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ORIGINAL ARTICLE

E. A. M. Graham · M. Tsokos · G. N. Rutty

Can post-mortem blood be used for DNA profiling after peri-mortem blood transfusion?

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Abstract The question of whether blood transfusions can affect DNA profiling is still a contentious issue throughout the forensic community. It is hypothesised that donor leucocytes present in the administered blood will be detected upon examination of recipient blood. In order to resolve this issue, a selection of theoretical experiments were carried out to determine how much donor DNA must be present for its detection in blood components. Five casework examples of material collected from individuals after massive transfusion, including a case of whole organ transplantation, were also investigated. The results indicated that filtration processes used during blood production do not allow the passage of enough donor leucocytes for detection using current forensic profiling techniques. No evidence of secondary profile alleles were found in any case, indicating that peri-mortem blood transfusion does not affect DNA profiling.

Keywords Blood transfusion · Post-mortem · Forensic DNA profiling · Short tandem repeats

E. A. M. Graham · G. N. Rutty (✉)
Forensic Pathology Unit,
University of Leicester,
Robert Kilpatrick Building,
Leicester Royal Infirmary,
Leicester, LE2 7LX, UK
e-mail: gnr3@le.ac.uk
Tel.: +49-116-2523221
Fax: +49-116-2523274

M. Tsokos
Institute of Legal Medicine,
University of Hamburg,
Butenfeld 34,
22529 Hamburg, Germany

Introduction

Within the United Kingdom, one is encouraged not to use blood for short tandem repeat (STR) profiling from cadavers who have received peri-mortem blood transfusions. Although there are no specific guidelines, the assumption is made that the profile generated will be derived from the transfused blood and not from the deceased. This assumption leads to the request for the submission of other biological samples including plucked hair or more commonly deep muscle, which increases the cost of profiling and the time required for samples to be processed.

Despite this practice, the literature does not support this assumption. A review of the literature identifies that previous authors have shown that blood transfusion does not affect the DNA profiling of living or dead individuals from blood. A summary of the literature is presented in Table 1 [1–8].

Because of the continuation of this practice and the persistence of this assumption, we have undertaken a study considering whether STR profiling of a cadaver can be performed from blood of recipients of massive peri-mortem blood transfusions. We have also considered the affect of organ transplantation and, by laboratory experimentation, considered the theoretical level of exogenous DNA that is required to be added to a blood sample before it affects the profile.

Materials and methods

Sample collection

Written informed consent was obtained from the immediate relatives of the deceased or the appropriate legal authority for the collection of post-mortem samples from five cadavers. All samples were collected from individuals who had undergone massive blood transfusion prior to death. Typically, 20 ml blood was obtained from the iliac vein as

Table 1 A summary of previously published papers considering the affect of blood transfusion on DNA profiling of the living and the dead

Reference	Number of patients	Type of blood product	Volume received (U)	Type of investigation performed	Result
[1]	12	Packed red blood cells/whole blood	1–25	Single and multi-locus probing	No effect
[2]	1	Packed red blood cells	12	PCR	No effect
[3]	10	Mixed	9–74	Southern blotting and PCR	No effect
[4]	1	Packed red blood cells	?	STR PCR	No effect
[5]	60	Packed red blood cells	2–50	PCR	No effect
[6]	5	Whole blood	>1	PCR	No effect
[7]	107	Mixed	>1	PCR and nested PCR	No significant effect
[8]	7	Whole blood	1–2	STR PCR	No effect

well as 5 g psoas muscle tissue and 20–30 plucked head hairs. Table 2 illustrates the history related to the donated samples.

DNA extraction

Blood

DNA was extracted from 200 μl post-mortem blood using the QIAamp DNA Blood Mini Kit (QIAGEN, West Sussex, UK) according to manufacturer's protocol.

Muscle and liver

DNA was extracted from approximately 25 mg muscle/liver tissue using the QIAamp DNA Tissue Mini Kit (QIAGEN) according to manufacturer's protocol.

Plucked head hair

Prior to DNA extraction, approximately ten hairs were transferred to a sterile 5-ml Falcon tube for removal of any external contaminants that may have been present. Hairs were washed twice with sterile water and twice with 70% ethanol. Hairs were cut into 1-cm lengths and placed into a sterile 1.5-ml Eppendorf tube containing 500 μl lysis buffer (75 mM NaCl, 25 mM EDTA, 10 mM Tris–HCl pH 7.5, 39 mM DTT, 1% SDS) and 10 μl proteinase K (10 mg/ml). The sample was incubated for 24 h at 37°C with occasional vortexing to aid digestion. DNA was then purified using phenol/chloroform/isoamylalcohol (25:24:1) followed by Microcon concentration (Millipore, Billerica, MA).

Red blood concentrates

Five units of out-of-date, leucocyte-depleted, red blood cells (RBCs) in additive solution were donated by the Haematology Department, Leicester Royal Infirmary, Leicester, UK, for use in this study.

Prior to DNA extraction, 2 ml RBC solution was transferred to a 5-ml Falcon tube and centrifuged at 2,500×*g* for 10 min to aid isolation of any leucocytes present in the sample. DNA was extracted from a 200-μl fraction taken from the upper portion of the centrifuged RBCs using the QIAamp DNA Blood Mini Kit (QIAGEN) according to manufacturer's protocol.

Theoretical levels of DNA required for detection in red blood cell concentrates

Human dermis fibroblasts were obtained from a live cell culture grown from a donated breast reduction surgical specimen. Cell counting was performed after staining with trypan blue. Cells were pelleted, resuspended in 100% ethanol and stored at −20°C before use. Control genomic DNA K562 was purchased from Promega (Madison, WI).

A serial dilution of both cells and genomic DNA was prepared and added to 200-μl aliquots of RBC concentrate. Dilutions theoretically containing 0, 1, 10, 100, 1,000 and 10,000 cells were added to each 200-μl aliquot of RBCs. Similarly, dilutions containing 0, 0.01, 0.1, 1, 10 and

Table 2 Case histories

Case	Complaint	Treatment	Time between treatment and death
1	Multiple stab wounds	~60 U packed red blood cells	None
2	Multiple stab wounds	~30 U packed red blood cells	None
3	Multiple stab wounds	~6 U packed red blood cells	None
4	Compartment syndrome	60 U packed red blood cells	18 days
5	Liver failure followed by myocardial infarction	Liver transplant	5 months

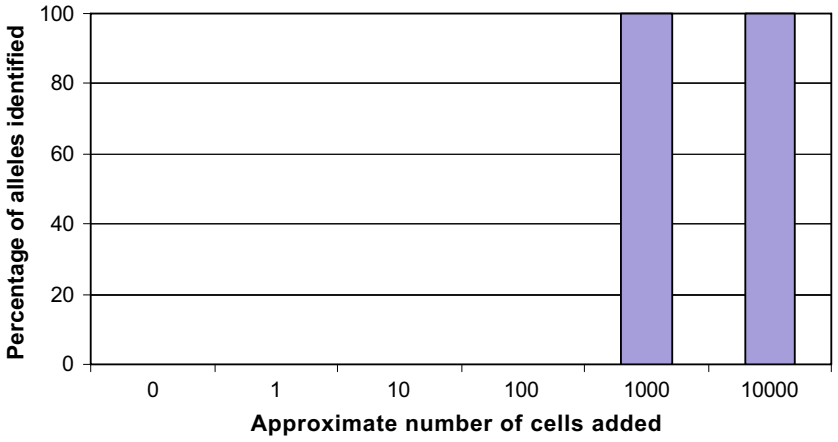

Fig. 1 Percentage of alleles called after addition of known amounts of whole cells to 200 μl RBC concentrate

100 ng free genomic DNA were added to blood cells. Each amount of cells/DNA was extracted in duplicate. The entire experiment was also duplicated so that each known amount of DNA/cells was extracted a total of four times. DNA was extracted using the QIAamp DNA Blood Mini Kit (QIA GEN) according to manufacturer's instructions and eluted in 100 μl buffer AE (QIAGEN).

DNA quantification

DNA was quantified using PicoGreen dsDNA Quantitation Reagent (Molecular Probes, Eugene, OR) according to manufacturer's protocol.

DNA profiling

Profiling of extracted DNA was carried out using the AmpF*l*STR SGM Plus PCR Amplification kit (Applied Biosystems, Foster City, CA) in a final reaction volume of 25 μl [9]. A total of 1 ng template DNA was added to each reaction. Polymerase chain reaction (PCR) products were separated and visualised on an ABI PRISM 377 DNA sequencer (Applied Biosystems). Fragment sizing was carried out using GeneScan software version 2.1 (Applied Biosystems), and allele designation was carried out using Genotyper software version 3.7.

Results

DNA extraction from packed red blood cells

DNA extraction was carried out on the surface 200 μl blood after centrifugation of 2-ml aliquots of out-of-date packed RBCs. Five units were obtained for this study, and experiments were carried out in duplicate for each unit. No DNA was detected by either quantification or STR profiling for any of the units.

Test for detectable levels of cells/free DNA in red blood cell concentrates

Experiments were carried out in order to quantify the number of cells or amount of 'free DNA' that would need to be carried over in order for a foreign profile to be detected by practiced DNA profiling techniques. Results of DNA profiling following addition of cells indicated that a level equivalent to 1,000 cells must be present for detection per 200 μl RBC concentrate (Fig. 1). When free genomic

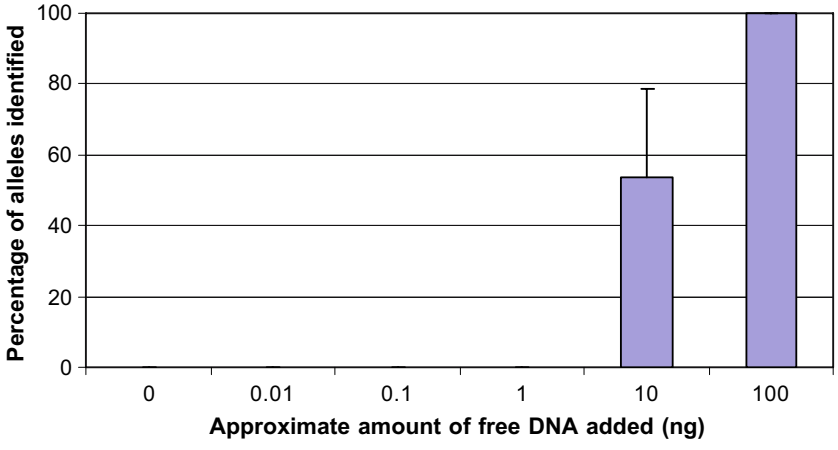

Fig. 2 Percentage of alleles called after addition of known amounts of free DNA to 200 μl RBC concentrate

Table 3 Results of STR profiling

Case	Material	STR loci										Amelogenin
		D3S1358	vWA	D16S539	D2S1179	D8S1179	D21S11	D18S51	D19S433	THO1	FGA	
1	Hair	15, 16	17, 17	13, 14	20, 23	13, 13	29, 33.2	12, 17	12, 13	6, 9.3	19, 21	X, Y
	Blood	15, 16	17, 17	13, 14	20, 23	13, 13	29, 33.2	12, 17	12, 13	6, 9.3	19, 21	X, Y
	Muscle	15, 16	17, 17	13, 14	20, 23	13, 13	29, 33.2	12, 17	12, 13	6, 9.3	19, 21	X, Y
2	Hair	13, 17	15, 17	11, 11	23, 24	12, 14	28, 32.2	15, 18	13, 15	9.3, 9.3	21, 22	X, Y
	Blood	13, 17	15, 17	11, 11	23, 24	12, 14	28, 32.2	15, 18	13, 15	9.3, 9.3	21, 22	X, Y
	Muscle	13, 17	15, 17	11, 11	23, 24	12, 14	28, 32.2	15, 18	13, 15	9.3, 9.3	21, 22	X, Y
3	Hair	14, 16	15, 17	12, 13	17, 21	13, 13	30, 31	13, 15	13, 15.2	6, 8	22, 23.2	X, Y
	Blood	14, 16	15, 17	12, 13	17, 21	13, 13	30, 31	13, 15	13, 15.2	6, 8	22, 23.2	X, Y
4	Hair	16, 16	17, 17	11, 12	21, 25	13, 13	29, 33	15, 16	12, 15.2	8, 9.3	22, 22	X, Y
	Blood	16, 16	17, 17	11, 12	21, 25	13, 13	29, 33	15, 16	12, 15.2	8, 9.3	22, 22	X, Y
5	Hair	15, 18	18, 18	12, 14	20, 22	10, 13	29, 31	14, 16	13, 14	7, 9.3	25, 25	X, X
	Blood	15, 18	18, 18	12, 14	20, 22	10, 13	29, 31	14, 16	13, 14	7, 9.3	25, 25	X, X
	Liver	15, 16, 18	14, 16, 18	10, 11, 12, 14	20, 22, 23	10, 12, 13, 14	29, 30, 31	13, 14, 16	13, 14, 16.2	7, 8, 9.3	20, 22, 25	X, X

DNA was added to RBC concentrate, on average, only a partial profile could be detected when 10 ng was added to 200 μl RBC concentrate and a full profile with the addition of 100 ng (Fig. 2).

DNA profiling of transfused patients

DNA profiling was carried out on DNA extracted from all materials listed in Table 2. Plucked head hair was collected to provide a reference profile to which post-transfusion materials could be compared. Hair was used for this purpose to dispose of the need for tracking down ante-mortem reference material. Hair is included in the discrete class of evidence types [10] that may be collected during the course of forensic investigation. It is solid and non-soluble in water or ethanol, so it can therefore be cleaned externally to ensure extracted DNA is from a single donor source. It has also been shown that DNA profiling of plucked head hairs is unaffected by many issues relevant to this investigation such as blood transfusion, bone marrow transplantation and foetal trafficking [11]. The results of DNA profiling of the samples from each case are given in Table 3.

In all five cases, the DNA profile obtained from post-transfusion blood sample was identical to the cadaver profile obtained from plucked head hairs. The exception in this study was that of the liver sample (case 5). The DNA profile obtained from this sample showed a mixture of two sources of DNA. A portion of this profile has identical alleles to that of the cadaver hair, leaving a complete second profile. Considering the previous literature and the results obtained in the other four cases, this second DNA profile is hypothesised to originate from the donor organ. However, information regarding the DNA profile of the donor was not available to the authors.

Discussion

Addition of cells/free DNA into red blood cell concentrates

It has been reported that filtration processes used in the fractionation of whole blood may allow leucocytes to pass through to the RBC fraction used for transfusion. According to the standards of the Blood Transfusion Service in the United Kingdom, all allogenic blood components with the exception of granulocytes must undergo filtration to achieve leucocyte depletion before use. It is specified that the leucocyte depletion should be to a minimum level of 99%, so that components should contain less than 5×10^6 leucocytes per unit (450 ml ± 10%) [12]. This is equivalent to approximately two cells per microlitre, a level that could not be detected using the DNA extraction and amplification techniques described in this report. It should be noted that all DNA profiling was undertaken following standard protocols, using 28 PCR cycles. The use of low copy number protocol as described by Gill [10] may allow for the detection of more contaminating DNA than is described in this report.

Results obtained from the theoretical contamination studies presented in this report found that between five and ten leucocytes must be present per microlitre of RBC concentrate for detection of donor DNA alleles using standard forensic protocols. It was observed by the authors that only partial DNA profiles could be generated when 10 ng free genomic DNA was added to 200 μl RBC concentrate before DNA extraction. Assuming 100% efficiency, a final concentration of 100 pg/μl would be expected after DNA extraction using the QIAamp DNA Blood Mini Kit (QIAGEN). This would result in a 500-pg template DNA being entered into the 25-μl PCR reaction and full profile generation. The observed failure to generate

full DNA profiles may be due to a decrease in DNA extraction efficiency caused by blocking of the QIAamp silica–gel membrane with excess erythrocyte-derived proteins. This has however not been proven at this time.

Although this investigation does not take into account the affects of recipient cells being introduced to post-transfusion blood, it clearly demonstrates the requirement for relatively large amounts of DNA or DNA-containing components to be present in a given blood product before donor profiles can be detected. This observation is further strengthened by the mixture detection limitations of forensic STR profiling kits such as the one used in this investigation, whereby a secondary profile must be present at a concentration greater than 10% of the total template DNA to be observed [13].

Peri-mortem blood transfusion and microchimerism

Despite a number of publications available that state that blood transfusion does not affect DNA profiling, there is still a general confusion surrounding this topic. By monitoring forensic Internet forums and through personal communication with colleagues, it has been noted by the authors that the question of blood transfusion interfering with cadavic forensic investigation is frequently asked and is not conclusively answered. Some confusion regarding single profile generation after blood transfusion may arise due to the numerous publications available to support the suggestion that microchimerism does result from blood transfusion [14–18] and that donor leucocytes can even persist in the recipients blood circulation for up to 25 years [17]. The observation of genotypes that do not arise from the recipient can however only be performed when the detection method is designed to be highly specific to the donor profile [19]. It must also be noted that the majority of such studies have been performed on female recipients of male, whole unfiltered blood. This allows for the design of highly specific PCR strategies to be directed at regions of the Y chromosome, alleviating problems caused by interference of recipient DNA [20].

We have performed DNA profiling of five cases in which the individual donor had received multiple blood transfusions and, in one case, a whole organ transplant, prior to death and sample collection. In no case did we find any evidence of microchimerism in post-transfusion blood. In each case, a full profile identical to that obtained from plucked head hair was observed. Our results are consistent with previous published works presented in Table 1 in which similar forensic type investigations have been carried out. Combining the eight listed publications in Table 1 with the results presented in this report, a total of 208 individuals have been tested for microchimerism following blood transfusion of 1–74 U of various blood components, including whole blood. The observation of a secondary profile has not been observed in any case reported to date.

A mixed profile was generated from DNA extracted from the liver of case 5. The authors anticipated this observation, and the mixture result can be explained by the continued proliferation of donor cells within the organ combined with colonisation by recipient cells. It is important to note that although a secondary (donor) profile was detected when the received organ was sampled, no secondary profile was observed in the circulating blood of the patient. A more thorough investigation into this particular aspect is required. The results obtained here however would suggest that whole organ transplantation, like blood transfusion, does not affect forensic DNA profiling.

There are several situations in which blood should not be collected at autopsy for identification or paternity verification purposes. It has been shown that bone marrow transfusion does result in the circulation of donor leucocytes in the recipients bloodstream for up to 5 years following treatment [11] and that it can be detected using techniques similar to those used in this report. In this particular report [11], donor alleles were also detected in the buccal mucosa of patients following bone marrow infusion. A mechanism for stem cell differentiation following bone marrow transplant resulting in the detection of microchimerism buccal cells of recipients has been suggested by Tran et al. [21]. Care must also be taken during the investigation of immuno-suppressed/incompetent patients who have received ante-mortem blood transfusion, including any individuals who have suffered or died of transfusion-associated graft-vs-host disease. This will include any patients who have undergone chemotherapy, radiotherapy and major surgery, neonates and individuals suffering from AIDS. In all of these cases, natural levels of leucocytes will be severely depleted, allowing the proliferation of any donor haematopoietic stem cells that are received during transfusion and detection of chimeric genotypes upon investigation [14–20]. Microchimerism in women after pregnancy has also been observed [22] but did not interfere with standard forensic Y chromosome testing so will not be considered as a potential problem in issues surrounding microchimerism following blood transfusion. Finally, it must also be noted that cases of naturally occurring microchimeric DNA profiles have been observed to be shared between triplets [23] and dizygotic twins [24]. This issue is however unrelated to the issues surrounding blood transfusion and organ transplantation and cannot be predicted at this time.

Recommendations

This study supports the current literature to allow blood samples from recipients of massive blood transfusions, be

they living or dead, to be collected for the purpose of DNA profiling. It also supports that blood can be collected from the recipients of whole organ transplants but recommends that further studies need to be conducted in this area. It draws attention to those cases where living or cadavic blood should not be collected due to the issue of microchimerism.

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