ASSESSMENT OF D-NEGATIVE BLOOD SUPPLY FOR RHD*DAU-POSITIVE INDIVIDUALS

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Background
Variant RHD alleles and anti-D immunization are more prevalent in Africans as compared to Europeans. The aberrant RHD alleles of the DAU (in German: "D Afrikanischen Ursprungs") cluster are considered to be one of the major causes of RHD polymorphism in the African population, with DAU-0 (RHD*10.00) being the most prevalent one. As suggested by a relatively high RhD similarity index of 0.74 for DAU-0 (1), D-positive transfusions may be considered for DAU-0 carriers. All other members of the DAU cluster, e.g. DAU-1 to DAU-7 (RHD*10.01-07, Fig. 1) may behave like partial Ds and are prone to rise anti-D upon transfusion of RhD positive blood. In order to prevent unnecessary RhD negative transfusion to DAU positive individuals, both parental RHD-haplotypes and type of the DAU allele present need to be assessed.

Methods
17 DAU-positive (1136C>T, all DAU-positive individuals available at our institute) individuals have been identified using commercial kits or by high throughput genotyping based on MALDI-TOF mass spectrometry. All samples were analysed for the presence of "Rhesus boxes" and RhD null alleles using commercial kits. RhD-sequencing was carried out for exons 4 to 8, allowing identification of all known DAU alleles. The origin of all 17 samples was heterogenous: Eleven of the individuals were patients, nine of them were assigned to presumptive African ancestry based on other RHD alleles (e.g. RHD*03N.01) or blood group antigens (e.g. FY*02N.01). Another six samples were identified by high throughput genotyping of approximately 5,500 regular blood donors of various areas of Switzerland.

Results
Genetic frequency of RhD 1136C>T was roughly measured to be about 1 among 1,000 regular blood donors in the Zurich area of Switzerland ("Caucasians", allele frequency: 0.0006). 16 of 17 individuals (94%) with DAU positivity (1136C>T) could have been transfused with RhD positive blood, given the most prevalent DAU-0 genotype is considered to behave like a "regular" RhD positive. PCR-SSP analysis and sequencing of RH D exons 4 to 8 of all 17 samples revealed various RHD genotypes: Five samples were heterozygous DAU-0/RHD, three were DAU-0/RHd, and one was homozygous for DAU-0/DAU-0. Sequencing displayed DAU-0 (1136C>T), in heterozygous combination with DAU-3 (n=3), RHdNull (other than the deletional type, n=2), and partial RHd (n=1), respectively. Genotyping of the remaining four samples revealed DAU-0.1/RHD, DAU-5/RHD, DAU-3/RHD and DAU-3/Rhd genotypes, respectively (Fig. 2).

Conclusion
In most DAU positive cases it seems appropriate to transfuse RhD positive blood, provided the complete RHD genotype is extensively assessed. E.g. in the presented study, demand for RhD negative supply would have been reduced from 5 of 17 (29%) to 1 of 17 (6%) by the presented analysis in DAU positive individuals. However, some DAU proposita (e.g. DAU-3/Rhd, or homozygous non-DAU-0/non-DAU-0) still require RhD negative blood in order to prevent anti-RhD immunization. For decision making, besides genotyping of RHD zygosity status in DAU positive individuals, the second parental allele may sufficiently be assessed by excluding common SNPs of non-DAU-0 alleles in exon 5, 697G>C/A (DAU-4, -5), in exon 6, 835G>A (DAU-3, -7) and in exon 7, 998G>A (DAU-2, -6, -7) of the RHD gene by respective and yet to establish PCR-SSPs.

References