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The effect on toxicology, biochemistry and immunology investigations by the use of targeted post-mortem computed tomography angiography☆

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ABSTRACT

It is recognised in autopsy practice that investigations such as toxicology can be affected by post-mortem change. Post-mortem computed tomography angiography (PMCT-A) involves the injection of contrast agents. This could cause dilution of a biological fluid sample or cause the circulation of blood after death by mechanical pumping, and thus has the potential to affect laboratory investigations. We undertook a small sample study to consider whether targeted PMCT-A had any significant effect on subsequent samples taken for biochemical, toxicological or immunological investigations. Although the results of our study do illustrate differences between the pre and post PMCT-A results, these differences are considered not to be of diagnostic significance and not due to the direct effect of targeted PMCT-A.

1. Introduction

Post-mortem computed tomography (PMCT) is being increasingly used to investigate death across the world. One of the main weaknesses of PMCT is the lack of cardio-pulmonary circulation, precluding contrast enhanced studies after intravenous injection of water soluble contrast agents, as used in clinical CT scanners. This is a weakness to both the investigation of the coronary arteries; the most common cause of sudden death, and the investigation of vascular injury resulting from trauma. To aid in the visualisation of the vessels there are three PMCT angiography (PMCT-A) techniques currently described within the literature; angiography at the time of terminal cardio-pulmonary resuscitation [1,2], whole body angiography [3–8] and targeted angiography [9–12]. The whole body angiography system uses lipid soluble iodinated contrast media, whereas the other two techniques use standard iodinated water soluble media. Each of the three systems vary depending upon cultural and religious requirements in the handling of the dead, cost and availability of equipment, and the purpose for undertaking the procedure i.e. looking for a vasculature leak within a body cavity verses targeting a specific organ or vessels to consider specific pathologies. Each has been proven to work and therefore it is up to the user to consider which is the most appropriate system to be applied for the circumstances and question in hand, for example the investigation of natural death, intra-operative complications, homicide or use in temporary mortuary mass fatality investigations.

It is recognised that investigations such as toxicology can be affected by post-mortem change [13]. Thus any PMCT-A system that could cause dilution of a biological fluid sample, such as by the introduction of liquid contrast medium, or cause the circulation of blood after death by mechanical pumping of contrast medium has the potential to affect laboratory investigations. Thus, for whole body PMCT-A techniques using lipid soluble contrast media and paraffin oil [3–8], all relevant fluid based samples are taken prior to the procedure. The effect of such systems on DNA identification has not been reported, but the previous work of Graham et al. on the effect of ante-mortem blood transfusion on subsequent cadaver blood based DNA identification, would suggest that such systems should not affect DNA identification [14].

We undertook a small sample study to consider whether targeted PMCT-A, as described by Saunders et al. [10], using the targeted injection of 900 mL of air and 300 mL of iodinated water soluble contrast media (15 mg iodine/mL) into the ascending aorta, had any significant effect on subsequent samples taken for biochemical, toxicological or immunological investigations. The results of our study illustrate that, although this specific form of PMCT-A does produce different pre and post PMCT-A results,

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these differences are considered not to be of diagnostic significance.

2. Materials and methods

This work is funded by a grant from the National Institute of Health Research (NIHR) under its Research for Innovation Speculation and Creativity (RISC) Programme, reference number RC-PG-0409-10052. The trial is being conducted within local guidelines with approval by the local research ethics committee (LREC 04/Q2501/64, UHL 09523) and supported by the local Coroner's offices.

2.1. Case selection

Sixteen cases were selected and consented for participation in a sub study to an ongoing study of the accuracy of PMCT-A as compared to autopsy in deaths reported to the HM coroner. All forms of natural and non-suspicious unnatural deaths, including decomposed bodies are included in the trail to represent the full spectrum of non forensic autopsies (where a forensic case is defined as a suspicious or homicide death). The only exclusion criteria were bodies weighing over 125 kg (due to body handling issues). Up to two PMCT-A cases are undertaken per day at our unit, which are selected by being the first coroner's reports that arrive to the fax machine and then the first two that are consented for the procedure. The trial consenter (an Advanced Nurse Practitioner with counselling experience) contacts the 'next of kin' on the day prior to the autopsy to request permission to enter the deceased into the trial. Informed consent is obtained by telephone with information leaflets sent to the next of kin on the next working day if requested. As toxicological, biochemical and immunological examinations are not routine investigations on non-forensic coronial autopsies in England and Wales, consent was acquired for both PMCT-A and biological fluid laboratory examinations.

2.2. PMCT angiography

The cases underwent PMCT-A as previously described by Saunders et al. [10]. As part of the procedure a catheter was inserted into the ascending aorta, just above the aortic valve, via a 'cut down' procedure in the left neck to expose the carotid artery. The PMCT-A procedure involves injecting 900 mL of air as 3 boluses of 300 mL over 30–40 s and then 300 mL of Urografin (150 diluted to 15 mg iodine/mL, Bayer Healthcare), as 2 boluses of 150 mL over 50 s. After the end of the procedure there is wide dispersal of both air and contrast media throughout the heart, pulmonary circulation and great vessels.

For each case, prior to PMCT-A, in the mortuary approximately 10 mL of venous blood was withdrawn under direct visualisation from the femoral vein and placed into a sterile tube without preservative and a sterile tube containing a potassium fluoride-EDTA preservative. 1 mL of vitreous humour was then withdrawn from both eyes (mixed sample to ensure sufficient volume of sample available for testing prior to and after PMCT-A). Following sampling and catheterisation the body was immediately taken to the department of radiology for PMCT-A. Following PMCT-A the body was returned to the mortuary and the sampling was repeated in each case, from each site, thus providing pre and post PMCT-A peripheral venous blood and vitreous humour samples. Urine was not sampled as urine is used in our toxicology laboratory to screen, not quantify drugs and as our methodology allows for screening and quantification upon the blood samples it was felt unnecessary for the purpose of this research to sample urine. Stomach contents were not considered as this would have required further incisions and the primary sample for drug quantification is blood, not stomach contents.

For all tests the within batch coefficient of variation (analytical CV) has previously been calculated in our lab equipment based on retesting a pooled sample. The CV only includes the effects of retesting the same sample, not resampling, which is expected to cause the most variation in post mortem testing.

2.3. Biochemistry

2.3.1. Vitreous humour sodium, potassium, urea, creatinine and glucose

The pre- and post-PMCT-A vitreous humour sodium, potassium, urea, creatinine and glucose concentrations were measured in the routine clinical biochemistry laboratory using a Siemens Advia 2400 analyser. The within-batch coefficient of variation (CV) for vitreous sodium, potassium, urea, creatinine and glucose was 1.5%, 1.0%, 9.8%, 8.7% and 11.7% respectively.

2.3.2. Beta-hydroxybutyrate

In the cases in which the pre- and post-PMCT-A preserved blood acetone levels were elevated (>1 mg/dL), plain blood beta-hydroxybutyrate levels were measured using a beta-hydroxybutyrate liquicolor kit reagent (STANBIO, US) according to the manufacturer's protocol. The within-batch CV for this method was 7.4%.

2.3.3. Glycated haemoglobin (HbA1c)

The pre- and post-PMCT-A plain blood HbA1C levels were measured in the routine clinical biochemistry laboratory using a using a TOSOH G7 analyser with a within-batch CV of 3%.

2.4. Immunology

2.4.1. Mast cell tryptase

Mast cell tryptase levels were determined in all pre- and post-PMCT-A plain blood samples in the routine clinical immunology laboratory using a UniCAP tryptase fluoroenzyme immunoassay with a within-batch CV of 12%.

2.5. Toxicology

2.5.1. Ethanol, acetone, methanol and isopropanol analysis

All pre- and post-PMCT-A preserved blood and vitreous samples were analysed for the presence of volatiles including ethanol, methanol, isopropanol and acetone. Samples were prepared for analysis following an adapted procedure [15] with the use of peak height ratios. The analysis was performed on an Agilent 6890 series gas chromatography with flame ionisation detector system. The pre- and post-PMCT-A samples were run in a single batch and the overall within-batch CV for ethanol was 4%, and for acetone, methanol and isopropanol was approximately 7%.

2.5.2. Blood drug screen

All pre- and post-PMCT-A plain blood samples were screened using an Agilent 6410 triple quadrupole tandem mass spectrometry system in multiple-reaction monitoring mode for 330 commonly encountered drugs in a forensic toxicology laboratory. The samples were extracted prior to analysis using a standard solid phase extraction procedure [15].

2.5.3. Blood drug quantitation

Drugs detected during screening were quantitated in both the pre- and post-PMCT-A plain blood samples in a single batch for each case. Following standard extraction procedures [15] blood paracetamol, caffeine and carbamazepine concentrations were measured using an Agilent 1100 series reverse phase high performance liquid chromatography (HPLC) with diode array detection. The within-batch CV for paracetamol, caffeine and carbamazepine were 7%, 8% and 6% respectively. All remaining blood drug levels were determined using an Agilent 6410 triple quadrupole tandem mass spectrometry system with an electrospray ionisation source. All samples were extracted prior to analysis using an adapted solid phase extraction procedure [15]. The within-batch CV for citalopram, gabapentin, free morphine, free codeine, free dihydrocodeine, metoclopramide, duloxetine, tramadol and gliclazide was 4.6, 5.0, 6.9, 6.1, 5.1, 5.8, 6.6, 6.5 and 6.0% respectively.

2.6. Statistical analysis

For biochemical analysis where levels are expected data was analysed in 2 ways. Firstly the mean difference between the pre and post procedure tests was calculated with 95% confidence intervals. A paired students '*t*' test was used to test for any statistical significance of the difference. Secondly the inter-subject coefficient of variation (CV%) was calculated (standard deviation expressed as percentage of the mean) and the within-subject CV% (WS-CV%) was calculated by taking the individual variances of the test retest results (expressed as a percentage of their mean value); WS-CV% is the square root of the average of the variances. It may be expected that the WS-CV% may be greater than the within batch (analytical) CV% previously calculated for the analytical tests as this value involved testing the same sample not resampling. However the WSD-CV% was compared with the analytical CV%. The WSF-CV% was also compared with the inter subject CV%. A within subject CV% that is close to or greater than the inter patient CV% would suggest a clinically significant variation.

The standard deviation (SD) for each biochemical, immunological and toxicological method was previously determined during routine laboratory validation procedures performed on post-mortem samples. For routine biochemistry and mast cell tryptase analysis the expected repeatability (95% confidence interval) between the pre-PMCT-A test and post-PMCT-A retest was calculated using 1.96 SD. The observed repeatability (mean within-subject SD, WSD) was calculated using the square root of the mean variance for each analyte. The observed repeatability was then compared to the expected repeatability for each analytical method to determine test–retest repeatability. Due to the limited number of drugs and volatiles detected in the toxicology cases, as a 'normal' level would not be expected, data was analysed differently. The difference between the pre and post-PMCT-A concentration was compared to the 95% repeatability value. This describes the range around the mean that 95% of values would be expected to fall due to variability in the analytical method. This value is created by taking the product of 1.96 × the expected within-batch CV (expressed as a fraction) × the mean of the pre and post test value [1.96 × CV%/100 × (pre + post test value)/2]. If the difference between the test retest value is less than or close to this value there is assumed to be no significant difference due to the procedure.95% confidence interval (±1.96SD) for each analyte to determine test-retest repeatability.

3. Results and discussion

A total of 16 cases were included the study. As the cases were not pre-selected 1 case was found to have no drugs present in