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Non-invasive determination of the fetal rhesus factor to prevent maternal rhesus sensitization[1]

Extract

[1] Translation of Chapters 1 to 6 of the final report *Nicht invasive Bestimmung des fetalen Rhesusfaktors zur Vermeidung einer mütterlichen Rhesussensibilisierung* (Version 1.0; Status: 20 March 2018). Please note: This translation is provided as a service by IQWiG to English-language readers. However, solely the German original text is absolutely authoritative and legally binding.

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This report was prepared in collaboration with external experts.

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Key statement

Research question

The aim of the present investigation was the benefit assessment of non-invasive testing for the fetal rhesus factor in Rhesus D (RhD)-negative pregnant women in combination with indication for targeted anti-D prophylaxis to prevent maternal rhesus sensitization in the framework of screening tests in accordance with the German maternity guidelines regarding patient-relevant outcomes.

The assessment was conducted in comparison with the currently used method of regular administration of anti-D prophylaxis in all RhD-negative pregnant women without prenatal testing for the fetal rhesus factor.

Conclusion

Diagnostic accuracy of the prenatal test

The analysis of 11 studies on diagnostic accuracy of non-invasive testing for the fetal rhesus factor showed that the test has a very high sensitivity of 99.9% (95% confidence interval [CI] [99.5; 100]) and a very high specificity of 99.1% (95% CI [98.4; 99.5]). 10 of the 11 studies had a high risk of bias; the pooled estimation of the diagnostic accuracy from all studies was comparable with that of the study with low bias, however. Prenatal and postnatal testing for the rhesus factor can be considered equivalent. It was observed in the present subgroup analyses that the prenatal test before 8 or 11 weeks of gestation showed markedly lower sensitivity in 2 of 3 studies. Regarding multiple pregnancies, there were 2 usable studies, from which no conclusion on the influence of this factor could be inferred, however.

Guiding antenatal anti-D prophylaxis

There were no controlled intervention studies investigating non-invasive testing for the fetal rhesus factor to guide anti-D prophylaxis. There were also no studies on the possible harm from non-indicated anti-D prophylaxis. No conclusions on the benefit of indicated antenatal anti-D prophylaxis could be drawn from 2 controlled intervention studies considered as supplementary information, which investigated the administration of antenatal anti-D prophylaxis (with a dosage that is not approved in Germany).

Hence, the patient-relevant benefit or harm of non-invasive testing for the fetal rhesus factor to guide antenatal anti-D prophylaxis is unclear. Due to the high sensitivity of the prenatal test, only a small number of anti-D prophylaxes erroneously not given before birth could be expected. The corresponding adverse effects would therefore be estimated as immeasurably small even if antenatal anti-D prophylaxis does have a benefit. Ethical, resource-based or economic advantages due to omission of non-indicated antenatal anti-D prophylaxes were not subject of this assessment.

Guiding postnatal anti-D prophylaxis

The above results on diagnostic accuracy of prenatal testing for the rhesus factor were based on the comparison with postnatal test results as the reference standard. However, 2 studies showed that the postnatal test produces false-negative results of a similar magnitude as the prenatal test, so that the measured values constitute a marginal underestimation of the true sensitivity of the prenatal test.

Overall, the effects of the implementation of the prenatal test by anti-D prophylaxes erroneously not given and the corresponding adverse effects can be considered immeasurably small. On the one hand, this applies to the testing strategy in which each woman whose child is tested RhD-positive in the prenatal and/or the postnatal test receives postnatal anti-D prophylaxis. On the other, this also applies to a testing strategy in which the prenatal test replaces the postnatal test, and is also used for the decision for or against postnatal anti-D prophylaxis.

In summary, there is no greater benefit or harm for guiding postnatal anti-D prophylaxis if non-invasive testing for the fetal rhesus factor supplements or replaces the currently used postnatal test, i.e. the prenatal test is equivalent to the postnatal test. The assessment of using the test to replace the postnatal test is subject to the condition of a corresponding quality assurance when implementing the prenatal test in Germany.

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1 Background

RhD-negative pregnant women can produce anti-D antibodies if they carry an RhD-positive child and fetal erythrocytes enter the maternal circulation. This process called sensitization leads to an incompatibility between maternal antibodies and fetal erythrocytes. This incompatibility usually manifests itself in a subsequent pregnancy with another RhD-positive child when maternal anti-D antibodies pass through the placenta into the fetal circulation, which can lead to a breakdown of fetal erythrocytes, resulting in serious fetal diseases such as anaemia and hydrops, and fetal death. In 25% to 35% of all cases of RhD incompatibility, the child has anaemia with hyperbilirubinaemia at birth, which can lead to kernicterus and brain damage. Neonatal hyperbilirubinaemia is treated with phototherapy and blood exchange if necessary. In another 20 to 25% of the cases of rhesus incompatibility, the fetus develops haemolytic anaemia while still in the womb, which can lead to hydrops fetalis with cardiac failure and fetal death. Depending on gestational age and severity of the condition, fetal anaemia can be treated with one or several blood transfusions via the umbilical cord vein [1].

According to the German maternity guidelines [2], a blood sample is taken from the pregnant woman as early as possible to determine the maternal blood group and rhesus factor and screen for antibodies. Another antibody screening test for all pregnant women is mandated between 24 and 27 weeks of gestation. RhD-negative pregnant women with no detectable anti-D antibodies receive a prophylactic standard dose (300 µg) of anti-D immunoglobulin between 28 and 30 weeks of gestation to prevent sensitization by catching any erythrocytes passing from the fetal to the maternal circulation in the period until birth. The rhesus factor of any child of an RhD-negative mother has to be determined immediately after birth. If the child is RhD-positive, the mother requires another standard dose of anti-D immunoglobulin within 72 hours after birth. This prophylaxis aims to promote rapid decomposition of RhD-positive erythrocytes that have passed into the maternal circulation particularly during birth to prevent development of anti-D antibodies in the mother. Anti-D prophylaxis is also administered after a miscarriage or a pregnancy termination [2] as well as after an abdominal trauma or other events with the possibility of fetal blood passing into the maternal circulation [1]. Human anti-D immunoglobulin from sensitized donors is used. Side effects cited for these preparations include allergic hypersensitivity reactions (rare) and skin reactions (uncommon). The possibility of transmission of pathogens when using drugs produced from human blood or plasma cannot be completely ruled out [3,4].

If the mother does not receive prophylaxis, sensitization occurs in 4 to 9% of the births of RhD-positive children, as well as in 4% of the cases of abortion curettage or vaginal bleeding, and in 2 to 5% of the cases of chorionic villus sampling or amniocentesis. In about 1% of the cases, there is spontaneous sensitization in the second and third trimenon [1].

The proportion of RhD-negative people in the total central-European population is 15%. About 12.5% of all European couples have a rhesus constellation during pregnancy, i.e. the mother is RhD-negative and the father is RhD-positive. Since some of the fathers are heterozygous and

do not pass on the RHD gene, the proportion of RhD-negative pregnant women with RhD-positive fetuses is reduced to about 8.2% [1]. Since in Germany the father's RhD status is not regularly determined, under the current procedure, a total of 6.8% of pregnant women receive an anti-D prophylaxis for which there is no indication. With 737 575 live births per year in 2015 [5], this affects about 50 000 pregnant women in Germany. Despite the described measures, sensitizations still occur in Germany. There is no exact information on the number of cases. The German Federal Statistical Office lists 300 cases in 2016 under Code P550 ("Rh isoimmunization in the fetus and the newborn") [6]. It is unclear, however, whether this only includes antibodies against RhD or also against further antigens of the Rh system.

The intervention to be assessed is a non-invasive molecular genetic test, which determines the fetal rhesus factor, for example by using cell-free fetal deoxyribonucleic acid (DNA) from the maternal plasma. If an RhD-negative pregnant woman has a negative test result, which means that she is carrying an RhD-negative child, non-indicated anti-D immunoglobulin can be dispensed with as a result of the new test. In addition, it is conceivable that the implementation of the additional test may improve adherence to anti-D prophylaxis as it offers women knowledge about their risk of immunization in their current pregnancy. This may lower the number of sensitizations [7].

The phenotypic expression of the RhD antigen on the erythrocyte membrane is controlled by the expression of the RHD gene. Non-invasive testing for the fetal RhD status by detection of one or several exons of the RHD gene in cell-free maternal plasma always constitutes only a prediction of the fetal phenotype. The fetal phenotype is not determined directly. Prediction of the phenotype may be flawed, e.g. in case of rare fetal gene variants. For reasons of better readability, these limitations are not always separately addressed in the following text.

2 Research question

The aim of the present investigation was the benefit assessment of non-invasive testing for the fetal rhesus factor in RhD-negative pregnant women in combination with indication for targeted anti-D prophylaxis to prevent maternal rhesus sensitization in the framework of screening tests in accordance with the German maternity guidelines regarding patient-relevant outcomes.

The assessment was conducted in comparison with the currently used method of regular administration of anti-D prophylaxis in all RhD-negative pregnant women without prenatal testing for the fetal rhesus factor.

3 Methods

Controlled intervention studies of the diagnostic-therapeutic chain were to be included in the benefit assessment. In case that such studies were not available or were not available in sufficient quality, an assessment of controlled intervention studies on the benefit or harm of anti-D prophylaxis as well as of studies on diagnostic accuracy as individual components of the diagnostic-therapeutic chain were mandated (linked evidence).

Controlled intervention studies of the diagnostic-therapeutic chain

The target population of the benefit assessment using controlled intervention studies of the diagnostic-therapeutic chain were RhD-negative pregnant women not sensitized to the RhD antigen. The experimental intervention was non-invasive molecular genetic prenatal testing for the fetal rhesus factor and regular omission of antenatal or ante- and postnatal anti-D prophylaxis if the test indicates an RhD-negative fetus. The comparator intervention was administration of an anti-D prophylaxis to all RhD-negative pregnant women.

The following patient-relevant outcomes were considered in the assessment:

- mortality
- occurrence of haemolytic anaemia of fetuses or newborns due to RhD incompatibility and related complications
- adverse events associated with the administration of antenatal anti-D prophylaxis
- health-related quality of life

Where meaningful and not further specified, all outcomes mentioned refer to pregnant women, mothers, fetuses and children.

If an assessment based on the outcome “occurrence of haemolytic anaemia of fetuses or newborns due to RhD incompatibility and related complications” was not possible, the following outcome was used:

- sensitization to the RhD-antigen as sufficiently valid surrogate for the patient-relevant outcome “occurrence of haemolytic anaemia of fetuses or newborns due to RhD incompatibility and related complications”

Randomized controlled trials (RCTs) were primarily to be included and, if necessary, also non-randomized, prospectively planned, controlled intervention studies of the diagnostic-therapeutic chain with concurrent control group and adequate control for confounders. There was no limitation regarding study duration.

The assessment was also to include studies with an enrichment design that only investigate effects in pregnant women with a certain test result (fetus RhD-positive or RhD-negative).

Controlled intervention studies on the benefit and harm of anti-D prophylaxis

Controlled intervention studies on the harm of non-indicated anti-D prophylaxis

Controlled intervention studies on the omission of anti-D prophylaxis were to be included to evaluate the harm (benefit of omission) of non-indicated anti-D prophylaxis. The target population were RhD-negative pregnant women and women after childbirth, each without sensitization to the RhD antigen. The comparator intervention was approval-compliant administration of anti-D prophylaxis. The patient-relevant outcomes mentioned above under "controlled intervention studies of the diagnostic-therapeutic chain" were to be considered (except for occurrence of haemolytic anaemia in fetuses or newborns and related complications). RCTs were primarily to be included and, if necessary, also controlled cohort studies (including retrospective studies or studies with historical comparison). There was no limitation regarding study duration.

Controlled intervention studies on the benefit of indicated antenatal anti-D prophylaxis

Controlled intervention studies on the administration of antenatal anti-D prophylaxis were to be included to evaluate the benefit (of administration) of indicated antenatal anti-D prophylaxis. The target population were RhD-negative pregnant women without sensitization to the RhD antigen. The comparator intervention was omission of antenatal anti-D prophylaxis. The patient-relevant outcomes mentioned above under "controlled intervention studies of the diagnostic-therapeutic chain" were to be considered. RCTs were primarily to be included and, if necessary, also controlled cohort studies with concurrent control group. There was no limitation regarding study duration.

Studies on diagnostic accuracy

According to the maternity guidelines, all RhD-negative pregnant women should receive antenatal anti-D prophylaxis. Now this commission requested the assessment of a prenatal test that should restrict treatment to women with indication. Reasons for the implementation of this test may not only be the benefit and harm of the anti-D prophylaxis, but also resource-based, economic or ethical aspects. Such reasons were not within the scope of the commission of this assessment, but their weighing is certainly influenced by the diagnostic accuracy of the test to be assessed. To account for this need for information, this report investigates the diagnostic accuracy of the prenatal test, irrespective of the result of the assessment of controlled intervention studies.

Form a patient's perspective, there is another reason to investigate diagnostic accuracy, however. The prenatal test could also be used in Germany to replace postnatal RhD testing. In this case, pregnant women with a false-negative result would also not receive postnatal anti-D prophylaxis, whose effect is indisputable, as a result of this test. This risk can only be estimated if the diagnostic accuracy of the prenatal test is known.

Studies with RhD-negative pregnant women without sensitization to the RhD antigen were included in the assessment. The index test was non-invasive molecular genetic prenatal testing

for the fetal rhesus factor. The reference test was postnatal testing for the child's rhesus factor. Prospectively planned cohort studies, from which personal data for the calculation of the diagnostic accuracy of the index test could be derived, were included.

In the commenting procedure on the preliminary report, doubts were expressed concerning the assumption of a 100% sensitivity of the postnatal test, which was used as reference for determining test accuracy. Against this background, the study pool on diagnostic accuracy was checked for studies that investigated the discordant results between the prenatal and the postnatal test regarding reasons for differences between both tests.

Information retrieval and reporting of results

A systematic search for primary literature was conducted in the following databases: MEDLINE, Embase, and the Cochrane Central Register of Controlled Trials. At the same time, a search for relevant systematic reviews was conducted in the databases MEDLINE, Embase, the Cochrane Database of Systematic Reviews, the Database of Abstracts of Reviews of Effects, and the Health Technology Assessment Database.

Systematic reviews and publicly available trial registries were also searched. Furthermore, publicly accessible documents from regulatory authorities, documents sent by the Federal Joint Committee (G-BA), and documents that had been provided in the hearing procedure were also screened. In addition, the authors of relevant study publications were contacted in order to clarify important questions.

The selection of relevant studies was performed by 2 reviewers independently of each other. Data were extracted into standardized tables. To evaluate the qualitative certainty of results, the risk of bias at study and outcome level was assessed and rated as low or high respectively. The results of the individual studies were described, organized by research questions and outcomes.

If the studies were comparable regarding the research question and relevant characteristics and no relevant heterogeneity was observed, the individual results were to be pooled quantitatively by means of meta-analyses.

For each outcome a conclusion on the evidence base of the (greater) benefit and (greater) harm was drawn in 4 levels with regard to the respective certainty of the conclusion: The data provide either "proof" (highest certainty), an "indication" (medium certainty), a "hint" (weakest certainty), or none of these 3 situations applies. The latter is the case if no data are available or the data available do not allow any of the other 3 conclusions to be drawn. In this case, the conclusion "There is no hint of (greater) benefit or (greater) harm" is drawn.

4 Results

4.1 Results of the information retrieval

The systematic literature search in bibliographical databases yielded 68 publications on 64 studies that fulfilled the criteria on study inclusion defined for this report. The last search was conducted on 26 September 2017.

3 additional relevant documents on 3 studies were identified by the search in further search sources.

2 studies were identified, the relevance of which could not be conclusively determined. Furthermore, one ongoing study was identified.

A total of 65 studies (71 documents) were identified as relevant for the research question of the present benefit assessment.

No controlled intervention studies of the diagnostic-therapeutic chain were available for the benefit assessment. Therefore, controlled intervention studies on the benefit or harm of anti-D prophylaxis and studies on diagnostic accuracy were used.

There were also no controlled intervention studies on the harm of non-indicated anti-D prophylaxis. Hence, only data on the benefit of indicated antenatal anti-D prophylaxis (2 studies, 2 documents) and test accuracy (63 studies, 69 documents) were available. The following presentation of the results is therefore limited to these 2 research questions.

The 2 studies on the benefit of antenatal anti-D prophylaxis did not use the dose approved in Germany, but only 1 third or 2 thirds of the approval-compliant 1500 International Units (IU). Since these 2 studies are the only data on this research question from RCTs or prospective controlled intervention studies, the results were considered as additional information.

4.2 Results on the benefit of antenatal anti-D prophylaxis

4.2.1 Characteristics of the studies on the benefit of indicated antenatal anti-D prophylaxis presented as additional information in the assessment

2 controlled intervention studies were considered as additional information on the benefit of antenatal anti-D prophylaxis.

The prospective controlled intervention study by **Huchet 1987** [8] included 1969 RhD-negative pregnant women from 23 maternity clinics from the Paris region. Pregnant women born in an odd year were allocated to the intervention group (n = 927), and pregnant women born in an even year were allocated to the control group (n = 955). Only the intervention group received an anti-D prophylaxis of 500 IU between 26 and 29 weeks of gestation, and between 32 and 36 weeks of gestation. Both groups received postnatal anti-D prophylaxis. A total of 1189 children were RhD-positive (intervention = 599; control = 590), which constituted the relevant

study population. The authors provided information on the sensitization status during pregnancy, at birth, and between 2 and 12 months after delivery. For the information at 12 months after birth, the rate of patients who discontinued the study was about 50%. Hence, only the sensitization to the RhD antigen at birth was considered for the present report.

The RCT by **Lee 1995** [9] included 2541 RhD-negative pregnant women into the multicentre study conducted in the United Kingdom. 513 participants with RhD-positive child from the intervention group, and 595 from the control group were analysed. Only the intervention group received an anti-D prophylaxis of 250 IU at 28 and 34 weeks of gestation. Both groups received postnatal anti-D prophylaxis. The authors provided information on the sensitization rate at birth and up to 12 months after birth. Again, the rate of patients who discontinued the study in the follow-up period was very high, so that birth was the only documentation time considered.

4.2.2 Available outcomes relevant for the assessment

The surrogate outcome "sensitization to the RhD antigen" was reported as the only relevant outcome in both studies used as additional information. No data were available on the outcomes "mortality", "haemolytic anaemia in fetuses or newborns" and "health-related quality of life". The studies also contained no information on adverse events from administration of antenatal anti-D prophylaxis and were therefore not relevant for the research question described in Chapter 3 (harm of anti-D prophylaxis).

4.2.3 Assessment of the risk of bias at study level and outcome level

The risk of bias at study level was rated as high for both studies on the benefit of indicated antenatal anti-D prophylaxis.

The study by Huchet 1987 [8] was not a randomized study because group allocation was based on the participants' year of birth. Observation of the groups was concurrent, but no information was provided on comparability of the groups, blinding of participants or treating staff, or sample size planning. The second study by Lee 1995 [9] was described as randomized, but there was no information on random sequence generation and allocation concealment. There was also no information on blinding of participants or treating staff. There are hints of selective reporting as the original sample size planning was deviated from. Participant flow was also non-transparent.

The high risk of bias at study level had a direct impact on the risk of bias at outcome level. In addition, both studies neither reported information on statistical methods nor adequately imputed missing data. This makes comprehensibility of the results notably more difficult.

4.2.4 Results on patient-relevant outcomes

Results on the outcome "sensitization"

The primarily planned meta-analysis of both studies presented as additional information according to the Knapp-Hartung method showed no statistically significant difference

regarding the number of women with sensitization to the RhD antigen at birth. The resulting effect estimation was very imprecise. Hence, different sensitivity analyses with varying meta-analytical methods were conducted, which also found no statistically significant difference. Overall, the benefit of antenatal anti-D prophylaxis regarding sensitizations at birth is unclear.

4.3 Summary of the results on antenatal anti-D prophylaxis

Neither controlled intervention studies on the diagnostic-therapeutic chain, nor controlled intervention studies on the harm of non-indicated anti-D prophylaxis were available. Hence, there were also no data on adverse events caused by anti-D prophylaxis. The benefit of antenatal anti-D prophylaxis also remains unclear on the basis of 2 studies on the administration of antenatal anti-D prophylaxis with a low, and hence not approval-compliant dose, presented as additional information. These studies also provided no data on adverse events.

4.4 Results on diagnostic accuracy

4.4.1 Characteristics of the studies on diagnostic accuracy included in the assessment

63 studies on diagnostic accuracy were included. All studies were prospective cohort studies.

Hence, there was comprehensive evidence on test accuracy of the prenatal test to be assessed, and also a large number of studies with comparatively low numbers of participants. Only the 11 largest of the studies included [10-20] were therefore assessed in this report. With a total of about 65 000 RhD-negative pregnant women analysed in the included studies, about 60 000, i.e. over 90% of the analysed study participants, were included in the assessment with the 11 largest studies.

The 11 studies analysed also included the studies de Haas 2016 and Müller 2008 [10,16], which investigated discordant results between the prenatal and postnatal test regarding reasons for the differences between both tests. Among all 63 relevant studies on diagnostic accuracy, there was one further study that also addressed this topic [21]. This study was not considered in the report due to its low number of participants (115).

All 11 studies considered were conducted in Western Europe. The index test in all studies was the testing for fetal RhD status by analysing cell-free circulating DNA from maternal plasma. It analysed different RHD exons using a real-time polymerase chain reaction (PCR) method. The reference test in all studies was postnatal serologic testing for the RhD antigen by analysing the umbilical cord blood or the blood of the newborn.

De Haas 2016 [10] describes the results of a national screening programme conducted in the Netherlands from July 2011 until October 2012. Data from von 25 789 RhD-negative pregnant women were analysed. The mean time point for taking samples for the PCR analysis was the 27th week of gestation. The authors used a test to detect RHD exon 5 and exon 7. The PCR protocol or the preset computer software algorithm in this study was changed in the meantime.

The study **Clausen 2014** [11] reports the results of 12 668 RhD-negative pregnant women. This national screening programme from all 5 regions of Denmark was conducted from January 2010 until December 2011. Prenatal testing was conducted at about 25 weeks of gestation with a PCR analysis for detecting the RHD exons 5, 7, or 10.

Haimila 2017 [12] describes the results of a national screening programme conducted in Finland from February 2014 until January 2016. 10 814 RhD-negative pregnant women had prenatal testing between 24 and 26 weeks of gestation. The authors used a PCR test to detect RHD exons 5 and 7.

Wikman 2012 [13] reports 3652 RhD-negative pregnant women tested in 83 centres in Sweden between September 2009 and May 2011. The median time point for the test to detect the RHD exon 4 was the 10th week of gestation.

Between 2009 and 2012, 7 birth centres in England participated in the study by **Chitty 2014** [14] with a total of 2288 analysed RhD-negative pregnant women. The median time point for the test was the 19th week of gestation. The authors used a PCR test to detect RHD exons 5 and 7. The authors referred to the methods published by Finning 2008 [15].

Finning 2008 [15] reports screening results of 1869 RhD-negative pregnant women analysed in Middle and Northern England. The median time point for a PCR test to detect the RHD exons 5 and 7 was the 28th week of gestation.

In a German study conducted by **Müller 2008** [16], 1022 RhD-negative pregnant women were recruited by 173 gynaecologists and analysed from the year 2006. The median time point for the index test was the 25th week of gestation using a PCR analysis for detecting the RHD exons 5 and 7. The publication focussed on the comparison of 2 DNA extraction methods.

The study by **Macher 2012** [17] investigated 1012 RhD-negative pregnant women relevant for this assessment. The study was conducted in Spain in 2010; the tests in the study were carried out between 10 and 28 weeks of gestation. A test for detecting RHD exons 5 and 7 was also used in this study.

Akolekar 2011 [18] describes the results of one study from the United Kingdom, for which no information was provided on the documentation time. The study included 586 RhD-negative pregnant women. The median time point of a PCR analysis to detect RHD exons 5 and 7 was the 12th week of gestation.

Minon 2008 [19] describes the results of an investigation in 545 RhD-negative pregnant women from Belgium between November 2002 and December 2006. The median time point for the PCR test for detecting RHD exons 4, 5 and 10 was the 17th week of gestation.

The smallest of the studies analysed here, **Soothill 2015** [20], investigated 499 pregnant women from 3 birth centres in England between April and September 2013. There was no information

on the week of gestation at the time point of conducting the test. The index test was a test for detecting RHD exons 5 and 7. The authors used the method published by Finning 2008 [15].

4.4.2 Available outcomes relevant for the assessment

Data for the benefit assessment were used from the 11 largest studies on diagnostic accuracy included [10-20]. Sensitivity and specificity could be assessed as measurement of diagnostic accuracy.

4.4.3 Assessment of the risk of bias at study level and outcome level

A high risk of bias was determined overall for 10 of 11 studies on diagnostic accuracy analysed. In 9 of 11 studies, the risk of bias of the index test was rated as unclear because there was no information whether the cut-off value of the index test, i.e. the threshold cycle (CT) value, was defined prospectively, which directly affected the final assessment. Partly no information on the size of this cut-off value was found. It was unclear for a large proportion of the studies whether index and reference test were analysed without knowledge of the respective result of the other test. Participant flow and timescales of the testing were assessed as problematic in 4 studies. In these studies, not all RhD-negative pregnant women had received the reference test, and not all women were included in the analysis. Concerns of applicability were rated as low in all studies.

4.4.4 Results on the outcomes of sensitivity and specificity of the prenatal test

From 63 included studies, the data from the 11 largest studies, and hence over 90% of the total population, were analysed. The meta-analysis showed very high values for the diagnostic accuracy of a non-invasive testing for the fetal rhesus factor in RhD-negative pregnant women using real-time PCR:

- sensitivity: 99.9% (95% CI [99.5%; 100%])
- specifity: 99.1% (95% CI [98.4%; 99.5%])

The results of the studies were homogeneous. The study Wikman 2012 [13] determined the lowest sensitivity with a value of 97.6% (95% CI [96.9%; 98.2%]). The lowest value for specifity was shown in the study de Haas 2016 [10]: 97.7% (95% CI [97.4%; 98.0%]). 10 of the 11 studies had a high risk of bias; the pooled estimation of the diagnostic accuracy from all studies was comparable with that of the study with low bias, however. In the studies with explicit information on the proportion of undeterminable samples, the proportions varied between 0.4 and 17.2%. In the publications that did not report undeterminable samples, it cannot be excluded that undeterminable samples were excluded without documenting this.

Usable data regarding multiple pregnancies were only available for 2 of the studies assessed. No conclusions on the influence of this factor on diagnostic accuracy of non-invasive testing for the fetal rhesus factor can be drawn from the information provided for only 31 pregnancies in total.

Reporting of the results was differentiated by gestational age in 3 studies. Whereas no influence of this factor was detected regarding specifity, the increased proportion of false-negative results became apparent in 2 of these studies in women in the early stage of pregnancy. The tests conducted before the 8th week of gestation in the study Wikman 2012 [13], for example, only had a sensitivity of 85.7% (95% CI [80.4%; 90.0%]). The values for sensitivity measured in the study Chitty 2014 [14] was notably lower in the group with tests before the 11th week of gestation compared with other weeks of gestation: 96.2% (95% CI [93.8%; 97.8%]).

4.4.5 Results on discordant test results between prenatal and postnatal test

It was noted in the commenting procedure that the postnatal test also produces a small number of false results. The following consideration of discordant test results between the prenatal and the postnatal test therefore focusses on the false classifications of the postnatal test detected in these investigations.

2 of the analysed studies [10,16] used an additional test to check the discordant results between prenatal and postnatal test. In de Haas 2016 [10], this test was performed using DNA fingerprinting with the stored maternal blood or plasma sample, or the umbilical cord blood sample. Müller 2008 [16] performed this investigation using another PCR analysis, if necessary followed by genotyping from cells of the oral mucous membrane of the newborn.

Out of 26 000 samples in the study de Haas 2016 [10], there were 10 sample mix-ups between mother and child in the delivery room. In addition, the postnatal test (serological testing) rated 22 newborns with RhD variants with weak antigen expression as RhD-negative in the study. These were identified as RhD-positive by the prenatal test (genotyping). These RhD variants could lead to sensitization of the woman [22] and are rated as RhD-positive also by the German clinical practice guideline (CPG) on haemotherapy [23]. The investigation showed 9 false-negative test results for the prenatal test.

In the study Müller 2008 [16] with a total of about 1000 tested participants, 3 samples that had been rated as RhD-positive in prenatal testing were rated as RhD-negative in postnatal testing. According to the authors, one sample was a mix-up between mother and child. 2 other samples were also RhD variants. Hence, the 3 results of the prenatal test were rated as true-positive and, correspondingly, the results of the postnatal test as false-negative. The investigation showed 1 or 2 false-negative test results in the prenatal test (magnetic tips or spin column method). The sensitivity of the postnatal test determined in these 2 studies was over 99%.

Since only these 2 studies were available on the discordant results, no meta-analysis was conducted.

The additional test conducted did not identify false-positive results in the postnatal test in either of both studies. In the prenatal test, 193 (de Haas 2016) and 3 or rather 7 (Müller 2008) false-positive results were detected.

In summary, both studies showed that both tests can be expected to have few false-negative test results. A proportion of the discordant test results was not due to a false result of the prenatal test, but of the postnatal test. Hence, the test accuracy of the prenatal test presented in Section 4.4.4 underestimates its true sensitivity, albeit to a small degree. This also means that postnatal testing produces a small number of false-negative classifications. If the testing strategy of treating discordant cases is adhered to, the use of the prenatal test could in principle prevent affected women from not receiving postnatal anti-D prophylaxis despite indication. With 32 of 25 789 (de Haas 2016) and 3 of 1022 (Müller 2008), the numbers provided on the false-negative test results in the postnatal test are vanishingly small, with the resulting number of additional haemolytic anaemias in fetuses or newborns being even smaller.

This low number of discordant test results also means that both tests can be considered as equivalent.

4.5 Studies of unclear relevance

2 completed studies and one ongoing study on diagnostic accuracy of unclear relevance were identified. These had no influence on the conclusion of the present benefit assessment, however.

4.6 Evidence map

Due to the clear evidence base, no table on the overview of the evidence is presented.

Neither studies on the diagnostic-therapeutic chain nor on the harm of anti-D prophylaxis were available for the benefit assessment. The 2 studies on the benefit of indicated antenatal anti-D prophylaxis on the outcome “sensitization” presented as additional information also showed no effect of this intervention. Hence, the benefit or harm of non-invasive testing for the fetal rhesus factor to guide antenatal anti-D prophylaxis is unclear.

The results of the studies on diagnostic accuracy of the prenatal test showed that this can be rated as very high, with a sensitivity of 99.9% (95% CI [99.5%; 100%]) and specificity of 99.1% (95% CI [98.4%; 99.5%]). 10 of the 11 studies had a high risk of bias, however; the pooled estimation of the diagnostic accuracy from all studies was comparable with that of the study with low bias. Prenatal and postnatal testing for the rhesus factor can be considered equivalent.

These results were based on calculations, in which postnatal RhD testing was the reference test. Investigations on discordant results of the prenatal and the postnatal test using a new investigation showed that the reference test used in this benefit assessment can also produce false-negative results. With 99%, the sensitivity of the postnatal test is of the same magnitude as the sensitivity of the prenatal test. This is an additional support for the assessment that both tests are interchangeable for the decision regarding postnatal anti-D prophylaxis.

5 Classification of the work result

Since the current maternity guidelines mandate treatment of all RhD-negative pregnant women with antenatal anti-D prophylaxis, the implementation of a prenatal test for identifying pregnant women with RhD-positive fetus has various potential effects. These effects are described in the following sections.

Omission of non-indicated anti-D prophylaxis

No relevant data on adverse events, i.e. on possible side effects of anti-D prophylaxis, were identified in the framework of this benefit assessment. It is currently assumed that no pathogens are transmitted when immunoglobulins are administered. Transmission of pathogens when using drugs produced from human blood or plasma cannot be completely ruled out, however. This also applies to currently unknown viruses and other pathogens [3,4]. Overall, the patient-relevant benefit of omission of non-indicated antenatal anti-D prophylaxis is unclear.

An implementation of the test would almost halve the number of anti-D prophylaxes in pregnant women as only about 8.2% of pregnant women would receive prophylaxis instead of the currently about 15% [1]. A total of about 50 000 antenatal prophylaxes could be saved per year. The costs of the preparations for anti-D prophylaxis and the costs of RhD testing would have to be balanced against each other. A detailed health economic evaluation of implementing the test was not part of the commission for this assessment, however.

Ethical questions, such as non-indicated drug administration and production of preparations for anti-D prophylaxis, were also not investigated. Anti-D immunoglobulin is derived from plasma pools of human donors. This requires the immunization of healthy donors, for whom emergency treatment with RhD-positive blood is no longer possible as a result. Anti-D immunoglobulin is not produced in Germany, which challenges the sense of the regulations of the German Transfusion Act regarding supply mandate, donor selection, and donor immunization [24]. The official reports of the Paul Ehrlich Institute [25] show that in 2011 a total of 97 kg of anti-D immunoglobulin was completely imported from non-European countries and sold in Germany.

Administration of antenatal anti-D prophylaxis according to indication

The present report could only use 2 studies presented as supplementary or additional information to investigate the question whether antenatal anti-D prophylaxis is principally useful. Based on these 2 studies with only very limited informative value, the benefit remains unclear. However, this should not be interpreted to mean that this prophylaxis should be removed from maternity care. Since additional antenatal anti-D prophylaxis presumably carries an at most minimal risk of harm, it seems to be acceptable to attach greater weight to the possible benefit, as has been stipulated for many years by the Federal Joint Committee (G-BA) in the maternity guidelines.

Influence on the adherence in indicated antenatal anti-D prophylaxis

It is conceivable that the implementation of the test and the resulting targeted use of indicated anti-D prophylaxis can improve the adherence of pregnant women. Data to support this assumption were not identified in the framework of this benefit assessment, however.

Danger of risk constellations without indicated antenatal anti-D prophylaxis

The present assessment identified no studies to prove the benefit or harm of antenatal anti-D prophylaxis. Hence, there was also no proof that the prenatal test could lead to harm in pregnant women with false-negative test result. This can also not be excluded on the basis of the available data, however. An estimation of corresponding potential harm is therefore conducted below.

There were 737 575 live births in Germany in 2015 [5]. The current approach ensures that all RhD-negative pregnant women with RhD-positive fetuses, i.e. 8.2% of the pregnant women (about 60 500), receive antenatal anti-D prophylaxis [1]. With about 60 500 pregnant women who have an indication in Germany per year, 22 to 319 cases would be expected to have a false-negative test result and do not receive prophylaxis; this calculation is based on the estimated sensitivity of 99.9% (95% CI [99.5; 100]).

Some of the pregnant women have a risk of sensitization if indicated antenatal anti-D prophylaxis is omitted. In the 2 studies presented as additional information, 13 sensitizations (about 1%) before birth were observed in a total of 1185 RhD-negative pregnant women in the control groups.

Assuming 22 to 319 pregnant women who, despite having an indication, would not receive antenatal anti-D prophylaxis due to a false-negative prenatal test in Germany, this would mean that an addition 0 to 3 cases of sensitization during pregnancy per year could be expected after implementation of the prenatal test.

Sensitizations can also occur despite administration of antenatal prophylaxis. After analysis of the studies it is unclear by how many the number of sensitizations would be reduced by the administration of antenatal anti-D prophylaxis. No sufficient data for a valid estimation of the effect could be found (see Section 4.2.4). When classifying the importance of potential additional sensitizations it should also be taken into account that, on the one hand, the proportion of women with subsequent pregnancy is about 50% and, on the other, not every sensitization leads to haemolytic anaemia of the fetus or newborn (in case of rhesus incompatibility, about 60% of the fetuses develop haemolytic anaemia while still in the womb [maximum assumption based on [1]). In theory, additional cases of haemolytic anaemia of fetuses or newborns due to omission of antenatal anti-D prophylaxis can therefore be expected after implementation of the prenatal test. The magnitude would probably be barely measurable, however.

Consequences of the testing strategy on the administration of postnatal anti-D prophylaxis

It was noted in the commenting procedure that 2 studies [10,16] showed that postnatal testing for the RhD status of the newborn can also produce false-negative results. These errors were partly caused by sample mix-ups between mother and child in the delivery room. It was explained in the commenting procedure that this is notably more unlikely in prenatal testing as only one sample (from the mother) is used. Furthermore, postnatal serological testing in the studies did not find RhD-negative variants with weak antigen expression and rated the samples as negative. These were identified by genotyping in the prenatal test, however. These RhD variants may lead to sensitization of the woman [22,26] and are therefore designated as RhD-positive also by the German CPG on haemotherapy [23]. In the oral hearing, such postnatal false-negative test results were mentioned as one of the possible causes for about 300 cases of RhD incompatibility per year that still occur in Germany despite prophylaxis.

The consequences of the 2 options for implementing the prenatal test regarding postnatal anti-D prophylaxis are addressed below.

Postnatal anti-D prophylaxis in case of at least one RhD-positive test result in the prenatal or postnatal test

The CPG on haemotherapy published by the German Medical Association in 2017 [23] considers antenatal anti-D prophylaxis in pregnant women as not required if the fetus is found to be RhD-negative in a validated procedure. It further recommends RhD typing after birth, preferably using umbilical cord blood.

The result of the prenatal test could be integrated into the postnatal testing strategy to identify women with indication for postnatal anti-D prophylaxis by rating all cases with discordance between the prenatal and the postnatal test as positive. Thus, the use of the prenatal test could in principle prevent affected women from not receiving postnatal anti-D prophylaxis despite indication. Since both prenatal and postnatal test have very high sensitivity, however, the magnitude of haemolytic anaemia of fetuses or newborns prevented by double testing would probably be barely measurable, and be at most in a single-digit range per year.

Postnatal anti-D prophylaxis for all women who tested RhD-positive in prenatal testing

2 studies [10,16] showed that the postnatal test produces false-negative results of a similar magnitude as the prenatal test. In both studies, there were even more false-negative results in the postnatal test than in the prenatal test (Section 4.4.5). The proportion of false-negative results both in the prenatal and in the postnatal test were in the parts per thousand range, and it can be assumed that the proportion of haemolytic anaemia in fetuses or newborns is even notably lower. Hence, replacing the postnatal test with the prenatal test would increase neither the rate of anti-D prophylaxis that is falsely withheld nor the rate of haemolytic anaemia of fetuses or newborns to a measurable extent.

In Denmark and the Netherlands, postnatal testing was replaced with prenatal testing alone, which then also guides postnatal anti-D prophylaxis [10,11]. It should be noted that, in these countries, the screening programme had been evaluated before abolishment of the postnatal test was recommended. It could be recommendable also for Germany to have an evaluation phase, in which the sensitivity of the non-invasive prenatal test within the health care structure in Germany is assessed, before deciding whether or not the postnatal test is completely dispensable.

Publication bias

The available data provide no indications of the presence of publication bias.

6 Conclusion

Diagnostic accuracy of the prenatal test

The analysis of 11 studies on diagnostic accuracy of non-invasive testing for the fetal rhesus factor showed that the test has a very high sensitivity of 99.9% (95% CI [99.5; 100]) and a very high specificity of 99.1% (95% CI [98.4; 99.5]). 10 of the 11 studies had a high risk of bias; the pooled estimation of the diagnostic accuracy from all studies was comparable with that of the study with low bias, however. Prenatal and postnatal testing for the rhesus factor can be considered equivalent. It was observed in the present subgroup analyses that the prenatal test before 8 or 11 weeks of gestation showed markedly lower sensitivity in 2 of 3 studies. Regarding multiple pregnancies, there were 2 usable studies, from which no conclusion on the influence of this factor could be inferred, however.

Guiding antenatal anti-D prophylaxis

There were no controlled intervention studies investigating non-invasive testing for the fetal rhesus factor to guide anti-D prophylaxis. There were also no studies on the possible harm from non-indicated anti-D prophylaxis. No conclusions on the benefit of indicated antenatal anti-D prophylaxis could be drawn from 2 controlled intervention studies on the administration of antenatal anti-D prophylaxis (with a dosage that is not approved in Germany), which were only considered as supplementary information.

Hence, the patient-relevant benefit or harm of non-invasive testing for the fetal rhesus factor to guide antenatal anti-D prophylaxis is unclear. Due to the high sensitivity of the prenatal test, only a small number of anti-D prophylaxes erroneously not given before birth could be expected. The corresponding adverse effects would therefore be estimated as immeasurably small even if antenatal anti-D prophylaxis does have a benefit. Ethical, resource-based or economic advantages due to omission of non-indicated antenatal anti-D prophylaxes were not subject of this assessment.

Guiding postnatal anti-D prophylaxis

The above results on diagnostic accuracy of prenatal testing for the rhesus factor were based on the comparison with postnatal test results as the reference standard. However, 2 studies showed that the postnatal test produces false-negative results of a similar magnitude as the prenatal test, so that the measured values constitute a marginal underestimation of the true sensitivity of the prenatal test.

Overall, the effects of the implementation of the prenatal test by anti-D prophylaxes erroneously not given and the corresponding adverse effects can be considered immeasurably small. On the one hand, this applies to the testing strategy in which each woman whose child is tested RhD-positive in the prenatal and/or the postnatal test receives postnatal anti-D prophylaxis. On the other, this also applies to a testing strategy in which the prenatal test replaces the postnatal test, and is also used for the decision for or against postnatal anti-D prophylaxis.

In summary, there is no greater benefit or harm for guiding postnatal anti-D prophylaxis if non-invasive testing for the fetal rhesus factor supplements or replaces the currently used postnatal test, i.e. the prenatal test is equivalent to the postnatal test. The assessment of using the test to replace the postnatal test is subject to the condition of a corresponding quality assurance when implementing the prenatal test in Germany.

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Please see full report for full reference list.

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