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Sub-Saharan African patients and blood donors in Flanders (Belgium): can they benefit from optimization of our transfusion policy?

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INTRODUCTION

People originating from sub-Saharan Africa (SS-Africa) are known to have more variant red blood cell (RBC) alleles.

The complexity is often underexplored in patients as well as donors.

This may result in an increased alloimmunisation risk, due to lack of information on the absence of high prevalence antigens in patients and the presence of low prevalence antigens in donors.

AIM

Improve RBC concentrate (RCC) selection for patients with hemoglobinopathies (such as sickle cell disease (SCD)) originating from SS-Africa by genotyping RHCE:

Importance of low (V RH:10, VS RH:20) and high (hrB RH:31, hrS RH:19) prevalence antigens.

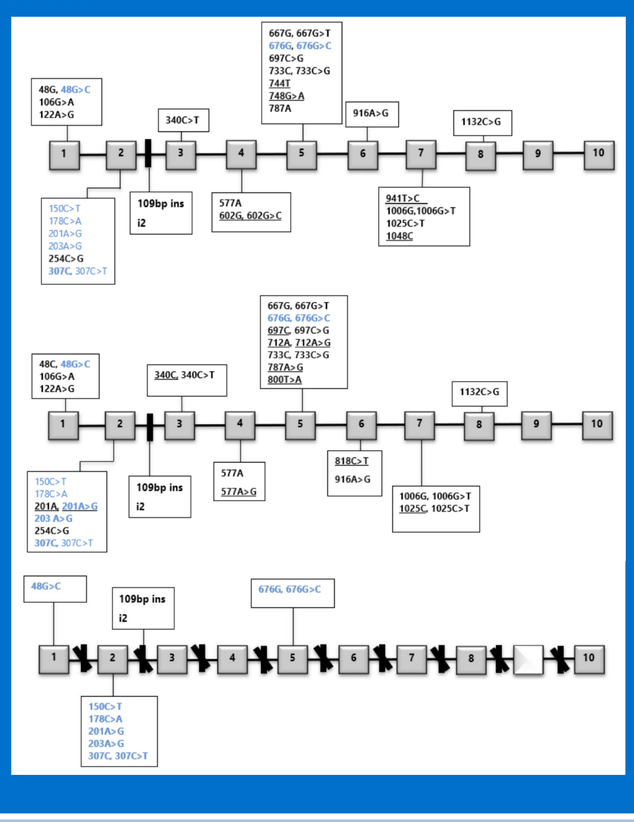
Key questions:

1) **Which RHCE variants do we need to discriminate?**
2) **Which test do we need to tackle this?**
3) **Is SNP testing sufficient or do we need NGS?**

METHODS

Cohort: SCD from SS-Afrika, proxy FYGATA

3 methods:



RESULTS

1) The relative frequency of the RHCE variants in our cohort is comparable to frequencies found in the studies of Chou et al, 2018 and Chang et al, 2020. The variants with a prevalence > 0,1% are considered relevant to discriminate, Figure 1.

45% had one RHCE variant allele and 32% was compound heterozygous.

RHCE*01.20.01, RHCE*01.20.03 and RHCE*01.20.09 comprise more than 30% of the total number of variants found in our cohort, Figure 1.
These alleles encode

- the extra low prevalence V (RH:10) and/or VS (RH:20) antigens, important to identify in donors.
- weak to negative high prevalence hrB (RH:31) antigen, which could lead to patient alloimmunisation.

These data illustrate the need to genotype both donors and patients originating from the SS-Africa region.

RHCE alleles		CHANG *et al* 2020 n= 884 %	CHOU *et al.* 2018 n= 587 %	Flemish cohort n = 43 %	Present as ambiguity
RHCE*01	ce	20,1	27,3	19,4	Yes
RHCE*01.20.01	ceVS.01	13,9	14,3	16,7	Yes
RHCE*02	Ce	11,7	13,6	13,9	Yes
RHCE*01.01	ce.01	22,7	19,3	11,1	Yes
RHCE*03	cE	10,2	10,0	6,9	Yes
RHCE*01.20.03	ceS	4,5	3,3	6,9	Yes
RHCE*01.06.01	ceAG	4,1	4,3	4,2	Yes
RHCE*01.20.09	ceVS.09	2,7	0,0	4,2	
RHCE*01.04.01	ceAR	0,06	0,3	4,2	
RHCE*01.02.01	ce.01.02	3	1,8	2,8	
RHCE*04	CE	0,11	0,0	2,8	Yes
RHCE*02:10	CeRN	0,0	0,2	2,8	
RHCE*03.04	cEIV	0,17	0,0	1,4	
RHCE*03.18	cE.18	0.0	0,0	1,4	Yes
RHCE*02:09	CeCX	0,0	0,0	1,4	
RHCE*01.20.02	ceVS.02	3,1	2,7	0,0	Yes
RHCE*01.07.01	ceMO	1,1	1,4	0,0	
RHCE*01.08	ceBI	0,2	0,3	0,0	
RHCE*01.20.06	ceCF	0,2	0,1	0,0	
RHCE*01.05.01	ceEK	0,17	0,0	0,0	
RHCE*01.20.05	ceVS.05	0,17	0,0	0,0	Yes
RHCE*01.03		0,06	0,0	0,0	
RHCE*01.20.04.02	ceTI type 2	0,06	0,1	0,0	Yes
RHCE*01.20.07	ceJAL	0,06	0,2	0,0	
RHCE*02.08.01	CeCW	0,06	0,0	0,0	
RHCE*02.22	Ce.22	0,06	0,0	0,0	
RHCE*02.30	Ce.30	0,06	0,0	0,0	

Figure 1: Frequencies of the RHCE alleles in the Flemish population.

Using the LinkSēq™ SNP test 30% of the results were ambiguous. In 20% it was not possible to identify the expression status of the relevant RHCE antigens CcEe and hrB: + / partial / weak / neg, Figure 2.

2) In 80% of all samples in our cohort SNP testing was sufficient to resolve the expression status of the RHCE antigens C, c, E, e, hrB (RH:31), hrS (RH:19), V (RH:10) and VS (RH:20).

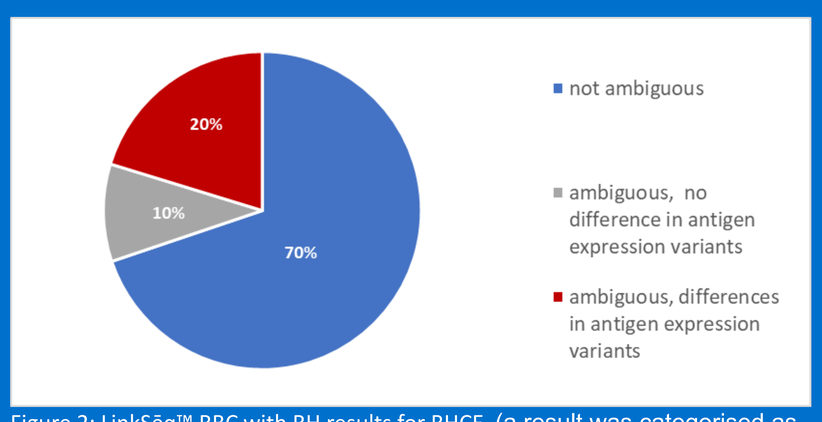


Figure 2: LinkSēq™ RBC with RH results for RHCE. (a result was categorised as ambiguous when 2 alleles with a prevalence > 0,001 found are possible).

CDE eXtend™ results were concordant with LinkSēq™, with a slightly higher ambiguity rate: 36% (missing SNP 941T>C = in development), Figure 3.

22 samples of our cohort with diverse RHCE variants were sequenced using the NGS CORE™ kit, inno-train, alpha trial, in development.

- 36% of the RHCE results (n=8) failed due allelic imbalances, crosstalk.
- The remaning 64% (n=14) confirmed the SNP test results.
- There was one sample where an extra SNP was found, that was not detected by both SNP tests.
- In only 2 samples NGS was able to resolve an ambiguity present in the SNP tests.

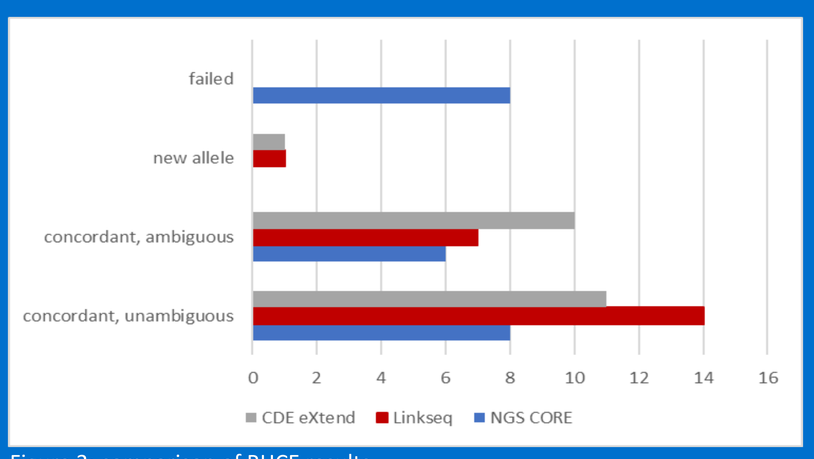


Figure 3: comparison of RHCE results

3) In this small cohort exon based NGS offered a limited added value for the identification of RHCE variants. The benefit of this NGS approach is the possibility of accurate high throughput sequencing for many different bloodgroup systems at once (NGS CORE, inno-train data not shown).

CONCLUSIONS

The prevalence of RHCE variants, as well as variants of other blood group systems (data not shown) is high in patients and donors in the Flemish SCD cohort originating from SS-Africa.

The most frequent RHCE variant alleles identified have different implications being present in a donor or in a patient, emphasising the need of extensive genotyping to realize the best donor-patient RHCE match.

Today extensive RHCE SNP testing allows to discriminate between the most relevant RHCE alleles and antigen variants.

In future, exon based NGS sequencing could offer advantages in a high throughput setting to identify an array of variants in multiple blood group systems. However, to resolve all phasis ambiguities, only a long read NGS approach could offer a solution.

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Belgian Red Cross

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CONTACT INFORMATION

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