difficult to characterize.

Therefore, this study aimed to determine the temporal evolution of biomarkers in the peri-operative period following SPKT, assess the inflammatory response of omentum in relation to elective major surgery and establish a correlation of serum biomarkers to clinical outcome.

2. Methods

2.1. Study centre

The study was undertaken at The Central Manchester University Hospitals NHS Trust and The University of Manchester. Appropriate ethical and Research and Development approvals were obtained. Recipient serum and omental biopsies were taken prospectively from SPKT recipients between November 2011 and March 2014.

2.2. Study design

This study was designed to:

1. Delineate the temporal evolution of IMs (IL-6 and -10, TNF-α, C-reactive protein (CRP), White Cell Count (WCC) and Amylase) and endocrine markers (C-peptide, insulin, glucagon and resistin) in the perioperative period following SPKT;
2. Evaluate specific factors which may affect the levels of inflammatory and endocrine markers;
3. Correlate biomarker levels with clinical outcomes.

All adult SPKT recipients were eligible for inclusion in the study. Serum samples were taken and processed for analysis on eight occasions in the peri-operative period (pre-operatively, immediately prior pancreas perfusion, 30 min post pancreas perfusion and at 6, 12, 24, 48 and 72 h post-transplantation) [38]. Two omental biopsies were taken intra-operatively, firstly on entering the abdomen and secondly prior to closing the abdomen. Specimens were processed in an automated processor and wax embedded for subsequent immunohistochemical analysis.

2.3. Serum sample analysis

Serum CRP, Amylase and WCC were measured prospectively by the biochemistry and haematology departments at the investigating unit. IL-6, IL-10 and TNF-α, were all measured in serum using ELISA development kits from R&D Systems (Abingdon, UK). Minimum detection limits were 1 pg/ml, 5 pg/ml and 2 pg/ml respectively. Readings below these minimal levels were considered as 0 for analysis. Insulin, C-peptide, glucagon and resistin were measured in bulk using a bioplex micro-array multi-bead based system (BioRad Life Science Group, USA). Minimum detection limits were 1.0 pg/ml, 14.5 pg/ml, 4.9 pg/ml and 1.3 pg/ml for insulin, C-peptide, glucagon, and resistin respectively. Beyond this level, the software imputes likely doses, by extrapolating the calibration curves.

2.4. Omental biopsy analysis

Two omental biopsies were taken intra-operatively by the operating surgeon, firstly on entering the abdomen and secondly prior to closing the abdomen. Samples were fixed in 4% formaldehyde then processed and wax embedded in an automated tissue processor. 7 μm sections were cut, mounted on positively charged slides (Superfrost®) and H&E staining was performed. Sections were cut and mounted as above, then dewaxed and rehydrated. Antigen retrieval was performed in heated 1 mM citric acid (pH6) and non-specific antigen binding was blocked in 10% goat serum in phosphate buffer solution (PBS). Sections were exposed to either anti-CD68 antibody (M1 macrophages, Dako) or anti-CD206 antibody (M2 macrophages, Abcam), diluted 1:100 in 1% goat serum in PBS, then detected using biotinlylated secondary antibody (Vector Labs) and the ABC system (Vector Labs). Counterstaining was performed with Mayer’s haematoxylin (Fisher Scientific) and sections were dehydrated then mounted in DPX reagent (Sigma). Slides were visualised on a Zeiss Axio Scope light microscope and quantified using ImageJ analysis software.