

A Systematic Review and Meta-Analysis of Non-Invasive Prenatal Diagnosis (NIPD) of Sickle Cell Disease (SCD)

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Abstract: *Introduction:* Sickle cell disease (SCD) is a genetically inherited, recessive mutation of the haemoglobin β S-gene. Each year, over 300,000 babies are born with SCD, which will have a significant impact on their quality of life and average life expectancy. Currently, for SCD to be tested prenatally, foetal DNA is extracted by amniocentesis, chorionic villus sampling or cordocentesis, and then analysed by polymerase chain reaction (PCR), for instance. These procedures increase the risk of foetal miscarriage by less than 0.5%. SCD may, however, be tested non-invasively using cell-free foetal DNA (cffDNA), which is extracted from maternal blood plasma. In this study, the current accuracy of using cffDNA testing for non-invasive prenatal diagnosis (NIPD) of SCD will be shown.

Methods: Using databases such as PubMed, Web of Science and Scopus, this study systematically reviewed existing studies pertaining to the use of cffDNA maternal blood samples for non-invasive prenatal testing (NIPT) or diagnosis (NIPD) for SCD in patients who were at risk of having a baby with SCD. The data collected from the systematic review of the studies was statistically analysed in the form of a meta-analysis, describing the proportion of correct diagnosis results for this method of prenatal testing.

Results: Of over 3,600 papers identified from the database searches, only five studies contained data pertaining to the use of cffDNA for prenatal testing of SCD and conformed to the inclusion criteria set out by this study. Collectively, these data showed an average of 81.30% accuracy of diagnosis when using cffDNA to test for SCD, with 18.70% of fetuses incorrectly diagnosed. These data were compiled as a Forest Plot meta-analysis.

Conclusion: CffDNA for non-invasive prenatal SCD diagnosis appears to have the potential to be an accurate technique for the testing of this genetic disease, despite not currently indicating a proportion of correct diagnosis results which would encourage the technique for clinical implementation. Whilst there are currently very limited data on the use of this technique for the specific testing of SCD, there is great opportunity for further research into the standardisation and clinical application of this procedure.

Keywords: Non-invasive, Prenatal, Diagnosis, Testing, Sickle cell disease.

INTRODUCTION

SCD is defined as a hereditary (autosomal recessive monogenic) haemoglobinopathy, which includes sickle cell anaemia (HbSS disease) and various compound heterozygous genotypes, for example, sickle cell HbSC disease or sickle cell β -thalassaemia disease (HbS β thal) characterised by chronic haemolytic anaemia and vaso-occlusive complications. It is globally amongst the most common genetic disorders, affecting approximately 30 million people [1-3]. SCD is associated with high lifetime morbidity and premature mortality, as described in the most recent Global Burden of Disease study. Approximately 7% of the world's populations are

healthy carriers of haemoglobinopathies, resulting in 300,000 newborns severely affected with SCD annually [4].

In South East London, close to 0.3% of newborns are affected by SCD, whereas the average in the United Kingdom is 0.05%, with SCD being the most common reason for invasive prenatal diagnostic testing, reporting approximately 350 cases each year [2, 5, 6].

Due to the severity of this disease and the limited treatment options, prenatal diagnosis by invasive testing is offered in many countries as part of a national prevention programme, with a procedure-related risk of miscarriage being reported as 0.05% for chorionic villus sampling and <0.5% for amniocentesis [5, 7, 8], which are the current gold standards for prenatal testing, and are approximately 100% accurate [7].

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The discovery of cffDNA in maternal blood circulation has led to the possibility of NIPT or NIPD mitigating the invasive procedure-related risk of miscarriage [5, 7]. This discovery has been applied successfully for RhD genotype, trisomy 21, 13 and 18 detection, as well as foetal sex determination (reporting 96.6% sensitivity and 98.6% specificity using this technique) [9, 10]. CffDNA is detectable very early during pregnancy, with the mean quantity of cffDNA during the first and second trimesters being approximately 10% of the total amount of cell-free DNA [2]. SCD may be tested non-invasively, therefore, patients may favour this technique as, apart from being safe, it is also accurate [2, 11]. This systematic manuscript review was undertaken to ascertain the available data to determine whether using cffDNA for NIPD of SCD has a sensitivity and specificity that can match the gold standard and could, therefore, potentially be used in a clinical setting.

METHODS

Search Strategy

A systematic review and meta-analysis was performed whilst adhering to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis guideline and Meta-Analysis of Observational Studies in Epidemiology criteria [12] and SEDATE guidelines [13]. This systematic review was registered a priori (Reference Number: CRD42016037239). The following databases were searched for previous work undertaken between January 1995 and April 2016. The databases researched were PubMed, Cochrane Library, Web of Science, Scopus, Google Scholar, Science Direct and Ovid (Limitations: research, reviews, author manuscripts); the search criteria are presented in Table 1. Abstracts and full text articles

were assessed by two authors (LS and EON) in order to select the articles that met the inclusion criteria. Further detailed investigations of the relevant original and review articles were undertaken to identify applicable studies. There were no language restrictions.

Study Selection

The studies incorporated in the systematic review met the following criteria:

- The study design was a cohort study;
- The exposure of interest was pregnant women at risk of having a baby with SCD or sickle cell trait;
- The studies contained specific data on the genetic testing of cffDNA from maternal plasma or serum, as part of an on-going study;
- The studies performed a data comparison to the gold standard, or tested for the genotype at birth;
- The selected studies describe results gained from each study compared to a control group, which presents as patients who are not at risk of having a SCD or sickle cell trait baby [14, 15].

Reviews, editorials, non-human studies, and published letters without data were excluded. No studies including data pertaining to twin studies using cffDNA for NIPD of SCD were included.

Study Characteristics

Study Outcome

The aim of this study was to perform a systematic review and subsequent meta-analysis on NIPD of SCD. All publications retrieved as a result of the search

Table 1: Key Phrases used for the Sophisticated Search of the Systematic Review, Determined by the Inclusion Criteria Presented in Study Selection Below

"Sickle cell disease" and "foetal testing"
"Sickle cell disease" and "prenatal" and "diagnosis" and "foetal blood"
"Sickle cell disease" or "sickle cell anaemia" and "prenatal" and "diagnosis" and "non-invasive"
"Sickle cell disease" and "prenatal" and "diagnosis" and "free foetal DNA"
"Sickle cell disease" and "prenatal" and "diagnosis" and "non-invasive" and "free foetal DNA"
"Sickle cell disease" and "detection" and "non-invasive" and "prenatal" and "cell-free DNA"
"Sickle cell disease" or "sickle cell anaemia" and "antenatal" and "diagnostic" and "minimally invasive" and "free foetal DNA"
"Sickle cell disease" and "prenatal" and "haemoglobinopathy" and "diagnosis" and "non-invasive"

strategies, previously outlined in the Study Selection, were systematically appraised to compile a list of manuscripts containing research data on the use of cffDNA for NIPD of SCD. A meta-analysis was then performed on the data retrieved from the final selection of manuscripts to determine the proportion of correct diagnosis results. Finally, a conclusion was drawn as to whether the level of accuracy and associated risk to the mother and foetus from tests using cffDNA technique should be recommended for clinical use, with regards to NIPD of SCD [15,16].

Intervention

The intervention for each of the studies analysed in this review was the technique for prenatal non-invasive SCD diagnosis, in the form of cffDNA, which was extracted from a maternal blood sample and tested for the sickle cell mutation, for example, by PCR [2, 15, 16].

Comparator

For each of the selected studies used in the meta-analysis, the result of the diagnosis using cffDNA was compared to the gold standard of testing, being either amniocentesis or chorionic villus sampling. In some cases, genetic testing to confirm foetal genotype was assessed at birth, as a secondary determination [16].

Study Design

The studies included in the meta-analysis were prospective cohort studies, therefore, the specific data on the genetic testing of cffDNA from maternal plasma or serum were collected as part of an on-going study, allowing for PCRs or single nucleotide polymorphism (SNP) analysis to be performed at the time of blood sampling. This was then followed up by comparison to the gold standard, or by testing of the genotype at birth. The results gained from each study were compared to a control group, which was presented as pregnant patients who were not at risk of having a SCD or sickle cell trait baby [14, 15].

Study Population

The study population selected for this systematic review were pregnant women, of whom either themselves, the father of the foetus, or both parents were a SCD carrier or SCD patient [14, 16, 17].

Data Extraction

Due to the limited appropriate data retrieved from the systematic review, those papers which contained

usable and relevant data to NIPD for SCD, whilst conforming to the restrictions outlined in Study Selection, were included in the meta-analysis. A total of 3,631 papers were identified and screened following the search stage during the systematic review, to which only five papers could be applied to the Newcastle-Ottawa Quality Assessment Scale (see Figure 1), as well as the QUADAS-II quality assessment guideline [18] (see Table 2), and, therefore, were included in the meta-analysis. The five studies described the relevant data retrieved from a total of 139 pregnant patients who were at risk of having a baby with SCD.

Quality Assessment

The manuscripts included in the systematic review were subjected to quality assessment scoring using the Newcastle-Ottawa Quality Assessment Scale for cohort studies [19] and the QUADAS-II assessment table, to determine the potential for any bias within the studies which were to be included in the meta-analysis. Those manuscripts to which the Newcastle-Ottawa Quality Assessment Scale could not be applied, due to the absence of relevant data, were excluded from the meta-analysis. Four papers and one abstract of a paper to which the quality assessment scale could be applied were used in the final data extraction stage. These manuscripts were studies by Cheung *et al.*, (1996) [20], Barrett *et al.*, (2012) [2], Phylipsen *et al.*, (2012) [21], Fielding *et al.*, (2013) [22] and Yenilmez *et al.*, (2013) [23]. The papers were scored as low, medium or high quality according to the QUADAS-II assessment table, and out of 8* according to the Newcastle-Ottawa Quality Assessment Scale, by their relevance to the answers of questions under three subheadings:

- Selection, whereby the study representatives, selection of participants, ascertainment and demonstration of exposure are determined;
- Comparability, whereby the use and propriety of a control group is assessed;
- Study Outcome, whereby the assessment of outcome, longevity of the study and follow up of the study are assessed.

The study with the highest quality score was that by Phylipsen *et al.*, (2013) [21], with 8*/8* (Medium QUADAS-II score). The study with the lowest score was the abstract of a paper by Fielding *et al.*, (2013) [22] with 3*/8* (Low QUADAS-II score), as the abstract

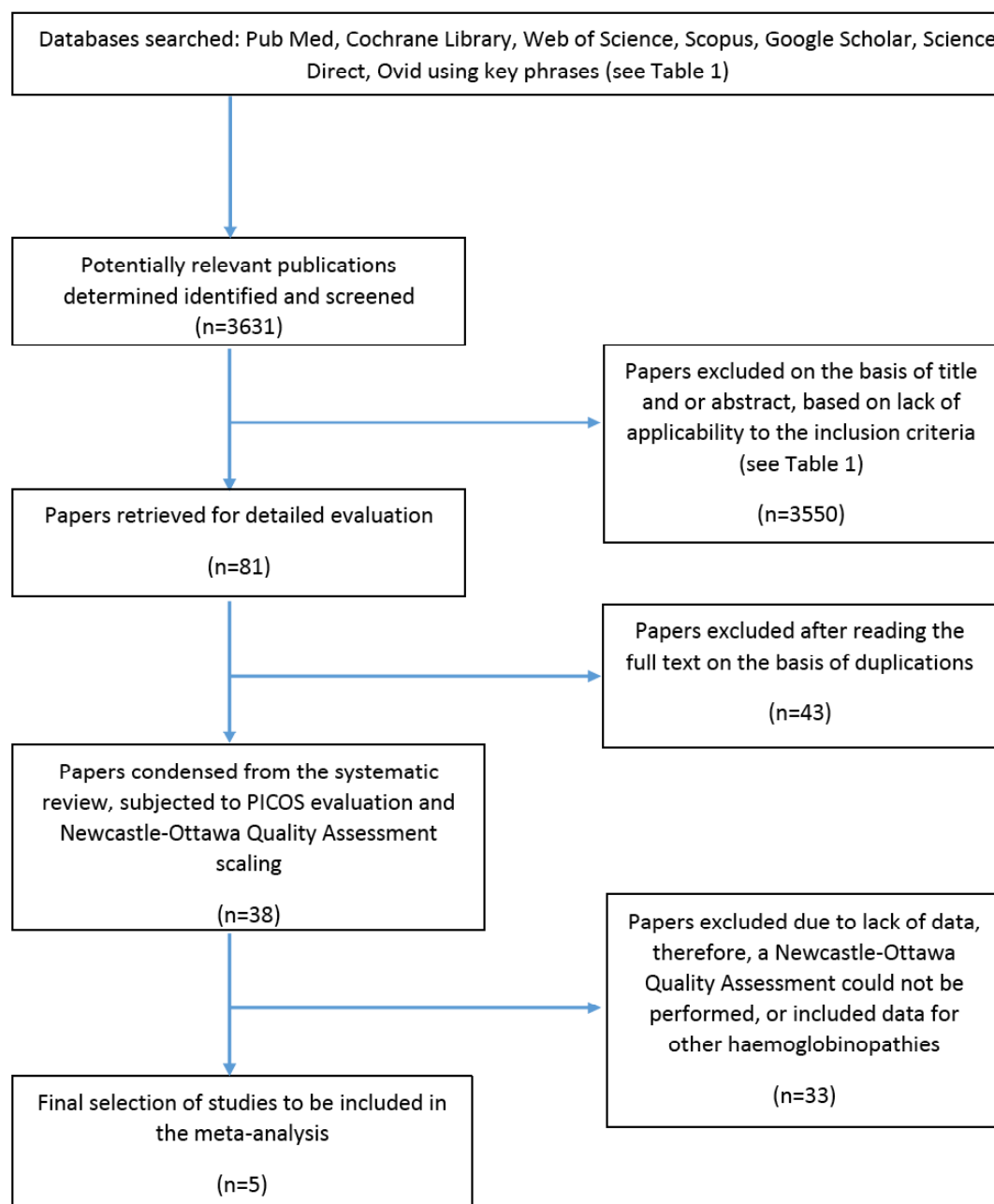


Figure 1: A flow diagram outlining the study selection process during the systematic paper review.

contained little information which would allow for the generation of a quality assessment score and was, therefore, of low quality for the purposes of this study. Overall, the quality of the studies used in the data synthesis was of a satisfactory standard, therefore, showed little bias. The risk of bias across these papers could be increased by the omission of false positive or false negative data, as well as the data from the controls. If the data from the control group was inaccurate, then the data retrieved from method and subsequent results would be inaccurate.

Statistical Analysis

It was originally intended that the data retrieved as part of the systematic review would be presented in the form of a meta-analysis and a subsequent Forest Plot, in order to determine the sensitivity and specificity of the use for NIPD for the determination of foetal SCD. However, the lack of homogeneity between the independent studies due to the uses of differing methods and techniques between the studies [24], as well as the inability to distinguish between the true positive and true negative values from the data

Table 2: A Table Detailing the Quality Assessment Scores and Criteria for the Five Studies from the Systematic Review which were Included in the Meta-Analysis. These were Based on the Newcastle-Ottawa Quality Assessment Scale and the QUADAS-II Guidelines

	Paper Author/ Year Published				
	Cheung <i>et al.</i> , (1996)	Barrett <i>et al.</i> , (2012)	Phylipsen <i>et al.</i> , (2012)	Fielding <i>et al.</i> , (2013)	Yenilmez <i>et al.</i> , (2013)
Newcastle-Ottawa Score	5*/8*	6*/8*	8*/8*	3*/8*	6*/8*
QUADAS-II score	Medium	Medium	Medium	Low	Medium
Describe methods of patient selection: Describe included patients (prior testing, presentation, intended use of index test and setting)	Laboratory setting, patients included those who were tested for prenatal diagnosis of sickle cell disease or β-thalassaemia, at the request of the patients.	Blood samples were collected from women who visited the Fetal Medicine Unit at University College Hospital NHS Foundation Trust, London for invasive diagnostic testing of SCD.	DNA samples for 7 subsets of different populations were genotyped by melting curve analysis. 13 patients were selected from those who were sent to the laboratory for carrier diagnostics of SCD.	Not described. Maternal plasma was extracted from pregnancies at risk of sickle cell disease.	Pregnant women admitted to the Department of Gynecology and Obstetrics and Department of Medical Biochemistry for prenatal diagnosis during a 2-year period, including those with SCD and β-thalassaemia mutations.
Was a consecutive or random sample of patients enrolled? (Yes/ No/ Unclear)	Yes	Yes	Yes	Unclear	Yes
Was a case-control design avoided? (Yes/ No/ Unclear)	Yes	Yes	Yes	Unclear	Yes
Did the study avoid inappropriate exclusions? (Yes/ No/ Unclear)	Unclear	Unclear	Unclear	Unclear	Unclear
Could the selection of patients have introduced bias? (High/ Low/ Unclear)	Low	Low	Low	Unclear	Low
Are there concerns that the included patients do not match the review question? (High/ Low/ Unclear)	Low	Low	Low	Low	Low
Describe the index test and how it was conducted and interpreted:	CffDNA by PCR: RBCs were lysed by boiling and 70 rounds of PCR amplification, with a pair of oligonucleotide probes which detected specific mutations. Single PCR markers flanked the mutations on bands of amplified β-globin genes.	CffDNA analysis using dPCR using TaqMan probes to discriminate between the wild-type haemoglobin A and the mutant haemoglobin S alleles.	CffDNA by melting curve analysis. Genomic DNA was isolated from leukocytes by Auto Pure LS robotic workstation, which was then centrifuged to remove plasma from leukocytes. This lead to the extraction of DNA with EZ1 advanced workstation. Primers which amplify 12 differet fragments on β-globin gene clusters covering 24 SNPs and 20 MCA probes to cover the SNPs. PAP is used to detect specific paternal allele in DNA isolated from maternal plasma resulting in primer pairs designed for 12 SNPs.	CffDNA extracted from maternal plasma was subsequently analysed by dPCR.	High resolution melting technique and quantification of cffDNA using real-time PCR were used to determine foetal genotype from cffDNA extracted from maternal plasma.

QUADAS-II Criteria

Were the index test results interpreted without knowledge of the results of the reference standard? (Yes/ No/ Unclear)	Yes	Yes	Yes	Unclear	Yes
If a threshold was used, was it pre-specified? (Yes/ No/ Unclear)	Yes	Yes	Yes	Yes	Yes
Could the conduct or interpretation of the index test have introduced bias? (High/ Low/ Unclear)	Low	Low	Low	Low	Low
Are there concerns that the index test, its conduct, or interpretation differ from the review question? (High/ Low/ Unclear)	Low	Low	Low	Low	Low
Describe the reference standard and how it was conducted and interpreted:	CVS was taken 20 days before SCD cffDNA test, and 20 days after β -thalassemia test. A Reverse dot blot analysis allowed normal/ mutant oligonucleotides to be immobilised on a filter, amplified and test DNA was hybridised. SCA only indicated normal oligonucleotides, therefore, no presence of disease or carrier status was identified.	Amniocentesis was performed as a gold standard against which to compare the accuracy of the test.	Foetal genomic DNA in question was confirmed using CVS testing or tested after birth, to confirm the PAP results, using direct sequencing analysis.	Described as conventional genetic testing.	Foetal DNA was tested against amplification refractory mutation system (ARMS)-PCR from samples taken by CVS.
Is the reference standard likely to correctly classify the target condition? (Yes/ No/ Unclear)	Yes	Yes	Yes	Unclear	Yes
Were the reference standard results interpreted without knowledge of the results of the index test? (Yes/ No/ Unclear)	Yes	Yes	Unclear	Unclear	Unclear
Could the reference standard, its conduct, or its interpretation have introduced bias? (High/ Low/ Unclear)	Low	Low	Low	Unclear	Low

Are there concerns that the target condition as defined by the reference standard does not match the review question? (High/ Low/ Unclear)	Low	Low	Low	Low	Low
Describe any patients who did not receive the index test(s) and/or reference standard or who were excluded from the 2x2 table (refer to flow diagram): Describe the time interval and any interventions between index test(s) and reference standard:	It is not described what happened to the non SCD patients included in the study (control patients). Only data pertaining to SCD or β -thalassaemia is described. Interval is described as CVS was taken 20 days before cffDNA test was performed for SCD, and 20 days after the test for β -thalassaemia.	None stated.	Two patients were unavailable for post-partum testing. No time intervals were stated.	N/A	All maternal blood samples were taken prior to CVS.
Was there an appropriate interval between index test(s) and reference standard? (Yes/ No/ Unclear)	Yes	Unclear	Yes	Unclear	Yes
Did all patients receive a reference standard? (Yes/ No/ Unclear)	Yes	Yes	No	Unclear	Yes
Did all patients receive the same reference standard? (Yes/ No/ Unclear)	Yes	Yes	No	Yes	Yes
Were all patients included in the analysis? (Yes/ No/ Unclear)	Yes	Yes	Yes	Unclear	Yes
Could the patient flow have introduced bias? (High/ Low/ Unclear)	Low	Low	Low	Unclear	Low

retrieved, lead to the determination that it was more appropriate to present the data as a Forest Plot meta-analysis (see Figure 2) indicating the proportion of correct diagnosis results for the use of cffDNA for the diagnosis of SCD in each study. The meta-analysis was carried out using Meta-DiSc, version 1.4 [25]. The proportions were compared using the DerSimonian and Laird approach, adapted for proportions [26].

RESULTS

In order to assess how accurate or relevant the evidence proffered by the data collected from the systematic review was, the validity of the studies was interrogated. The evidence presented in the studies was deemed to be of good strength, as each paper described a moderate success rate. Despite some papers containing a smaller number of participants

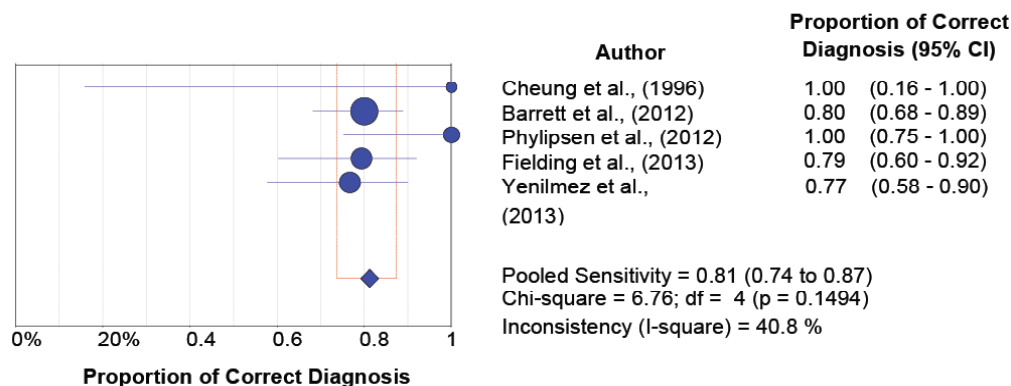


Figure 2: A Forest Plot Meta-Analysis describing the Proportion of Correct diagnoses from the data detailed in the studies retrieved from the systematic review.

than others, the combined research indicated that the use of cffDNA for NIPD for SCD has the potential for a high degree of accuracy and consistency [15], despite not yet indicating an accuracy that would allow this technique to be used in clinical practice.

From the systematic review that was performed, four manuscripts and one abstract of a paper were identified to contain data on NIPD for SCD (see Table 3). As shown in Table 3, the correct diagnosis from use of cffDNA to test prenatally for SCD ranged from 76.67% to 100% (with a weighted average of 81.30%). However, two studies (Cheung *et al.*, (1998) [20] and Phylipsen *et al.*, (2012) [23] yielded a 100% success rate. These two studies were made up of two and thirteen test subjects respectively, therefore, the high percentage accuracy and low cohort size have an

impact on the reliability of the average percentage accuracy of the data. That being said, the remaining three studies with the largest cohorts still returned an individual accuracy of over 75%. The incorrect data, for the purpose of this meta-analysis, indicates the incorrect and unclassified data retrieved from the studies. The incorrect diagnosis rate ranged from 0% to 23.33%, with a weighted average of 18.70%. The study with the highest inaccuracy percentage was that by Yenilmez *et al.*, (2013) [23], with 23.33% inaccuracy. Despite describing the correct determination of sickle cell mutations in 30/30 fetuses using high resolution melting (HRM) analysis (which was then confirmed by chorionic villus sampling), the technique did not distinguish between fetuses with SCD (7/30) and those with sickle cell trait (23/30).

Table 3: Results Detailed in the Manuscripts Retrieved from the Systematic Review

Study Name	Author	Correct Result	Correct Diagnosis	Incorrect Result*	Incorrect Diagnosis*
Prenatal diagnosis of sickle cell anaemia and thalassaemia by analysis of foetal cells in maternal blood	Cheung <i>et al.</i> , 1996	2/2	100.00	0	0.00
Digital PCR Analysis of Maternal Plasma for Non-invasive Detection of Sickle Cell Anaemia	Barrett <i>et al.</i> , 2012	52/65	80.00	13/65	20.00
Non-invasive prenatal diagnosis of beta-thalassaemia and sickle cell disease using pyrophosphorolysis-activated polymerisation and melting curve analysis	Phylipsen <i>et al.</i> , 2012	13/13	100.00	0	0.00
Implementation of noninvasive prenatal diagnosis for single gene disorders into clinical practice - PCR-RED, dPCR or NGS?	Fielding <i>et al.</i> , 2013	23/29	79.31	6/29	20.69
Non-invasive prenatal diagnosis experience in Çukurova Region of Southern Turkey: detecting paternal mutations of sickle cell anaemia and β -thalassaemia in cell-free foetal DNA using high-resolution melting analysis	Yenilmez <i>et al.</i> , 2013	23/30	76.67	7/30	23.33
Total Weighted Average (Percent):			81.30		18.70

*In this table, the data classified by each of the studies as incorrect or unclassified results have been combined under one subheading of "Incorrect Result" or "Incorrect Diagnosis", in order to easily distinguish between those results which produced a correct foetal diagnosis and those which did not.

As can be seen in Figure 2, the study by Barrett *et al.*, (2012) [2] shows a smaller confidence interval than the study by Cheung *et al.*, (1996) [20], due to the larger study size by Barrett *et al.*, (2012) [2]. Furthermore, the final result of the pooled sensitivity indicates a result which is closest to the result from the study by Barrett *et al.*, (2012) [2], thereby indicating that the results gained from this study describe the data which is closest to the true sensitivity of using cffDNA to test for SCD.

DISCUSSION

Summary of Evidence

The analysis of data collected during the systematic review shows that currently NIPD for SCD achieves approximately 81.30% accurate diagnosis and 18.70% inaccurate diagnosis, which is significantly less than the current accuracy of the gold standard for prenatal testing. Therefore, extensive future research to enhance the accuracy of this technique will render invasive testing for this condition obsolete, and mitigate procedure related risk of miscarriage.

Despite the use of cffDNA for NIPD for SCD having first been suggested over 20 years ago (as was seen from the study by Cheung *et al.*, (1996) [20]), there are very few studies detailing specific data on the accuracy and reliability of cffDNA as a diagnostic tool for SCD. As a result, out of over 3,600 papers scanned for this systematic review, only four full manuscripts and one paper abstract were found to contain any usable and relevant data. Therefore, the need for further research into this field is absolute. Further research will be able to design novel techniques for detection and analysis of cffDNA, in order to most accurately diagnose the foetal genotype.

Limitations

During the formation of this systematic review, it was evident that an insufficient number of clinical trials producing data regarding the use of NIPD for SCD have been undertaken, as of the date of submission of this study. Furthermore, there was significant heterogeneity with regards to the methods of cffDNA retrieval and testing [16] between the studies found during the systematic review. For example, Phylipsen *et al.*, (2012) [21] and Yenilmez *et al.*, (2013) [23] used SNPs to detect gene mutations, whereas Cheung *et al.*, (1998) [20], Barrett *et al.*, (2012) [2] and Fielding *et al.*, (2013) [22], used PCR. Consequently, until there is a standardised method by which this technique is

performed, there may be no detailed statistical analysis performed on the available data [16], in order to find the true strength of evidence present for the sensitivity and specificity for the use of cffDNA for NIPD of SCD [27], and thus generate a true odds ratio, risk ratio, confidence interval and p-value [16].

The variability between participants, interventions and outcomes results in clinical heterogeneity, or clinical diversity. The combination of the clinical heterogeneity, variability in study design and risk of bias gives rise to the statistical heterogeneity, as seen between the retrieved studies [16], which may cause inconsistencies and inaccuracies in the overall data. There are several factors which may contribute towards statistical heterogeneity of data collected between the studies. These include:

- The varying quality of the studies, caused by discrepancies in the detailing of each of the studies. In particular, the abstract of a paper by Fielding *et al.*, (2013) [22] had a low Newcastle-Ottawa Quality Assessment score and QUADAS-II score, as it contained little information about the study, where as the paper by Phylipsen *et al.*, (2012) [21] had very high quality scores, as it reported a more detailed description of the study;
- The study sizes, ranging from 2 to 52 participants in the study. The paper by Cheung *et al.*, (1996) [20] only has two test subjects, therefore, despite 100% accuracy rating this may be a less accurate or reliable result when included in a pooled average of results;
- The paper by Cheung *et al.*, [20] was published in 1996, therefore, may use obsolete or aged methods for cffDNA extraction and analysis;
- The differing methods of isolating foetal DNA from maternal blood and the following techniques for DNA analysis;
- The differing genetic backgrounds of the participants and, therefore, the different genetic variation of SCD, which would consequently have different SNPs, thereby requiring different identification markers [21];
- The experiments were performed by separate research groups, which would result in different sets of random and systematic errors, and would subsequently alter the accuracy of the data collected.

Implications for Clinical Practice

These results, therefore, indicate that with further research, the use of cffDNA for NIPD of SCD has the potential to be an accurate technique for clinical testing, replacing the current invasive methods of testing, such as amniocentesis and chorionic villus sampling. This would diminish the potential harm caused to the mother and foetus during genetic testing.

Additionally, the cost for NIPD in general is very expensive. It has been reported that NIPD in China may cost between \$487 and \$587, whereas amniocentesis costs approximately \$326, and in Brazil, NIPD may cost \$1,492, whereas amniocentesis costs \$426, on average [25]. Therefore, unlike in countries with subsidised health care, such as the United Kingdom, it is personally very expensive for the parents to undergo a non-invasive procedure in order to test for genetic abnormalities and, therefore, this may impact the acceptance of this procedure to be used in the clinic and may deter patients from undergoing this procedure [25]. Furthermore, when compared to the cost of post-natal testing, of which reports state some techniques can cost as little as \$0.50 per test [28], it is obvious that further research needs to be performed in order to increase efficiency of NIPD testing at a minimal cost, so that it may be best applied to the global population.

CONCLUSION

CffDNA for non-invasive prenatal SCD diagnosis appears to have the potential to be an accurate technique for the testing of this genetic disease, despite not currently indicating a proportion of correct diagnosis results, which would encourage the technique for clinical implementation. Whilst there are currently very limited data on the use of this technique for the specific testing of SCD, there is great opportunity for further research into the standardisation and clinical application of this procedure. Therefore, it is imperative that a reliable, sensitive and specific method for prenatal testing of an unborn foetus for this disease is developed, so the parents of the foetus can be prepared to deal with this lifelong disease. Furthermore, low cost but high quality testing without risking the health of the mother and unborn foetus in the process is absolutely necessary. The use of cffDNA for NIPD of SCD has the potential to be an optimal alternative to amniocentesis, chorionic villus sampling or cordocentesis with regards to accuracy, as well as maternal and foetal safety of these procedures,

however, more research needs to be performed in order to fully determine the sensitivity and specificity of this test, before it can become the preferred method of prenatal diagnosis for SCD.

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