The Influence of Infused Erythrocytes on the Detection of Individual Membrane-, Enzyme- and DNA- Systems

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SUMMARY

We report on a case of affiliation. The putative father has passed away before a blood sample having been taken . Serological examinations were complicated by the fact that the patient had been infused with concentrates of erythrocytes. In such cases not only the red cell markers but also DNA bands can be falsified. Most likely the use of single locus probes and determination of plasma protein polymorphisms may lead to conclusive results.

INTRODUCTION

In rarer cases of affiliation or identification serological examinations have to be taken on blood samples from individuals having been treated with blood transfusions. It is known that in such cases serological markers can be falsified (1,2,3,4). Usually the results can be verified by a second blood test (three months later). If the patient has passed away in the meantime such control examination is not possible. Determination of plasma protein polymorphisms and DNA fingerprinting promise the most meaningful results. But DNA patterns can also be falsified. Dependend on cleansing the donor's blood concentrates of erythrocytes can contain white blood cells.

MATERIAL & METHODS

Blood samples were taken from patients which had to undergo an operation with expected higher loss of blood. Sampling was carried out just before and immediately after operation. The in-vitro experiments were carried out on pints of stored blood.

CASUISTIC

A four-year-old girl wanted to be accepted as illegitimate child of a sixty-year-old man who had died some days before. Blood sampling was carried out in the cemetery. The corpse had attained an advanced status of putrefaction. Samples could be taken from the heart blood. It was known that the putative father had been treated with 27 concentrates of red cells over a period of two weeks. 20 conventional serological markers could be determined, biostatistical evaluation of them all led to W = 72.33 % (likelihood in accordance to Hummel). Separate evaluation of the not affected markers (ABO, Gm, Km, PI, D) resulted in W = 72,95 %. DNA fingerprinting was carried out with the single locus probes MS 43, 3'HVR, G3 and YNH 24. The definite results (no additional bands) led to likelihood of more than 99.9 %.

RESULTS AND DISCUSSION

Depending on the number of infusions and intervals of blood extraction pints of stored blood can influence serological examinations on the patients blood. For the examiner it is important to know what kind of stored blood had been infused. Today concentrates of red cells are prefered (except: heart-lung machine etc.!). Therefore plasma protein polymorphisms can unambigously be interpreted in such instances. Due to importance of compatibility the donor's red cell antigens have to agree with the patient's blood on ABO-, Kell-, Duffy-

Advances in Forensic Haemogenetics 4
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and Rhesus- (at least: D) system. The remaining red cell antigens and the iso enzymes have to be carefully interpreted. On what scale the serological blood pucture can change is shown in table 1. The five patients were treated with 2-24 transfusions and 0-5 additional markers were detected after operation. The number of alterations is surely dependend on frequency of the marker concerned. For example: patient 2 (frequency of the marker combination : 1 in 19267) can have alterations less likely than patient 4 (1 in 27780). The frequency of the marker M is about 80%. There is a good chance to get an additional marker M (patient 2) because most of the donors show this mark.
Conclusive evidential value is expected from genetic fingerprinting. But concentrated erythrocytes can contain white cells and therefore foreign DNA. Washed and unwashed concentrates are available. Furthermore the anaesthetist himself can carry out a bedside filtration on unwashed red cells. The content of DNA in unwashed concentrates seems to be relatively comparable to native blood (presumable above 50%). Fig.1 shows a titration mixture of native blood and concentrated red cells. Using multilocus probes it may become difficult to identify additional foreign bands. The same titration mixture is shown in fig.2. Using singlelocus probes the examiner can detect additional bands (except : homozygous). In practice he will recognize the foreign bands by different density, especially because of rarer use of unwashed concentrates.
Furthermore the survival time of foreign white cells is of importance. To our estimation it may be short. Examinations on these problems and the effect of bedside filtration of unwashed concentrates are already under way. We will report on the results as soon as possible.

CONCLUSIONS

In cases of blood transfusion it is of importance to know what kind and what number of transfusions has been carried out. Typing of plasma protein polymorphisms and the tranfusion-relevant red cell markers is a useful examination. Concentrates of erythrocytes (mostly used kind of transfusion) can contain DNA. Therefore one should give singlelocus probes preference. Foreign DNA bands can be detected considerably easier. However in practice this phenomen will take a secondary role. The chance of identical DNA bands is small. Foreign DNA will result in smear only. Principally the medical file has to be requested. Interpretation of the symptoms can be useful (immune reactions, neoformation of red and white cells).

marker	patient 0 14 r.c.c.		patient 1 21 r.c.c. 3 p.		patient 2 2 r.c.c.		patient 3 4 r.c.c.		patient 4 7 r.c.c.	
	ante	post	ante	post	ante	post	ante	post	ante	post
ABO	A1	A1A2	B	B	A1	A1	0	0	B	B
MNSs	Ns	MNSs	MNSs	MNSs	MSs	MNSs	MNSs	MNSs	MNSs	MNSs
Rhesus	CcDEe	CcDEe	cDEe	CcDEe	cDEe	CcDEe	CcDe	CcDe	CcDEe	CcDEe
Kell	K-	K-	K-	K-	K+	K+	K-	K-	K-	K-
Duffy	a+b-	a+b+	a+b+	a+b+	a-b+	a+b+	a-b+	a+b+	a+b+	a+b+
P	P-	P+	P+	P+	P+	P+	P+	P+	P+	P+
Kidd	a+b+	a+b+	a+b-	a+b+	a+b+	a+b+	a-b+	a+b+	a+b+	a+b+
Lutheran	a+	a+	a-	a-	a-	a-	a-	a-	a-	a-
Colton	b-	b-	b-	b-	b-	b-	b-	b-	b+	b+

r.c.c.: red cell concentrate p: pints of stored native blood

TAB.1 ALTERATIONS OF RED CELL MARKERS BY TRANSFUSION

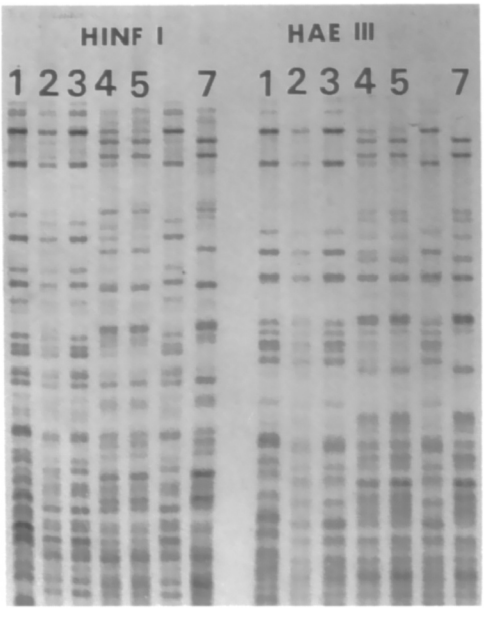

FIG.1 ALTERATION OF DNA BANDS BY TRANSFUSION - IN VITRO EXPERIMENTS , MZ 1.3
Left to right;
Blood : red cell concentrate
Lane 1: 10 to 0
Lane 2: 9 to 1
Lane 3: 7 to 3
Lane 4: 5 to 5
Lane 5: 3 to 7
Lane 7: 0 to 10

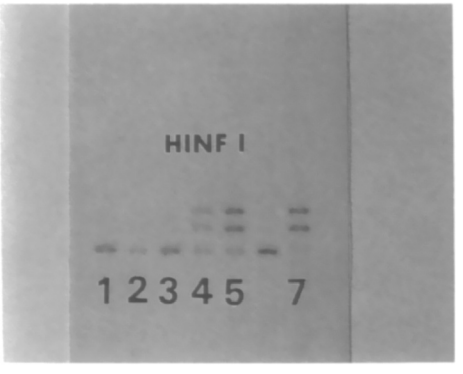

FIG.2 ALTERATION OF DNA BANDS BY TRANSFUSION - IN VITRO EXPERIMENTS , NICE MS43 A
Left to right;
Blood : red cell concentrate
Lane 1: 10 to 0
Lane 2: 9 to 1
Lane 3: 7 to 3
Lane 4: 5 to 5
Lane 5: 3 to 7
Lane 7: 0 to 10

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