

The effect on cadaver blood DNA identification by the use of targeted and whole body post-mortem computed tomography angiography

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Abstract Post-mortem computed tomography angiography (PMCTA) involves the injection of contrast agents. This could have both a dilution effect on biological fluid samples and could affect subsequent post-contrast analytical laboratory processes. We undertook a small sample study of 10 targeted and 10 whole body PMCTA cases to consider whether or not these two methods of PMCTA could affect post-PMCTA cadaver blood based DNA identification. We used standard methodology to examine DNA from blood samples obtained before and after the PMCTA procedure. We illustrate that neither of these PMCTA methods had an effect on the alleles called following short tandem repeat based DNA profiling, and therefore the ability to undertake post-PMCTA blood based DNA identification.

Keywords Targeted · Whole body · Post-mortem · Computed tomography · Angiography · DNA

Introduction

Post-mortem computed tomography angiography (PMCTA) [1] has become an established method used in the

investigation of death across the world. Currently there are three techniques described in the literature; angiography at the time of terminal cardio-pulmonary resuscitation (CPR) [2, 3], whole body angiography [4–14], and targeted angiography [15–17]. Different countries use different approaches including the use of different contrast medium depending upon the population's cultural and religious needs, cost, availability of equipment, and the underlying reason for undertaking the procedure, for example the search for a vascular leak in a post-operative death or the targeted investigation of coronary artery pathology. All three systems have been shown to be effective and therefore it is up to the users to apply the most appropriate method to the case in hand.

For targeted PMCTA Rutty et al. recently demonstrated that, although differences were experienced between pre- and post-PMCTA toxicological, biochemical, and immunological laboratory results, these differences were not considered to be of diagnostic significance and were not obviously related to the direct effect of targeted PMCTA [18]. In this paper they noted that any possible effect of PMCTA on cadaver blood based DNA identification remained unknown, although they hypothesized that targeted PMCTA should also not affect this, as ante-mortem blood transfusion does not preclude the use of cadaver blood for blood based DNA identification [19].

We undertook a small sample study to directly test whether cadaver blood based DNA identification is effected by the use of PMCTA. As terminal CPR angiography is not undertaken within our country and PEG and water soluble contrast medium is also not used to date within the United Kingdom (UK), we confined the study to the consideration of both targeted and whole body systems as described by Robinson et al. [16] and Grabherr et al. [9].

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Materials and methods

Case selection

Twenty deaths referred to and authorized by HM coroner for autopsy examination were selected and consented by the next-of-kin for participation in this substudy of an ongoing study of the accuracy of PMCTA, using both targeted and whole body angiography, compared to invasive autopsy. All forms of natural and non-suspicious unnatural deaths, including decomposed bodies, are included in the study to represent the full spectrum of non forensic autopsies (where a forensic case is defined as a suspicious or homicide death). The only exclusions are those with a known transmittable disease that could place those undertaking the procedures at risk (for example tuberculosis, HIV, or hepatitis C) or cadavers whose weight (over 210 kg) or dimension (shoulder width of 65 cm and above) preclude them from being scanned. The cases were selected by using the first suitable coroner's request arriving to the secure department fax machine on a study day. The trial consentor (an Advanced Nurse Practitioner) undertook the consenting process as reported by Saunders et al. [20]. The first 10 cases consented for the substudy underwent targeted PMCTA and the next 10 cases had whole body PMCTA. Consent was also obtained for

pre- and post-PMCTA blood sampling for DNA identification in all cases, as the identifications of the deceased were all known to HM coroner.

PMCTA sampling

The first 10 cases underwent targeted PMCTA using air (negative) and Urografin® (positive) contrast mediums in volumes and by the delivery system described by Robinson et al. [16]. The next 10 underwent whole body PMCTA using a mixture of Angiofil® and paraffin delivered using a Virtangio® machine (Fumedica AG, Switzerland) as described by Grabherr et al. [9]. For each case, prior to PMCTA, venous blood was withdrawn in the mortuary under direct visualization from the femoral vein and placed into a labeled sterile tube containing EDTA preservative (EDTA KE/4.9 ml monovette, Sarstedt, Nümbrecht, Germany). The volumes of blood acquired for each case are listed in Table 1. Following sampling either the left carotid artery or right femoral vessels were cannulated (PMCTA technique dependent) and then the body was taken immediately to the department of radiology for targeted or whole body PMCTA. Following PMCTA the body was returned to the mortuary where a repeat blood sample was taken as before. Both samples were placed inside an

Table 1 Summary of technical information relating to each blood sample used during this project

Case no.	PMCTA technique	Volume of blood (ml)		DNA quantitation result (ng/ul)		Concordant DNA profile? (Y/N)
		Pre	Post	Pre	Post	
1	Targeted	4.5	3.0	32.4	110.6	Y
2	Targeted	4.5	2.5	103.3	134.6	Y
3	Targeted	6.0	5.0	36.9	38.9	Y
4	Targeted	5.0	4.5	41.7	31.3	Y
5	Targeted	6.0	5.0	60.8	45.6	Y
6	Targeted	3.0	5.0	95.6	58.6	Y
7	Targeted	4.0	4.5	87.6	110.2	Y
8	Targeted	5.0	5.0	40.9	31.5	Y
9	Targeted	6.0	5.0	103.0	46.8	Y
10	Targeted	5.0	6.5	165.2	171.0	Y
11	Whole body	5.0	0.5	78.0	118.6	Y
12	Whole body	5.0	4.5	55.6	29.4	Y
13	Whole body	5.0	<0.2	86.2	42.6	Y
14	Whole body	5.0	1.0	23.9	11.0	Y
15	Whole body	5.0	<0.2	40.3	7.4	Y
16	Whole body	5.0	2.0	78.8	20.9	Y
17	Whole body	5.5	3.0	27.2	43.7	Y
18	Whole body	5.0	<0.2	207.2	28.2	Y
19	Whole body	6.0	0.3	48.9	16.6	Y
20	Whole body	6.0	0.5	61.3	11.7	Y

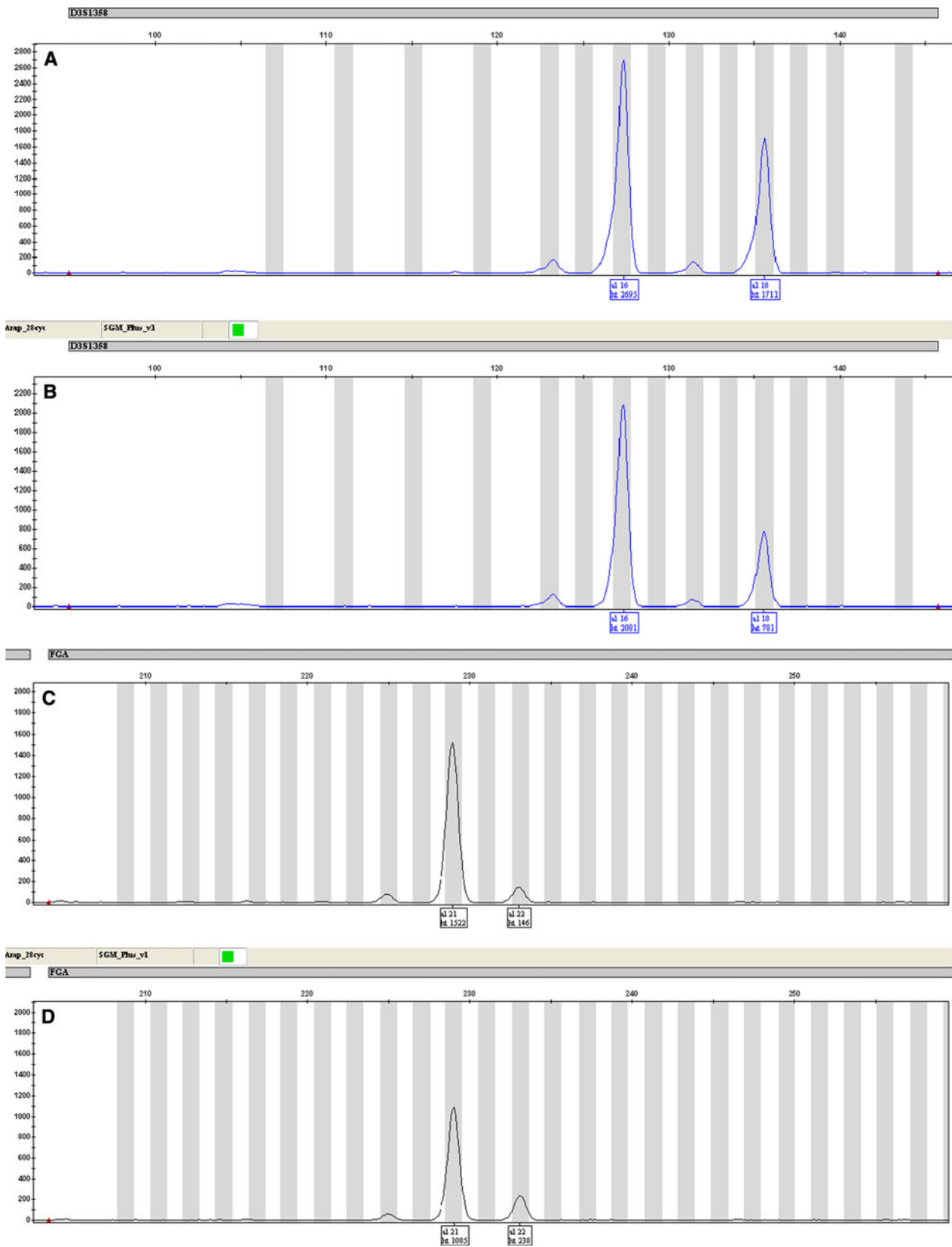


Fig. 1 Heterozygote imbalance observed for Case 11’s SGM plus DNA profile at D3S1358 (a pre-treatment, b post-treatment) and FGA (c pre-treatment, d post-treatment)

evidence bag which was sealed and placed into a freezer at -27°C for batch analysis.

DNA analysis

DNA was extracted from liquid whole blood using the QIAamp DNA Blood Mini kit (Qiagen, West Sussex, UK) following the manufacturer's protocol for blood/body fluids. Post-treatment samples comprising a blood-oil mixture were centrifuged at 1,000 rpm for 2 min to separate the fluids. Oil was carefully removed by pipette to allow for recovery of blood with minimal oil carry-over. Whenever possible, 200 μl blood was used for DNA extraction. When this was not possible due to smaller volumes of blood being collected for some post-treatment samples, the volume was made up to 200 μl using sterile phosphate buffered saline (PBS). DNA was eluted in 100 μl buffer AE. Negative DNA extraction controls were processed with every batch and consisted on 200 μl sterile PBS in place of liquid blood.

The quantity and purity of recovered DNA was determined by spectrophotometry using the Nanodrop-1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). Each DNA extract was quantified in duplicate

using 1.5 μl of the elute sample. The mean quantity was calculated to allow for DNA extracts to be normalized to 0.4 ng/ μl for PCR amplification using the SGM plus PCR Amplification kit (Life Technologies Corporation, Paisley, UK) in a total reaction volume of 12.5 μl and was amplified for 28 cycles. Negative DNA extraction controls were quantified and amplified undiluted. PCR products were visualized using the 3130 Genetic Analyser and genotyping was carried out using GeneMapper ID v3.2 software (Life Technologies Corporation, Paisley, UK).

Following DNA profile analysis extreme heterozygous imbalance was observed at two Short Tandem Repeat (STR) loci for both pre- and post-treatment samples from one patient (case 5). To further investigate this result a second STR amplification kit, the PowerPlex ESI16 System (Promega Corporation, Madison, WI, USA) was employed. Approximately 600 pg of extracted DNA was entered into a 12.5 μl reaction and PCR amplification was carried out for 30 cycles. Again, PCR products were visualized using the 3130 Genetic Analyser and genotyping was carried out using GeneMapper ID v3.2 software (Life Technologies Corporation, Paisley, UK) according to manufactures protocols.

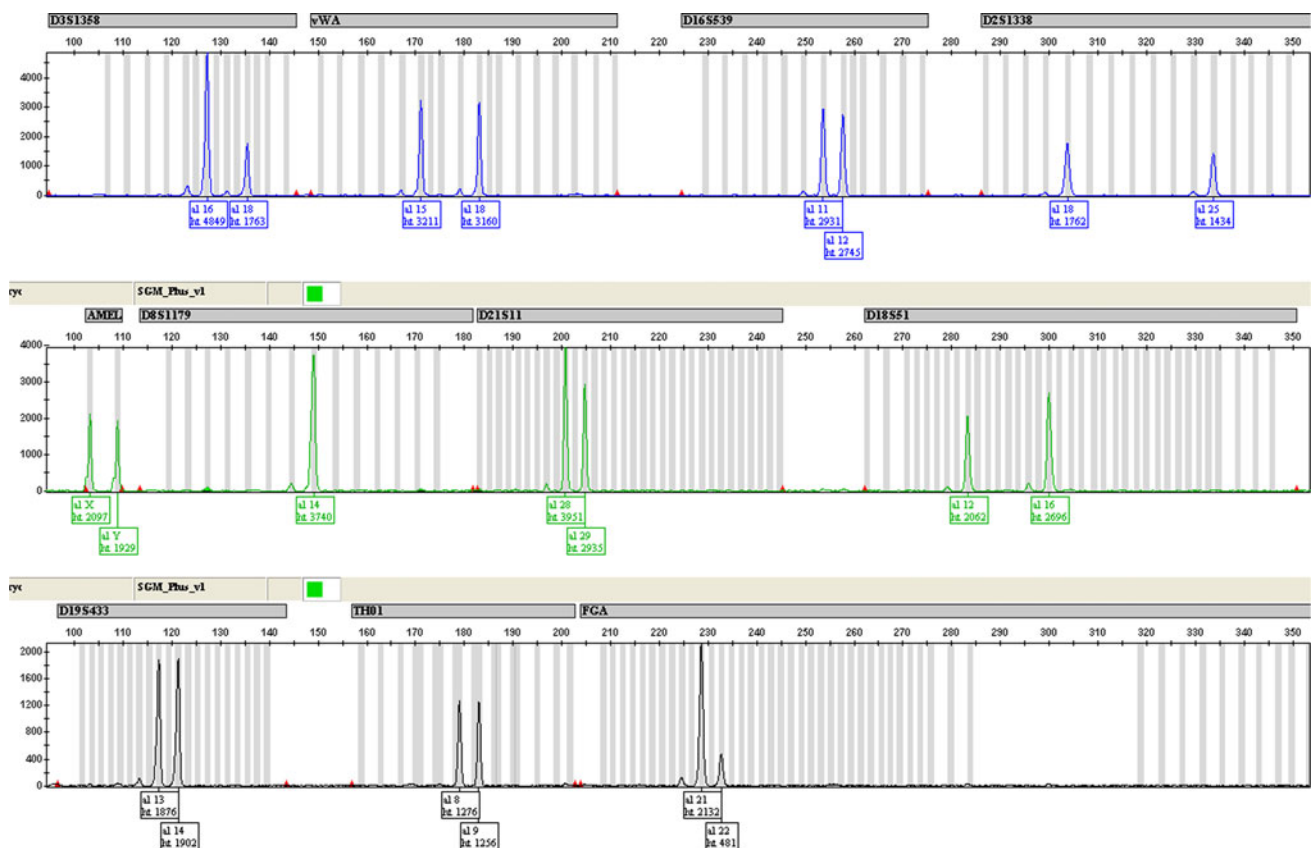


Fig. 2 Full SGM plus STR profile for Case 11's post-treatment sample. Balanced amplification, whereby heterozygote allele peak heights were within 60 % of each other was observed at all other STR loci

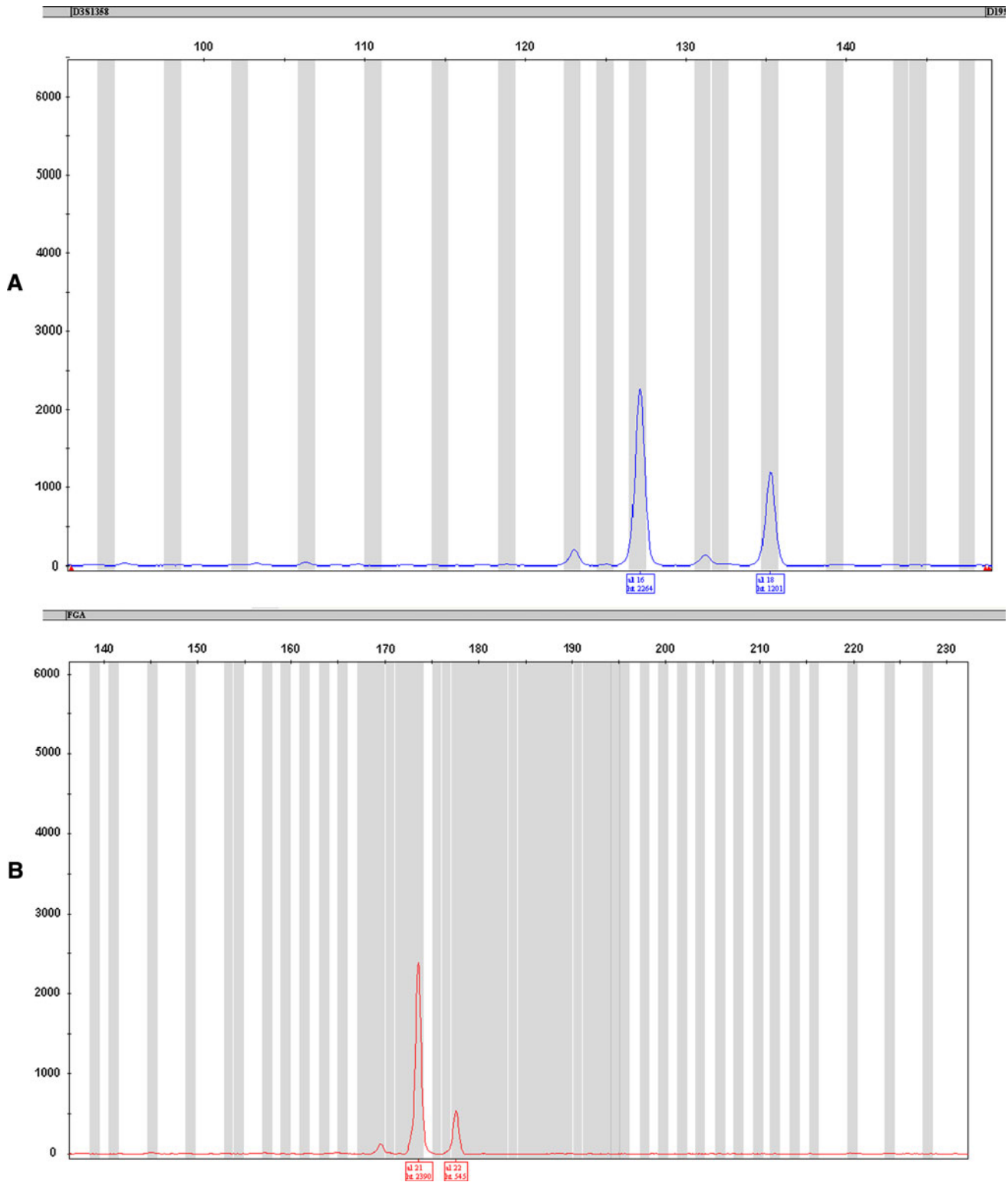


Fig. 3 Heterozygote imbalance observed for Case 11’s ESI DNA profile at D3S1358 (a) and FGA (b)

Results and discussion

A total of 20 cases were included in the substudy. Although difficulty was experienced in acquiring a comparable

volumes of post-PMCTA blood samples when using whole body PMCTA [9] (Table 1), the volume acquired was sufficient for full DNA profiles to be generated from all pre- and post-PMCTA blood samples. Complete

concordance between each paired pre- and post-treatment DNA profile was observed indicating that the use of targeted and whole body PMCTA, using the methods of Robinson [16] and Grabherr [9] does not affect STR profiling results (Table 1).

A limitation of this study was that the effect of the use of PEG with water soluble contrast medium was not investigated as this approach is currently not used, to our knowledge, within the UK in relation to adult PMCTA. However, as neither of the two approaches investigated affected the ability to generate a full DNA profile and as large volume blood transfusion has also previously been reported not to affect this investigation either [19] we are of the opinion that, although untested, the use of PEG should also not affect DNA profiling. Our opinion is based on the underlying principle that both large volume transfusion and the whole body angiography system tested add a large volume of non-self-fluid/oil to the intravascular space, as would the use of PEG, and yet do not affect DNA profiling. We are also not aware of an inhibitor within the PEG system that could affect DNA profiling and recognize PEG itself to be a PCR enhancer [21].

DNA profiles generated from one donor displayed heterozygous imbalance at two STR loci (Case 11, D3S1358 and FGA; Fig. 1) for both pre- and post-treatment samples. A review of Case 11's anonymous autopsy report revealed that the deceased had been suffering from diffuse large B cell lymphoma which can cause microsatellite imbalance and loss of heterozygosity at STR markers [22]. A high quantity of DNA was recovered from both samples (78.0 and 118.6 ng/ μ l respectively) so that an optimal amount of DNA could be entered into the SGM plus amplification reaction. The average 260:280 ratio of both Case 11 samples was 1.9 indicating that the recovered DNA was pure and free from potential PCR inhibitors. No other artefacts were observed in the DNA profile (Fig. 2). To confirm this result and to screen for potential primer binding site mutations, DNA extracted from both "case 11" blood samples was amplified using a different STR amplification kit, with different PCR primer sequences. The same heterozygous imbalance was observed in the resulting ESI16 DNA profiles (Fig. 3; Table 2).

Summary

Our results illustrate that both targeted and whole body PMCTA have no observable affect on cadaver blood based DNA identification. This is an important observation as it does not matter whether or not a blood sample for DNA identification is taken prior to, or after PMCTA using these systems. We also hypothesize that as the Virtangio[®] system involves the introduction of several liters of paraffin

Table 2 STR DNA profiling results for Case 11, pre- and post-treatment blood samples provided identical alleles calls so are not listed separately

Case	D3S1358	vWA	D16S539	D2S1338	D8S1179	D21S11	D18S51	D19S433	TH01	FGA	D22S1045	D1S1656	D10S1248	D2S441	D12S391
11	16, 18	15, 18	11,12	18, 25	14, 14	28, 29	12, 16	13, 14	8, 9	21, 22	15, 16	12, 17.3	13, 13	10, 14	20, 22

Due to the primer sets included in each of the products used, the first ten loci listed were amplified with both the SGM plus and ESI PCR amplification kits, the final five were amplified with ESI only

as the carrier agent for the contrast agent Angiofil[®], then CPR PMCTA methods would not affect DNA identification either.

Key points

1. Pre- and post-PMCTA using targeted [16] and Angiofil[®] based whole body [9] PMCTA does not affect STR profiling results.
2. The volume of blood available post whole body [9] PMCTA is reduced compared to that of targeted [16] PMCTA. This does not however preclude post whole body [9] PMCTA DNA STR profiling.

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Conflict of interest This study has an associated patent pending related to the development of a cadaver specific angiography catheter and the protocol related to its use.

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