Protocol: Utilising VAMS® technology to monitor tacrolimus and creatinine

Title: Utilising volumetric absorptive microsampling (VAMS®) technology to monitor tacrolimus and creatinine concentrations in adult renal transplant patients

Study Team & Collaborations:

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Background *(Literature review):*

Tacrolimus is an immunosuppressant which acts by inhibition of calcineurin. It's typically used as a first line drug to prevent allograft rejection in renal transplant patients (1). With pharmacokinetic variability between patients and a narrow therapeutic range, therapeutic drug monitoring (TDM) is essential to support graft survival. Low levels of tacrolimus risk organ rejection, whilst toxic levels risk nephrotoxicity and other adverse effects including neurotoxicity, electrolyte disturbances and hyperglycaemia (2). Clinical guidelines suggest target tacrolimus ranges, but levels are determined by renal clinicians and are patient specific, depending on individual circumstances, as well as the time since transplantation (3).

Renal transplant patients require lifelong monitoring of immunosuppressant levels and renal function to assess graft kidney stability. Renal function is monitored by measuring serum creatinine and urine protein excretion, levels of which determine the need to perform a biopsy if graft dysfunction is suspected (3). Creatinine is the product of creatine phosphate catabolism in muscle. Its endogenous production at a constant rate and clearance by glomerular filtration makes it a useful marker of graft function, with increasing levels indicating a reduced glomerular filtration rate and thus some element of renal disease (4). Creatinine is measured enzymatically on the Roche platform, as part of a urea and electrolyte panel.

The Renal department is currently managing just under 1000 patients with a functioning kidney transplant, with this number increasing annually. There are 80-100 transplants per year and a current waitlist of just under 300 patients. Following successful transplantation, every patient requires lifelong monitoring of transplant function. This is not only a long-term commitment for these patients, but also the renal department in providing the aftercare. Current practice for post-transplant monitoring involves regular venepuncture to collect a venous blood sample for tacrolimus measurement and a 'dialysis profile' (urea and electrolytes, bicarbonate, CRP). Clinical guidelines recommend monitoring tacrolimus levels by trough sample 3 times a week initially. Frequency decreases over time to every 4-6 weeks at 6-12 months and then every 3-6 months at 12 months post-transplant, unless adjustments are made to the dosage which warrants more frequent

monitoring. Serum creatinine should be measured at each clinic visit, as well as urine dipstick analysis for urine protein (3). Thus, the frequency of biochemical monitoring depends on patient circumstances, but once a steady state is achieved, the monitoring window increases.

The COVID-19 pandemic put considerable strain on patient care, with limited access to healthcare settings (including phlebotomy services) due to increased infection control measures. Transplant patients are already immunocompromised and often have comorbidities, so it is important to minimise unnecessary exposure to high-risk environments, including hospital settings. When presenting with COVID-19, solid organ transplant patients have an increased 30-day mortality compared to non-transplant patients, and they are also more likely to require mechanical ventilation and critical care (5). Furthermore, COVID-19 has been noted as a causative factor in graft rejection and subsequent loss (6). The risks associated with COVID-19 infection are applicable to various other disease groups, including patients undergoing cancer treatment (7). This highlights a requirement to protect these vulnerable groups in the hope of a more favourable patient outcome. To overcome some of the challenges imposed by COVID-19 and alleviate the associated risks for renal transplant patients, virtual appointments (by telephone or video consultation) were utilised. This enabled patients to attend their appointment anywhere, providing greater flexibility to both the patient and clinician. However, there is little benefit to these virtual appointments if the patient is still required to attend a healthcare setting for blood sample collection. Hence, alternative approaches to blood sample collection were explored.

A promising microsampling device has been growing in popularity. Neoteryx (Torrance, CA 90501, USA) has developed Mitra® devices with VAMS® (volumetric absorptive microsampling) technology. Patients collect a capillary blood sample by finger prick and send it to the laboratory for testing. Studies have shown results correlate well with those obtained from a venous blood sample collected by venepuncture (8, 9). There has been a positive bias noted for capillary tacrolimus concentrations, but this could be overcome by establishing new target ranges for patients (10). This method of sample collection is already being utilised by 2 Trusts within the UK to support transplant monitoring in paediatric patients.

The technology will be trialled in a small group of adult renal transplant patients with the intention to implement the blood collection strategy into routine clinical practice. This has the potential to significantly improve the patient pathway for renal transplant patients and reduce the economic burden. Reduced demand on blood clinics will increase availability for patients requiring renal transplant work-up, but also improve access to these services for patients from other departments. Remote blood collection, in addition to continued utilisation of virtual consultations frees up clinic availability for pre-transplant patients, whilst also reducing administration requirements for transplant nurses, who can better direct their time towards the patient. This strategy should empower the patient, thereby improving compliance and making care more efficient, all whilst greatly minimizing patient risk by removing the requirement for patients to regularly attend a hospital setting for their lifelong aftercare.

Research Question:

To compare tacrolimus and creatinine concentrations in venous samples collected by venepuncture and capillary samples collected using volumetric absorptive microsampling technology.

Objectives and Specific Aims:

- To develop and validate an LC-MS/MS method for tacrolimus and creatinine measurement in adult renal transplant patients.

- To compare tacrolimus and creatinine concentrations in venous samples collected by venepuncture and capillary samples collected using Mitra® devices.
- To implement home blood sample collection into routine clinical practice for renal transplant patients.
- To publish the study findings to encourage uptake of remote blood sample collection using VAMS® technology.

Rationale and Significance of Study:

The main advantage of this collection method is that it allows the remote collection of a blood sample. This reduces patient attendance at blood clinics and resultant strains on phlebotomy staff and clinics, eliminates administration requirements for transplant nurses and saves both the patient and the NHS time and money. Of most importance is the great flexibility and convenience this collection device offers, as patients can collect samples at anytime and anywhere, providing greater freedom compared to current procedures.

Patient adherence to immunosuppressants is a key factor in potential graft rejection, with lack of immunosuppression leading to graft loss, even years after transplantation (12). Due to the increased flexibility offered by the Mitra® device, it is anticipated that this collection method will improve patient compliance with TDM. Better timed sample collection is also expected (ie. a 'true' trough sample) as patients are not subject to blood clinic operating hours. Consequently, potentially more frequent and better-timed sample collections will be more clinically useful.

Importantly for patient management, is the ability to calculate AUC (area under the curve). This is not currently possible with current protocols as regular samples would be required which inconveniences the patient. AUC targeted therapeutic monitoring of tacrolimus may be more clinically informative than current pre-dose tacrolimus measurements and be a better indicator of immunosuppressant efficacy (13).

It is also worth noting the current issues encountered with venepuncture for venous blood collection. Increased patient anxiety occurs because of needle phobia (although uncommon) and if the patient is difficult to bleed. The latter of which can result in lengthy appointments whilst the required samples are obtained. In comparison, capillary blood sampling is minimally invasive, and a much smaller volume of blood is required (particularly useful for the elderly). However, a well-known issue associated with capillary blood is the volumetric haematocrit bias and poor blood spot quality, resulting in non-homogenous samples and inaccurate results (14). This issue is alleviated using Mitra® devices because a defined blood volume is collected.

VAMS® technology has been trialled in other TDM settings including cardiovascular (15). It is anticipated that this study will highlight the good correlation of results obtained from the different sample collection methods and ease of use of the collection device. Thereby supporting the implementation of Mitra® devices for blood collection into routine clinical practice, not just for TDM but also for other analytes such as HbA1c. Additionally, there is the potential to automate sample extraction, optimising laboratory workflow.

Research Design

Recruitment of Participants

A minimum of 50 adult renal transplant patients (>18 years old) taking tacrolimus as part of their immunosuppressant regime will be recruited from December 2022 when attending their routine outpatient follow up appointment. A patient information sheet will be distributed to the patients.

Written consent will be obtained from all participants.

Methodology

Sample collection

Patients attending blood clinic for routine venepuncture will also have a finger prick and 10µl blood will be collected using the Mitra® device (Neoteryx), according to manufacturer's instructions. The usual venous EDTA and serum samples will be collected by venepuncture by trained phlebotomists/nurses. Tacrolimus and creatinine are measured in venous blood samples (whole blood and serum, respectively) as part of routine patient care therefore these will be processed as normal, and results reported. The paired capillary samples collected using the Mitra® device will be batched and run when appropriate on the LC-MS/MS. Mitra® devices will be stored at -20°C prior to analysis. Any incorrectly filled Mitra® devices (over or under filled) which would affect sample quality will be rejected.

Sample analysis

Venous EDTA whole blood samples (collected by venepuncture) and capillary whole blood samples (collected by VAMS®) will be analysed for tacrolimus using a validated LC-MS/MS assay in routine clinical use in the lab using the Waters ACQUITY UPLC system and Xevo TQD MS. Additionally, samples from the UK NEQAS immunosuppressants scheme will be analysed as above for tacrolimus. Capillary whole blood samples (collected by VAMS®) and serum samples will be analysed for creatinine using an LC-MS/MS method which will be developed based on a previously published method (11). For the method comparison, serum samples (collected by venepuncture) will be analysed for creatinine using an enzymatic method on the Roche platform. Additionally, samples from the UK NEQAS clinical chemistry scheme will be analysed as above for creatinine. All sample analysis will be performed in an ISO 15189 accredited laboratory.

Creatinine method evaluation

Method comparison will be performed by comparing creatinine results obtained by enzymatic assay on the Roche platform with results obtained by LC-MS/MS. A minimum of 50 patient samples will be analysed, as well as EQA samples. Within and between batch precision will be performed by loading Mitra® devices with IQC sample. 3 levels of IQC will be run a minimum of 5 times within the same analytical run and between analytical runs, on different days. Precision studies will be used to determine the LLOQ. Low patient pools will be prepared to give appropriate concentrations. Samples will be analysed 10 times within the same analytical run and 10 times between runs.

Linearity will be determined by performing dilutions of the creatinine standard material to prepare an extended standard curve. To assess recovery, blank samples will undergo sample preparation steps and will be spiked with the 3 different levels of creatinine QC. Carryover will be assessed by analysing an alternating high creatinine concentration sample with repeated analysis of a low creatinine or blank sample.

Sample collection comparison

To compare results collected by venepuncture and results collected by VAMS® sampling, a minimum of 50 paired patient samples will be analysed for tacrolimus and creatinine by LC/MS-MS. 20 EQA samples from the NEQAS immunosuppressant scheme will be analysed for tacrolimus and 20 EQA samples from the NEQAS clinical chemistry scheme will be analysed for creatinine by LC-MS/MS. To compare creatinine methods (enzymatic and LC-MS/MS), creatinine samples will also be analysed using an enzymatic assay on the Roche platform.

To test the stability of whole blood collected by VAMS®, 5 samples will be stored at varying temperatures for 7, 14 and 21 days. The samples will be stored at -20°C, 4°C, room temperature and

37°C. This will test the effect of temperature extremes which may occur due to postal delivery of the Mitra® devices. The stability of post extraction samples will also be tested by storing the 96 well plate at 4°C post analysis and re-assaying at 24 and 48 hours post extraction. To investigate the effect of different haematocrit concentrations, blood cells and plasma will be mixed 20:80, 40:60, 60:40 and 80:20 to obtain 4 different haematocrit levels (20%, 40%, 60% and 80%).

Deriving a new reference range
If the LC-MS/MS tacrolimus and/or creatinine method shows a clinically significant bias compared to current methods, this will be confirmed by obtaining further patient comparison data. If confirmed, the reference range will need to be reviewed and amended accordingly. Any bias identified by Bland-Altman analysis will be used to derive a new reference range for patients being monitoring using blood collected using the Mitra® devices.

Data Analysis
Statistical analysis will be performed based on CSLI recommendations and departmental protocols.

Study population characteristics (including patient age, tacrolimus dose, time since transplantation, haematocrit) will be expressed as median (range).

Creatinine method evaluation
Precision: The following statistics will be calculated for within and between batch precision: mean, SD and %CV. A CV less than 20% will be deemed acceptable.

Accuracy: All samples will be analysed in duplicate and the results averaged. Passing-Bablok regression and Pearson correlation coefficient will be used to compare and assess correlation of the results obtained from each method. Bland-Altman analysis will assess agreement and determine presence of any bias between the results obtained from the different methods. 95% confidence intervals will be calculated. Statistical analysis will be performed using the Analyse-It add-in for Microsoft Excel.

Linearity: The diluted creatinine standards will be analysed in duplicate and plotted against the concentration to give the r^2 value. Means should be within 15% of each other to confirm linearity.

LLOQ: Mean, SD and %CV will be calculated. LLOQ will be defined as the level with a between batch precision of <15%.

Recovery: Each level of QC will be run in triplicate and the average calculated, with the recovery being expressed as a percentage with an acceptability of 100±20%.

Carryover: The % carryover from high to low concentrations will be determined. Differences of ±15% will be deemed acceptable.

Sample collection comparison
All samples will be analysed in duplicate and the results averaged. Passing-Bablok regression and Pearson correlation coefficient will be used to compare and assess correlation of the results of venous and capillary samples. Bland-Altman analysis will assess agreement and determine presence of any bias between the results obtained from venous and capillary sample collection. 95% confidence intervals will be calculated. Statistical analysis will be performed using the Analyse-It add-in for Microsoft Excel.

Stability: Samples will be analysed in duplicate and the results averaged. Means of each group will be

calculated and compared to baseline. Differences of ±15% will be deemed acceptable.

Effect of haematocrit concentration: The 4 samples will be analysed in duplicate and the results averaged. Differences of 15% will be deemed acceptable and this would confirm samples collected using Mitra® devices are unaffected by differing haematocrit concentrations.

Definition of the end of study:

The trial will end once enough samples have been collected to cover the required range of tacrolimus and creatinine concentrations for the assay. This will be a minimum of 50 samples. Only a single sample is required from each patient and there will be no follow up of the same patient. Patients will continue to be monitored post-transplant and have blood samples taken throughout the time of the study as part of the routine care, but they will not be included in the study.

Dissemination:

The study results will be reported and disseminated by writing an internal report, submitting to peer reviewed scientific journals and presenting at conferences.
The participants themselves will be informed of the results by the clinical care team at appointments following study completion. If the collection devices are successful, they'll be introduced into routine clinical care. Posters will also be produced and displayed in the outpatient areas, so the results are clearly visible.

Handling of participant data:

This is a single centre study taking place in an NHS organisation in England. The Chief Investigator and Sponsor will follow local Trust data protection protocols and general data protection regulations (GDPR) to ensure data protection and patient confidentiality.

Data collected from sample analysis will not contain identifiable patient information and will be kept within the laboratory until the project write up is complete. Access to Pathology requires a swipe card and only laboratory employees and signed in visitors are granted access. Clinical notes are stored electronically and a username and password are required for access. Clinical information will only be accessed by the clinical care team who would normally have access anyway as part of routine patient care. Personal data will be stored on NHS computers. Access to NHS computers is restricted to authorised personnel only and requires a username and password unique to an individual.

The venous blood samples will be processed as normal and results reported so routine patient care is unaffected. The Mitra collection device samples will be run in batches. Patient identifiable information will be removed in order to ensure patient confidentiality. Data collected from sample analysis will not contain identifiable patient information and will be kept within the laboratory until the project write up is complete.

Data will be analysed in the Trust Biochemistry laboratory by the research team. The data collected from the study will all be anonymized and thus no identifiable personal information will be used during data analysis and presentation. The data will be stored and destroyed according to local Trust and research policies once the required document retention time has passed. No data will be exported outside the UK

Upon study completion, all documentation will be archived according to Trust Research procedures,

in an off-site archiving facility for 15 years. Any anonymised data will be kept in password protected electronic folders in case of further research. After the retention period, data will be destroyed according to local protocol regarding confidential waste. Electronic records are erased or rendered unrecoverable.

Ethical issues:

The project is awaiting ethical review (IRAS No. 322562).

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