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*Original articles*

# Serological findings and efficiency of DNA profiling in transfused patients and their significance for identity and paternity tests

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**Summary.** The problems of testing patients or the deceased using classical blood group systems for identity or paternity purposes after multiple transfusions are well known. In a paternity case where the putative father received multiple transfusions it was possible to determine some classical blood group systems. The investigation showed no incompatibility between the putative father and the child and led to a probability of paternity of 72.33%. Using the DNA technique (single locus probes) it could be demonstrated that the DNA patterns were not influenced by the transfusion and the possibility of paternity reached 99.9%. For a further consideration of this problem classical blood group systems and DNA systems were investigated in 12 patients before and after multiple transfusions. It was possible to determine the changes due to transfusion in the classical systems but changes in the DNA pattern were not observed.

**Key words:** Paternity – Identity – Transfusion – DNA profiling

**Zusammenfassung.** Die Problematik bei serologischen Identitäts- und Vaterschaftsuntersuchungen an Blutproben von Patienten oder Verstorbenen, die Polytransfusionen erhalten haben, ist bekannt. In einem zu berichtenden Vaterschaftsfall aus unserer Laborpraxis konnten am Leichenblut einige klassische Blutgruppensysteme bestimmt werden. Es ergab sich keine Ausschlußkonstellation und eine Vaterschaftsplausibilität von W = 72,33%. Der zusätzliche Einsatz von Singlelocus-Sonden führte zu einer Vaterschaftsplausibilität von W = 99,9%. Hinweise auf einen Einfluß der Transfusionen ergaben sich nicht. Um diese Befunde im größeren Rahmen abzusichern, führten wir entsprechende Untersuchungen (klassische Systeme und DNA) an 12 Patienten vor und nach Transfusionen durch. Es fanden sich zwar transfusionsbedingte Veränderungen in den klassischen Systemen, ein Einfluß auf die DNA-Untersuchungen ergab sich jedoch in keinem Fall.

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**Table 1.** Blood and blood component conserves and their characteristics

| Type of conserve | Characteristics |
|---|---|
| 1. Whole blood conserve | Contains all components of native blood |
| 2. Fresh whole blood conserve | Like 1., transfusion within 72 hours |
| 3. Erythrocyte concentrate | Taken from whole blood conserves, the Buffy coat is not removed |
| 4. Leucocyte reduced concentrate | |
| 4.1 Leucocyte poor | $<1.2 \times 10^9$ leucocytes per unit |
| 4.2 Leucocyte free | $<0.1 \times 10^9$ leucocytes per unit |
| 5. Washed erythrocyte concentrate | Produced by washing with NaCl, content of plasma protein 0.5 to 1 g per dl |
| 6. Erythrocyte resuspension | The original volume of the conserve is restored with a resuspension solution |
| 7. Thrombocyte concentrate | Produced by using a cell separator, contamination with leucocytes is unavoidable |
| 8. Granulocyte concentrate | The use is extremely limited, contamination with other cells is possible |
| 9. Frozen fresh plasma | Substitution of coagulation factors, normally combined with erythrocyte concentrates |

**Schlüsselwörter:** Vaterschaft – Identität – Transfusion – DNA-Untersuchungen

## Introduction

The German guidelines for blood grouping experts advise, that under normal circumstances, there must be a period of 3 months between transfusion and blood sampling for serological testing to avoid difficulties in diagnosing and to avoid potentially incorrect results [16]. Occasionally cases occur after multiple transfusions where serological tests were necessary to determine identity or paternity and the only blood available is from the recipient. Depending on various factors, e.g. the patient's disease, many different preparations can be applied in practice (Table 1). The preparation techniques of these blood component conserves and the procedures and indications of their application are regulated on a national basis [16]. Stimulated by an actual case the effect of multiple transfusions on conventional systems and especially on DNA profiling was investigated.

## Case report

A 62-year-old man who died after intensive care was involved as putative father in a paternity case. Difficulties were caused by the relatives not only refusing blood samples but also keeping secret the whereabouts of the body. Thus a sample could only be taken immediately before the burial and since the body had been stored at room temperature putrefaction had already occurred. The hospital reports showed that transfusion therapy with a total of 27 erythrocyte concentrates had been carried out within the last 2 weeks before death. Conventional blood grouping led to inconclusive results in at least 5 out of 26 systems, but no exclusion was observed and the probability of paternity was 72.33%. Therefore DNA tests were carried out using the single locus probes MS 43a, 3'HVR, G3 and YNH24 and the multi locus probe 33.15. In all DNA systems no exclusions were found and no additional bands were present that could have indicated the presence of mixtures of DNA from different sources. A careful evaluation resulted in a probability of paternity of 99.9%. The results appeared to be conclusive,

**Table 2.** Serological findings in transfused patients ($n$ = 12) – red cell antigens and enzymes

| Time | MNSs | Rhesus | Kell | Duffy | Kidd | Co | acP | PGM1 | AK | GPT | GLO |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Patient No. 01 – Substitution: 21 rcc, 3 wbb* | | | | | | | | | | | |
| Ante | MNSs | cD.Ee | K– | a+b+ | a+b– | b– | BC | a1 | 1 | 2 | 2 |
| Post | | **C**cD.Ee | | | a+**b+** | | | | | 2–**1** | 2–**1** |
| 5 days | | c**D**.Ee | | | a+b– | | | | | 2 | 2 |
| *Patient No. 02 – Substitution: 2 rcc* | | | | | | | | | | | |
| Ante | MSs | cD.Ee | K– | a–b+ | a+b+ | b– | AB | a2a1 | 1 | 1 | 2 |
| Post | M**N**Ss | **C**cD.Ee | | **a+**b+ | | | | | | 2–**1** | |
| *Patient No. 03 – Substitution: 4 rcc* | | | | | | | | | | | |
| Ante | MNSs | CcD.Ee | K– | a–b+ | a–b+ | b– | B | a1 | 1 | 1 | 2–1 |
| Post | | | | a+b+ | a+b+ | | B**C** | | | 2–1 | |
| *Patient No. 04 – Substitution: 7 rcc* | | | | | | | | | | | |
| Ante | MNSs | CcD.Ee | K– | a+b+ | a+b+ | b– | AB | a1 | 1 | 1 | 2–1 |
| Post | | | K+ | | | | | | | | |
| *Patient No. 06 – Substitution: 25 rcc* | | | | | | | | | | | |
| Ante | MNss | CD.e | K– | a–b+ | a–b+ | b– | A | a1 | 1 | 2 | 2–1 |
| Post | MN**S**s | CD.**E**e | | **a+**b+ | **a+**b+ | | A**B** | **a2**a1 | **2–**1 | 2–**1** | |
| 12 days | MN**S**s | CD.**E**e | | **a+**b+ | **a+**b+ | | A**B** | a1 | **2–**1 | 2–**1** | |
| *Patient No. 07 – Substitution: 3 rcc* | | | | | | | | | | | |
| Ante | MNSS | CDe | K– | a–b+ | a+b+ | b– | BC | a2a1 | 1 | 1 | 2–1 |
| Post | MNS**s** | | | **a+**b+ | | **b+** | | | | | |
| *Patient No. 08 – Substitution: 1 rcc* | | | | | | | | | | | |
| Ante | MNSs | CcD.e | K– | a+b+ | a+b– | b– | AB | a2a1 | 2–1 | 2 | 2–1 |
| Post | | | | | | | | | | 2–1 | |
| *Patient No. 11 – Substitution: 4 rcc* | | | | | | | | | | | |
| Ante | MSS | CDe | K– | a–b+ | a+b– | b– | A | a4a1 | 1 | 2 | 1 |
| Post | M**N**S**s** | C**c**D**E**e | | **a+**b+ | a+**b+** | | A**B** | | | | 2–1 |

Ante: Before transfusion; post: after transfusion; details of time: days after operation; bold: changing results
rcc: Red cell concentrates; wbb: whole blood bottles

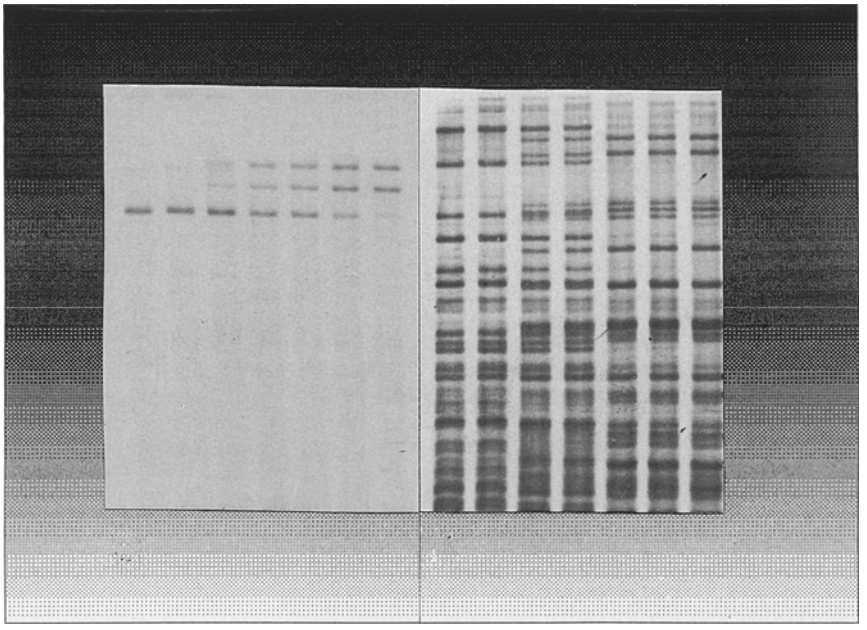

**Fig. 1.** In-vitro experiments on the content of DNA in Standard packed cells: Titration (vol. red cells: vol. blood) 10:0, 9:1, 7:3, 5:5, 3:7, 1:9, 0:10; a) NICE MS 43A, b) MZ 1.3 digoxigenin; Restriction enzyme *Hinf* I

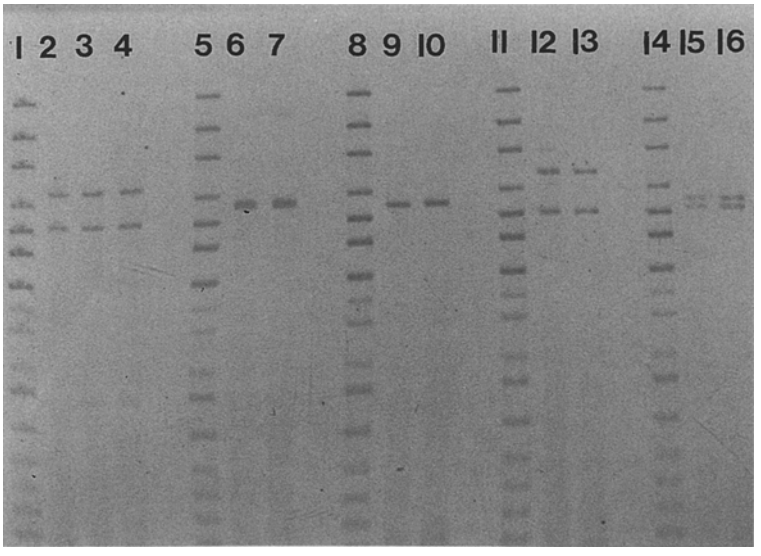


**Fig. 2.** DNA fingerprinting on blood samples from transfused patients ($n$ = 11; 1–5 red cell filtrated concentrates); probe NICE MS 43A; restriction enzyme Hinf I; from left to right: *Lane 1–4*: GIBCO BRL DNA analysis marker, patient 1 (4 concentrates) before, after transfusion, 4 days later; *Lane 5–7*: GIBCO BRL DNA analysis marker, patient 2 (5 concentrates) before and after transfusion; *Lane 8–10*: GIBCO BRL DNA analysis marker, patient 3 (4 concentrates) before and after transfusion; *Lane 11–13*: GIBCO BRL DNA analysis marker, patient 4 (2 concentrates) before and after transfusion; *Lane 14–16*: GIBCO BRL DNA analysis marker, patient 5 (2 concentrates) before and after transfusion

but further investigations and a survey of the literature was carried out because no publications were available about this specific medico-legal aspect of transfusion medicine.

## Material and methods

Blood samples were taken from 12 patients before surgery where the necessity of blood replacement was to be expected as well as after the transfusions and, if possible, a few days after the operation. The number and the type of transfusion were carefully recorded. For in-vitro simulation of a transfusion, standard packed erythrocytes and blood from the local blood bank were mixed together in different proportions before DNA testing. Erythrocyte concentrates were filtered (Erypur Prestomat or Erypurbedside-filter, Pall-RC 100) according to the manufacturers instructions and the DNA concentration was measured. In the 12 patients the serological tests in the conventional systems were carried out by routine laboratory methods. The DNA profiling was based on methods described previously [8]. The multilocus probe MZ 1.3 digoxigenin (Biotest) and the single locus probes pYNH 24 (Promega) and Nice MS 43A (Cellmark) were used with Hinf I as the restriction enzyme.

## Results

Of the 12 patients, 5 received only 1 or 2 units of red cell concentrates (patient Nos. 02, 05, 08, 09, 12). In the other cases between 3 and 25 red cell concentrates were transfused. Patient No. 01 received additionally 3 whole blood bottles.

*Conventional systems:*
*erythrocyte membrane- and iso-enzyme systems* (Table 2)

The testing in the ABO, Rh, MNS, Duffy, Kell, Pl, Kidd, Lu and Co systems gave different types due to transfusion in 7 cases (total number of changes 15). The investigation of 9 iso-enzyme systems (acP, PGM1, AK, ADA,

GPT, EsD, 6-PGD, GLO and PGP) showed additional bands after transfusion in 6 cases (total number of changes 15). In one case (patient No. 08) only an isolated change in the GPT system was observed. Three out of the 4 cases without changes in phenotype received only 1 or 2 units. In 3 patients the blood groups were determined again some days after the operation (patient No. 01, 06, 10). In case 01 the changes in blood groups were not permanent after 5 days. In patient No. 06 the patterns were changed directly after transfusion in 8 systems, 12 days later in only 7. In case 10 additional bands could not be observed neither immediately after transfusion nor 9 days later.

*DNA content in standard packed cells*

To demonstrate the influence of the DNA content of standard packed cells on the DNA pattern of the recipient, blood and standard packed cells were mixed together in different proportions. The DNA testing gave mixed patterns of the "donor" and the "recipient" of different intensity due to the mixing ratio (Fig. 1). In lane 6 a proportion similar to a transfusion of 2 units is demonstrated.

*DNA-profiling*

A test series ($n$ = 10) with the filtration system Optima bedside (Organon Teknika) confirmed that no DNA could be found in the filtered sample by routine methods after filtration. In the 12 patients investigated after transfusions no additional bands could be demonstrated using MZ 1.3, MS 43a and pYNH 24 (Fig. 2). This result was independent of the number of conserves given and in case No. 01 also the transfusion of 3 whole blood bottles.

## Discussion

For blood transfusion the donor and the recipient are only normally tested for compatibility in the ABO system and for the Rhesus factor D. In some cases Kidd and Kell/Cellano are also determined to avoid a possible immunization or delayed transfusion reactions. This means that there are uncertainties in evaluation with relatively high expectations of probabilities of exclusion and inclusion in forensic questions. Mixed results (characteristics of donor and recipient) can not necessarily be recognized as such. After transfusion of only a few units partial and total agglutinations should be discernible. But the various difficulties resulting from the quality of the antisera are known to the experienced examiner (so-called mixed field). In the present study the additional agglutinations found after transfusion were weak. In the isoenzyme systems additional bands with lower intensity may indicate mixed results, but in the PGM1 system for example, additional bands may be masked by the typical repeat bands. So a clear definition (patient or donor) would appear to be not entirely unproblematical.

In cases of multi-transfusions the conserves can be assumed to come from a variety of donors. The distribution of serological characteristics is also valid for the population of blood donors. Certain common characteristics may be transfused in a concentrated form and so additive characteristics may be relatively strongly expressed. But the results of the present study in the conventional systems indicate that there is no clear correlation between the number of transfused conserves and the quantity and quality of mixed results. Even after transfusion of 1 or 2 erythrocyte concentrates changes may be seen, while after massive transfusion there may be relatively minor serological changes. Certainly the time period between transfusion and taking the blood sample is another influencing factor. While leucocytes and thrombocytes are short-lived cells, erythrocytes can survive up to 3 months under storage conditions. After transfusion the survival time may be influenced by a faster decomposition of foreign erythrocytes due to incompatibility with the host organism. So in case No. 01 the serological changes observed after transfusion regressed totally up to the 5th day. In patient No. 06 a renewed consultation with the hospital proved that there had been additional transfusions before the last sampling.

Summing up the erythrocyte membrane and the isoenzyme systems it has to be concluded that the power of evidence of such results is very limited after a transfusion of whole blood preserves and all types of erythrocyte concentrates. After multitransfusion there can only be a limited evaluation even for homozygous results. In any particular case a specific discussion of single results would appear to be possible for inclusion in the expertise but is open to criticism. The contamination of blood conserves with leucocytes cannot be neglected. For forensic purposes this is especially important because leucocytes are the main source of possible DNA contamination of blood conserves and the number of leucocytes is directly related to the DNA content of the preparation. Höcker [6, 7] reported that the amount of leucocytes in standard erythrocyte concentrates is nearly the same as in blood. For washed erythrocyte concentrates there is a reduction only by a factor of 6. This corresponds with the results of the in vitro simulation of a standard packed cell transfusion (Fig. 1). To avoid non-haemolytical transfusion reactions and the formation of antibodies, new methods for the preparation of blood component conserves have been especially established to reduce the amount of leucocytes [2–5, 14, 17, 19, 20]. Due to new techniques in leucocyte filtration the absorption rate has reached nearly 100% [1, 4, 6, 7, 9–13, 15, 18, 21, 22]. The DNA profiling in the present study did not show any additionally bands after transfusion of up to 25 red cell concentrates. This shows that DNA profiling is the best method in cases of multi-transfused patients.

## Conclusions

1. When testing blood samples of transfused patients for identity or paternity all serological systems can be subject to change.
2. Due to modern methods in the preparation of blood conserves the amount of donor DNA in the conserves is so small, that in practice it does not have to be taken into account. Therefore DNA profiling is the method of choice.

3. In cases of deceased patients tissue specimens should be additionally sampled as control material for DNA profiling. In living persons roots of hairs and/or saliva samples are suitable.

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