Title

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Journal

Transfusion, 59(10)

Authors

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Publication Date

2019-10-01

DOI

10.1111/trf.15479

Peer reviewed

HHS Public Access

Author manuscript

Transfusion. Author manuscript; available in PMC 2020 October 01.

Published in final edited form as:

Transfusion. 2019 October ; 59(10): 3228–3235. doi:10.1111/trf.15479.

Diversity of *RH* and transfusion support in Brazilian Sickle Cell Disease patients with unexplained Rh antibodies

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Abstract

Background: Genetic diversity in the *RH* genes among Sickle Cell Disease (SCD) patients is well described, but not yet extensively explored in populations of racially diverse origin. Transfusion support is complicated in patients who develop unexpected Rh antibodies. Our goal was to describe *RH* variation in a large cohort of Brazilian SCD patients exhibiting unexpected Rh antibodies (antibodies against RH antigens to which the patient is phenotypically positive) and to evaluate the impact of using the patient's *RH* genotype to guide transfusion support.

Methods and Materials: Patients within the Recipient Epidemiology and Evaluation Donor Study (REDS)-III Brazil SCD cohort with unexpected Rh antibodies were selected for study. *RHD* and *RHCE* exons and flanking introns were sequenced by targeted next generation sequencing.

Results: Fifty-four patients with 64 unexplained Rh antibodies were studied. The majority could not be definitively classified as auto or alloantibodies using serologic methods. The most common altered *RH* were: *RHD*DIIIa* and *RHD*DAR (RHD* locus) and *RHCE*ce48C, RHCE*ce733G and RHCE*ceS* (*RHCE* locus). In 53.1% of the cases (34/64), patients demonstrated only conventional alleles encoding the target antigen: 5/12 anti-D (41.7%), 10/12 anti-C (83.3%), 18/38 anti-e (47.4%) and 1/1 anti-E (100%).

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The authors declare no conflicts of interest

Conclusion: *RHD* variation in this SCD cohort differs from that reported for African-Americans, with increased prevalence of *RHD*DAR* and underrepresentation of the DAU cluster. Many unexplained Rh antibodies were found in patients with conventional RH allele(s) only. *RH* genotyping was useful to guide transfusion to determine which patients could potentially benefit from receiving *RH* genotyped donor units.

Introduction

Alloimmunization is a serious transfusion complication, as antibodies to RBC antigens can cause delays in the identification of compatible blood, hemolytic disease of the fetus/newborn and post-transfusion hemolytic reactions, which can be severe or even fatal. Patients with SCD are particularly prone to alloantibody development because of multiple factors including frequency of exposures and antigenic differences between blood donors and SCD recipients, reflecting race disparity between these two groups[1–3]. The background inflammatory pathophysiology of SCD may also contribute to increased alloimmunization, enhancing antigen-presentation and stimulating a B-cell response[4,5]. Prospective transfusion of antigen-matched RBC units before the development of alloantibodies is the most effective prophylaxis, reducing the risks of alloantibody formation and minimizing the occurrence of post-transfusion hemolytic reactions[6–8]. However, the occurrence of transfusions outside centers with a phenotypic matching policy, as well as the prevalence of *RHD* and *RHCE* variation among SCD patients and minority blood donors, are important factors contributing to prophylaxis failure[9].

The complexity and diversity of *RH* in SCD patients make the Rh phenotype difficult to define by routine serological methods. In recent reports, approximately 85% of patients with SCD treated with chronic transfusion therapy exhibit at least one RHD / RHCE altered allele and this variation at the *RH* locus also extends to blood donors of African descent[10,11]. Most transfusion protocols do not consider *RH* genetic variation at the beginning of the transfusion protocol, therefore alloimmunization events due to *RH* variants can occur despite serologic phenotype-matched transfusions[9,12].

While there are many reports in the literature outlining the benefits of prospectively transfusing patients with SCD who are negative for the most relevant RBC antigens (C,c,E,e; K; Jka, Jkb; Fya, Fyb; S,s) with antigen-negative donor units[2,7,8,13] , there is no consensus regarding the benefit of *RH* genotyping prior to initiating a transfusion protocol or of the feasibility of selecting *RH* variant-matched units when genetic variation is detected. The major problem is that the likelihood or risk of formation of an Rh antibody in individuals with altered Rh proteins when exposed to conventional Rh proteins is not precisely known. Significant resources could be needlessly used by providing *RH* genotype-matched units, which would be difficult to find, when the risk of alloimmunization is not clear[14].

Patients who present with unexplained or unexpected Rh antibodies pose a transfusion dilemma. Efforts to determine whether the antibody is an auto or alloantibody are often inconclusive as patients have been recently transfused. Our goal was to describe the diversity of *RH* alleles in a large, multi-center cohort of Brazilian SCD patients exhibiting unexpected Rh antibodies, defined here as antibodies against RH antigens to which the patient is

phenotypically positive, by direct sequencing of *RHD* and *RHCE* coding regions. A secondary goal was to develop a transfusion protocol for the patients with Rh antibodies that were associated with inheritance of *RH* genetic variants and those associated with conventional alleles that considers which patients would be predicted to benefit from the transfusion with *RH* genotype-matched units to optimize transfusion outcomes and potentially avoid further Rh alloimmunization or delayed hemolytic transfusion reactions.

Methods

Patient recruitment and sample selection

The details of the REDS-III Brazilian SCD cohort have been previously reported[15]. Patients were randomly selected to be eligible for the REDSIII cohort from the active SCD patient population (clinical visit within the last 3 years) at four transfusion centers in six cities in Brazil: HEMOPE (Recife); Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Instituto da Criança (São Paulo); HEMORIO (Rio de Janeiro) and HEMOMINAS (Belo Horizonte, Juiz de Fora and Montes Claros). Patients were enrolled from November 2013 to March 2015. A total of 2,793 patients with SCD were enrolled, and 2,272 (81%) had been transfused. RBC antibody and patient RBC phenotype data were extracted from each participating center's electronic blood bank records.

Fifty-four patients within the REDS-III cohort were selected for the present study based on having a history of unexplained Rh-antibodies, i.e., their RBCs are phenotypically positive for a Rh antigen (D, C, c, E, e) with plasma reactive antibodies identified to have the same specificity. The antibodies were classified as autoantibodies, alloantibodies or indeterminate based on the records of the immunohematology reference laboratories at each site. The antibody was classified as autoantibody if it reacted with the patients' own RBCs and if it had been either recovered in the eluate or removed by autoadsorption in the absence of recent transfusions (less than 120 days from the last transfusion). The classification of the antibodies as alloantibodies was based on the following criteria: negative direct antiglobulin test (DAT), negative auto-control and last transfusion more than 120 days apart from the serological investigation. The cases in which the serological investigation was inconclusive, especially due to recent transfusions, the antibodies were classified as indeterminate.

DNA extraction, PCR amplification and Next Generation Sequencing-based assay (NGS)

Genomic DNA was isolated from EDTA blood samples using the commercial QIAamp Genomic kit in the QIAsymphony equipment (Qiagen, Valencia, CA, USA), following manufacturer's instructions. Samples were *RHD* and *RHCE* genotyped by targeted next-generation sequencing (NGS) as previously reported[16]. All *RHD* and *RHCE* exons and flanking intron regions were amplified as described using gene-specific primers[17].

Library construction was performed as described[16]. In brief, amplicons were quantified, diluted and mixed into two equimolar pools (*RHD* and *RHCE*) for each sample. Pools were subjected to mechanical fragmentation and the fragments sizing 200-bp were selected. Ion Xpress Barcode adapters were added to each pool. Emulsion polymerase chain reaction and Massively Parallel DNA Sequencing were performed on the Ion Torrent Personal Genome

Machine (Ion Torrent, San Francisco, CA, USA) using Ion PGM 200 Sequencing Kit and Ion 318 Chip. Data analysis was performed by Ion Reporter Software version 5.0. For each RH gene, a custom workflow was created to mask the homologous RH gene to avoid ambiguous read mapping. Samples with a change in *RHD* were tested for *RHD* zygosity using allele-specific primers designed to detect the common *RHD* deletion[18]. In cases of possible r'S haplotype, allele specific amplification was performed to confirm the presence of the hybrid *RHD*DIIIa-CE(4–7)-D* allele.

Results

Overall cohort

There were 392 alloimmunized patients in the REDS-III cohort (14% of 2793). This included 269 patients who made a total of 348 RH antibodies (142 anti-E, 108 anti-C, 45 anti-e, 31 anti-D and 22 anti-c). Fifty-four patients were Rh antigen positive but demonstrated the corresponding Rh antibody in their plasma (n= 64 antibodies) and were included in the study. These included 12 patients whose RBCs were D+ with anti-D in the plasma (Table 1), 12 who typed C+ but anti-C was identified in the plasma and 1 who was c + with anti-c identified (Table 2), as well as 1 E+ patient with anti-E (Table 3) and 38 with RBCs typing e+ with anti-e plasma reactivity (Table 3). Of the included patients, 49 (90.7%) were HbSS, 3 (5.6%) were HbSC and 2 (3.7%) were HbSβ0.

Unexpected anti-D

In the 12 patients typing as D+ but presenting with unexpected anti-D, 8 different variant alleles were identified (Table 1): *RHD*DAR1, RHD*DAR2, RHD*DAR3*, *RHD*DIIIa* (n=2), *RHD*DVII*, *RHD*541T*, *RHD*ψ* and *RHD*DIVa*. The serological classification of the unexpected anti-D (alloantibody, autoantibody or indeterminate) determined by the referring hospital is also shown. Six of 12 individuals had *RHD* encoding known, or presumed based on serologic alloreactivity (*RHD*541T)*, partial D phenotypes explaining the production of anti-D. Five (41.7%) had only conventional alleles (homozygous or hemizygous), and one was homozygous with one conventional *RHD* and one allele encoding a partial D phenotype, *RHD*DVII* (Table 1). Of the *RHD* evaluated in these 12 samples, 8 encoded known partial D or variant D phenotypes and 6 conventional D. There were 4 deleted *RHD*, 1 *RHD*ψ* (not encoding D antigen), and 5 samples in which the *RHD* was either deleted or conventional and in *trans* to a normal *RHD*.

Altered RHCE alleles were also detected in most of the patients with unexpected anti-D (83.3%, 10/12) with many presumed *in cis* to altered *RHD* (Table 1) as has been previously reported. Altered RHCE alleles in descending order of frequency included: *RHCE*ce48C* (n=3), *RHCE*ceAR* (n=2), *RHCE*ce733G* (n=2), *RHCE*ce48C,733G* (n=1), *RHCE*ceTI* (n=1), *RHCE*ceS* (n=1), *RHCE*ce48C,733G,1006T* (n=1) and *RHCE*cE48C* (n=1). The presumed altered RH haplotypes in this subset of patients with anti-D were *RHD*DAR / RHCE*ceAR* (n=2), *RHD*DIIIa / RHCE*ceS* (n=1), *RHD*DIIIa / RHCE*733G* (n=1), *RHD*DIVa / RHCE*ceTI* (n=1) and *RHD*541T / RHCE*ce48C,733G,1006T* (n=1). One patient with *RHD*DVII / D* exhibited concurrent anti-e but had conventional *RHCE*Ce/ce*. .

Unexpected anti-C and anti-c

Twelve patients with C positive RBCs had anti-C in the plasma and one c+ patient had anti-c, as shown in Table 2 with the serological classification. Among the 12 with anti-C, 10 (83.3%) inherited one or more conventional *RHCE*Ce*, but for two the C+ RBC phenotype was due to inheritance of the hybrid allele encoding partial C antigen *(RHD*DIIIa-CE(4–7)-D)* with known risk for clinically significant allo anti-C[19]. In the 10 patients with anti-C, despite having conventional *RHCE*Ce*, 1 had altered *RHD* (**DUC2*) and 2 had altered *RHCE*ce* (**ceAG* and **ce733G)*. A total of 9 patients in this group also had anti-e, despite the presence of conventional *RHCE*ce* in six. The patient with anti-c had two altered *RHCE*ce* encoding partial c and partial e and associated with a hr^B- phenotype (*RHCE*ceS/ce733G*) and also had the hybrid *RHD*DIIIa-CE(4–7)-D* encoding partial C antigen.

Unexpected anti-e and anti-E

Of the 38 e+ individuals with unexpected anti-e shown in Table 3 along with the serologic classification, 17 (44.7%) exhibited only conventional *RHCE* (**Ce, *ce*, and/or **cE*), and 8 (21.1%) had one conventional *RHCE*ce in trans* to an altered allele including **ceS, *ce48C, *ce733G, *ceTI or *Ce122G*. In contrast, 12 of the 38 (31.6%) (Table 3, top) had either two altered *RHCE*ce* (n=4), or one altered *RHCE*ce* in *trans* to altered *RHCE*cE48C* (n=3), or to conventional *RHCE*cE* (n=2), or to *RHCE*Ce* (n=3). Eleven of the 38 (28.9%) patients with anti-e demonstrated additional unexplained antibodies noted in Tables 1 and 2. Of the 76 RHCE alleles in 38 patients with anti-e, 28 were altered RHCE alleles (36.8%) and 48 were conventional alleles (63.2%) (Table3). In three, anti-f (anti-ce) was suspected associated with genotypes *RHCE*Ce/ceAG* (n=1) and *RHCE*Ce/ce733G* (n=2). Only one patient had unexpected anti-E, but *RH* genotyping revealed conventional RHCE alleles, *RHCE*Ce/cE* (Table3).

In summary, in this patient population with unexplained Rh antibodies the variant RHCE alleles in descending order of frequency included: *RHCE*ce48C* (11/28), *RHCE*ce733G* (4/28), *RHCE*ce48C,733G* (3/28), *RHCE*ceS* (3/28), *RHCE*cE48C* (3/28), *RHCE*ceMO* (1/28), *RHCE*ceAG* (1/28), *RHCE*Ce122G* (1/28) and *RHCE*ceTI* (1/28).

Discussion

In this study, patients with unexpected Rh antibodies (defined as antibodies against RH antigens for which the patient RBCs type as positive) from a large cohort of Brazilian SCD patients were *RH* genotyped and demonstrated that: 1) The most common altered RH alleles were: *RHD*DIIIa* and *RHD*DAR (RHD* locus) and *RHCE*ce48C, RHCE*ce733G and RHCE*ceS* (*RHCE* locus); 2) Patients who had an unexpected Rh antibody identified in the plasma often had one or more conventional RH alleles encoding the antigen under evaluation (34/64, 53.1%), consistent with other reports[10,21,22]; and 3) the number of Rh-antibodies that could not be classified as auto or as alloantibodies by the referring laboratory using serologic methods was high (71.9%; 46/64), and 4) *RH* direct sequencing was effective in identifying *RH* variants in patients with unexpected Rh antibodies. The *RH* genotype information can be used to guide the transfusion support of this highly transfused population

when the clinical significance of the unexpected Rh antibody is unknown and the serologic investigation to determine allo or auto reactivity is inconclusive or uncertain.

The specific alleles associated with anti-D identified in our studied population of RH-alloimmunized Brazilian SCD patients differs somewhat from reports of *RH* diversity in SCD patients of different ethnic backgrounds. In this Brazilian cohort, *RHD*DAR* and *RHD*DIIIa* were the most common altered *RHD*, while the *DAU* allele cluster was underrepresented. In studies of SCD patients of African-American origin, the *DAU* allele cluster represents the largest proportion of *RHD* variation[10,20]. DAU alleles identified in previous SCD cohorts, such as *RHD*DAU0*, are not strongly associated with anti-D development, while *RHD*DIIIa* and *RHD*DAR* carriers are known to be at risk for clinically significant anti-D[14,21]. We compared the RH genotype results to that of previous Brazilian SCD and blood donor cohorts including both Rh-alloimmunized and non-Rh alloimmunized individuals[22–24]. Consistent with our data, *RHD*DAR* and *RHD*DIIIa* were prevalent among Rh-alloimmunized SCD patients, and *RHD*DAU* were not commonly found. When non-alloimmunized SCD patients and blood donors were studied, the higher frequency of *RHD*DAR* and the relatively lower frequency of *RHD*DAU* persisted. The *RHD* variation distribution identified in our present cohort reflects the Brazilian distribution of *RHD*.

The *RHCE* alleles identified in these patients with unexplained Rh antibodies were similar to those previously encountered in African-American and African-Caribbean SCD patients[10,20]. *RHCE*ce48C*, *RHCE*ce733G* and *RHCE*ceS* were the most prevalent altered alleles, either as compound heterozygotes or in *trans* to a conventional RHCE alleles. *RHD*DIIIa-CE(4–7)-D* in cis to *RHCE*ceS* (fS haplotype type 1) was also seen in 2/12 C+ patients with anti-C , similar to what was found in African-American and African-Caribbean patients who were RhC-alloimmunized[10,25,26]. As reported previously, *RHCE*Ce* is more common in Brazilian patients with SCD than in African American[10,27].

Transfusion for patients with unexplained Rh antibodies based on the RH genotype

A secondary goal was to consider how information on the RH genotype could inform a transfusion protocol for the patients with unexplained Rh antibodies (defined above) that also considers which patients might be better transfused with *RH* genotype-matched units if possible. In the present study, 6 anti-D and 2 anti-C (8/64, 12.5%) were confirmed by genotyping to be associated with clinically significant partial D phenotypes (DIIIa, DIVa, DAR) or with a partial C phenotype, with clear indication for transfusion with RhD negative or RhC negative units (or alternatively *RH* genotype-matched units), respectively, despite the observation that the serologic workup was indeterminate for anti-D associated with *RHD*DIVa* and for both samples with partial anti-C. Six anti-D and 10 anti-C, with indeterminate or auto reactivity patterns were in patients confirmed to have one or more conventional *RHD* or *RHCE*Ce* and therefore not predicted to be at risk for clinically significant allo anti-D or anti-C, respectively. The only unexpected anti-c, indeterminate by serologic investigation, was associated with inheritance of two altered *RHCE*ce* encoding a hr^B- phenotype with potential for clinically significant alloantibody production. Transfusion

with *RH*-genotype matched units would be indicated if decreased survival of c+ RBCs is observed (Table 2).

Anti-e identified in patients with e+ RBCs is not uncommon in this patient population. Among 38 samples with anti-e, one demonstrated allo-reactivity, 30 were indeterminate, and 7 were classified as auto-reactive. *RH* genotyping found only conventional alleles in 17, and one conventional allele in 14 and as such 31 of 38 are not predicted to be at risk for allo-anti-e. Genotyping confirmed that the anti-e with characteristics of an alloantibody was associated with partial e antigen encoded by *RHCE*ceMO/cE*, while anti-e associated with partial e encoded by *RHCE*ceS/cE* was indeterminate in serologic testing, and anti-e in two samples with partial e encoded by *RHCE*ce48C, 733/cE48C* were autoreactive. The inheritance of *RHCE*cE in trans* allows transfusion of e- donor units for these patients without risk for anti-E. The risk for anti-E in patients with *RHCE*cE48C* requires further study.

*RHCE*ce48C* is a common allele in this population and was identified in 23.7% of samples with anti-e. Two were homozygous *RHCE*ce48C/ce48C*, and one example each *in trans to *ce733G, *ce48C, 733G*, or **cE48C*. *RHCE*ce48C* encodes weak e antigen without evidence of epitope loss and, in the present cohort, most (77.8%) of the antibodies associated with *RHCE*ce48C* were indeterminant and could not be serologically classified as auto or alloantibodies. The clinical significance of anti-e identified in patients with this allele requires further study[28]. In all but 5 patients in this cohort, *RHCE*ce48C* was in *trans* to conventional *RHCE* and patients could be transfused with units homozygous for the conventional allele.

This study confirms the challenges in multiply transfused patients to determine if unexplained Rh antibodies are allo or autoantibodies and shows how RH genotyping can guide transfusion therapy. Serologic auto reactivity is often used as a surrogate to predict clinically significance. However, sufficient serum or plasma and pre-transfusion autologous RBCs are needed, but patient samples are almost always not adequate in volume to do multiple adsorptions and patients often have been recently transfused. Testing is time consuming, and laborious methods are required to separate transfused from patient cells and are often not successful. Complex adsorption studies are only available in high complexity reference laboratories, and results and interpretations can be subjective and subject to dilution of the antibody reactivity.

One limitation of the present study is that the data do not give information on the likelihood of alloimmunization associated with specific alleles as the numbers of specific RH genotypes are small and data regarding the frequency of the identified RH variant alleles in our non-immunized patient population was not collected. Determination of risk for alloimmunization would require a much larger longitudinal study with follow-up on outcomes on *RH* genotyped patients. The number of different RH allele combinations alone precludes any conclusions about likelihood of alloimmunization.

Future Perspectives

The strategy of identifying *RH* variants prior to the beginning of the transfusion protocol and prospectively providing *RH* genotype-matched transfusions would be anticipated to reduce Rh alloimmunization[9]. However, this strategy would require having enough donors and there are cost considerations associated with genotyping enough donors to meet the transfusion needs of recipients with Rh variant phenotypes. Additionally, the risk for an immune response to conventional Rh antigen in patients with altered alleles is not always known[14], especially for e antigen. Further studies are needed to define the immunogenicity and clinical relevance of antibodies associated with *RH* variation, realizing these may be patient- or episode- specific. Recently, Chou S et al.[28] have evaluated the feasibility of supplying a cohort of African-American SCD patients on chronic transfusion therapy with *RH* genotype-matched units and have demonstrated that providing *RH* genotype and K-matched units prophylactically for all transfusions would require 25% additional donations compared to serologic CEK-matching when units from donors of African ancestry are selected[28].

In conclusion, this study performed RH genotyping on Brazilian SCD patients with unexpected Rh antibodies. The majority of serological investigations performed in local reference laboratories were inconclusive as to allo or auto characteristics mainly due to the fact that the patients were heavily transfused, a not uncommon occurrence and a significant confounding factor when attempting to select units for transfusion. The majority of the Rh antibodies identified in the studied cohort was associated with inheritance of only conventional RH alleles or were associated with inheritance of one conventional RH allele. These patients are not predicted to be at risk for clinically significant allo reactivity and can be transfused with donor units matched to their conventional RH allele(s). A small number of unexpected Rh antibodies were associated with inheritance of RH variants known to encode partial Rh antigens of clinical significance (D, C) and potential significance (hr^B). Although more studies are needed including clinical outcomes to determine the significance for transfusion associated with inheritance of some RH variant alleles with the selection of units based on RH genotyping, we show here that *RHD* and *RHCE* genotyping is helpful to begin to develop transfusion protocols and allocate use of *RH* genotyped unit resources.

Acknowledgements

This work was supported by Grant #**2014/50250–6**, São Paulo Research Foundation (FAPESP) and NHLBI Recipient and Evaluation Donor Study (REDS)-III. CMW is supported by the Doris Duke Innovations in Clinical Research Award 2015133

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Table 1.

RH genotyping results of patients with SCD exhibiting unexpected anti-D

Antibody Specificity	**Concurrent Antibodies**	***RH* genotype**				**Serological classification of the Rh-antibody**
		RHD		***RHCE***		
D+ patients without conventional *RHD*[#]		Allele1	Allele2	Allele1	Allele2	
(n=6)		*RHD***DAR1.02*	deleted *RHD*	*RHCE***ceAR*	*RHCE***ce*	*Auto anti-D*
		*RHD***DIVa*	deleted *RHD*	*RHCE***ceTI*	*RHCE***ce*	*Indeterminate*
		*RHD***541T**	deleted *RHD*	*RHCE***ce48C,733G,1006T*	*RHCE***ce*	*Allo anti-D*
		*RHD***DAR2.00*	*RHD***DAR3.01*	*RHCE***ceAR*	*RHCE***ce*	*Allo anti-D*[@]
		*RHD***DIIIa*	deleted *RHD*	*RHCE***ceS*	*RHCE***ce*	*Allo anti-D*
		*RHD***DIIIa*	*RHD** Ψ	*RHCE***ce733G*	*RHCE***ce*	*Allo anti-D*
D+ patients with one or more conventional *RHD*						
(n=6)	anti-e	*RHD***DVII*	*RHD*	*RHCE***Ce*	*RHCE***ce*	*Indeterminate*
		RHD	*RHD* or deleted D[ǂ]	*RHCE***Ce*	*RHCE***ce*	*Indeterminate*
		RHD	*RHD* or deleted D[ǂ]	*RHCE***ce48C*	*RHCE***cE*	*Indeterminate*
		RHD	*RHD* or deleted D[ǂ]	*RHCE***ce48C*	*RHCE***cE48C*	*Indeterminate*
		RHD	*RHD* or deleted D[ǂ]	*RHCE***ce48C*	*RHCE***ce48C,733G*	*Indeterminate*
		RHD	*RHD* or deleted *D*[ǂ]	*RHCE***ce*	*RHCE***ce733G*	*Auto anti-D*

[#] Use of RhD negative blood for transfusion indicated.

* Allele has not been described previously.

[@] adsorption of the antibody with *RHD*DAR3.01* RBCs was not possible.

[ǂ] *RHD* zygosity was not performed in samples with no alterations on *RHD* genotyping as the presumed phenotype would not change.

Transfusion. Author manuscript; available in PMC 2020 October 01.

Table2.

RH genotyping results of patients with SCD exhibiting unexpected anti-C or anti-c.

Antibody Specificity	Concurrent Antibodies	Number of Samples	*RH* genotype				Serological classification of the Rh-antibody
			RHCE		*RHD*		
C+ patients with anti-C without conventional *RHCE*Ce*[#]			Allele1	Allele2	Allele1	Allele2	
(n=2)	anti-e	1	*RHCE*ceS*	*RHCE*ce*	*RHD*DIIIa-CE(4–7)-D*	*RHD*	*Indeterminate*
	anti-e	1	*RHCE*ceS*	*RHCE*cE*	*RHD*DIIIa-CE(4–7)-D*	*RHD*	*Indeterminate*
C+ patients with anti-C with one conventional *RHCE*Ce*							
(n=10)	anti-e	5	*RHCE*Ce*	*RHCE*ce*	Without mutation		*Auto anti-C (1) Indeterminate (4)*
		2	*RHCE*Ce*	*RHCE*ce*	Without mutation		*Indeterminate (2)*
	anti-e	1	*RHCE*Ce*	*RHCE*cE*	*RHD*DUC2*	*RHD*	*Indeterminate*
		1	*RHCE*Ce*	*RHCE*ceAG*	Without mutation		*Auto anti-C*
	anti-e	1	*RHCE*Ce*	*RHCE*ce733G*	Without mutation		*Auto anti-C*
c+ patients with anti-c without conventional *RHCE*ce*							
(n=1)		1	*RHCE*ceS*	*RHCE*ce733G*	*RHD*DIIIa-CE(4–7)-D*	*RHD*	*Indeterminate*

The numbers in parenthesis represent the samples with the described classification.

[#]Use of C negative blood for transfusion indicated.

Table 3.

RH genotyping results of patients with SCD exhibiting unexpected anti-e or anti-E.

Antibody Specificity	**Concurrent Antibodies**	**Number of samples**	***RH* genotype**				**Serological classification of the Rh-antibody**
			RHCE		***RHD***		
e+ patients with anti-e without conventional *RHCE*ce*			Allele1	Allele2	Allele2	Allele2	
(n=12)		2[@]	*RHCE*ce48C*	*RHCE*ce48C*	Without mutation		*Indeterminate (2)*
		1[@]	*RHCE*ce48C*	*RHCE*ce733G*	Without mutation		*Auto anti-e*
		1[@]	*RHCE*ce48C*	*RHCE*ce48C,733G*	Without mutation		*Indeterminate*
		1[%]	*RHCE*ce48C*	*RHCE*cE48C*	Without mutation		*Indeterminate*
		2[%#]	*RHCE*ce48C,733G*	*RHCE*cE48C*	Without mutation		*Auto anti-e (2)*
		1[#]	*RHCE*ceMO*	*RHCE*cE*	Without mutation		*Allo anti-e*
	anti-C	1[#]	*RHCE*ceS*	*RHCE*cE*	*RHD*DIIIa-CE(4–7)-D*	*RHD*	*Indeterminate*
		1	*RHCE*Ce*	*RHCE*ceAG*	Without mutation		*Indeterminate*
	anti-C	1	*RHCE*Ce*	*RHCE*ce733G*	Without mutation		*Auto anti-e*
		1	*RHCE*Ce*	*RHCE*ce733G*	Without mutation		*Indeterminate*
e+ patients with anti-e and one conventional *RHCE*ce* or with *RHCE*Ce* either homozygous or heterozygote to *RHCE*cE*							
(n=26)		2	*RHCE*Ce*	*RHCE*Ce*	Without mutation		*Auto anti-e (1)* *Indeterminate (1)*
	anti-C	5	*RHCE*Ce*	*RHCE*ce*	Without mutation		*Auto anti-e (1)* *Indeterminate (4)*
		3	*RHCE*Ce*	*RHCE*ce*	Without mutation		*Indeterminate (3)*
	anti-D	1	*RHCE*Ce*	*RHCE*ce*	*RHD*DVII*	*RHD*	*Indeterminate*
		2	*RHCE*Ce*	*RHCE*cE*	Without mutation		*Indeterminate (2)*
		1	*RHCE*Ce*	*RHCE*cE*	Without mutation		*Indeterminate*
	anti-C	1	*RHCE*Ce*	*RHCE*cE*	*RHD*DUC2*	*RHD*	*Indeterminate*
		1	*RHCE*ce*	*RHCE*ce*	Without mutation		*Indeterminate*

Antibody Specificity	Concurrent Antibodies	Number of samples	*RH* genotype *RHCE*		*RHD*		Serological classification of the Rh-antibody
		1	*RHCE*ce*	*RHCE*cE*	Without mutation		*Indeterminate*
		1	*RHCE*ce48C*	*RHCE*ce*	*RHD*DAU3*	*deleted D*	*Indeterminate*
		3	*RHCE*ce48C*	*RHCE*ce*	Without mutation		*Auto anti-e(1) Indeterminate (2)*
		1	*RHCE*Ce122G*	*RHCE*ce*	*RHD*DAR3.01*	*deleted D*	*Auto anti-e*
	anti-C	1	*RHCE*ceS*	*RHCE*ce*	*RHD*DIIIa-CE(4–7)-D*	*RHD*	*Indeterminate*
		1	*RHCE*ceS*	*RHCE*ce*	*RHD*DIIIa-CE(4–7)-D*	*deleted D*	*Indeterminate*
		1	*RHCE*ceTI*	*RHCE*ce*	Without mutation		*Indeterminate*
		1	*RHCE*ce733G*	*RHCE*ce*	Without mutation		*Indeterminate*
E+ patient with anti-E (n=1)		1	*RHCE*cE*	*RHCE*Ce*	Without mutation		*Indeterminate*

[@] At risk for allo anti-E if transfused with e-; *RH* genotype-matching for transfusion may be required if decreased survival of e+ transfused RBCs is observed

[%] *Possibly* at risk for allo anti-E if transfused with e-; *RH* genotype-matching for transfusion may be required if decreased survival of E+ or e+ transfused RBCs is observed.

[#] Use of e- blood for transfusion indicated; predicted to have hr^{B}- phenotype.