

Cohort Study Potential PURL Review Form PURL Jam Version

PURLs Surveillance System Family Physicians Inquiries Network

SECTION 1: Identifying Information for Nominated Potential PURL [to be completed by PURLs Project Manager]

A. Citation: de Haas M, Thurik FF, van der Ploeg CP, Veldhuisen B, Hirschberg H, Soussan AA, Woortmeijer H, Abbink F, Page-Christiaens GC, Scheffer PG, Ellen van der Schoot C. Sensitivity of fetal RHD screening for safe guidance of targeted anti-D immunoglobulin prophylaxis: prospective cohort study of a nationwide programme in the Netherlands. *BMJ*. 2016 Nov 7;355:i5789. doi: 10.1136/bmj.i5789. PubMed PMID: 27821701; PubMed Central PMCID: PMC5098549.

B. Link to PubMed abstract: <https://www.ncbi.nlm.nih.gov/pubmed/?term=27821701>

C. First date published study available to readers: 11/7/2016

D. PubMed ID: 27821701

E. Nominated By: Kate Rowland

F. Institutional Affiliation of Nominator: Rush Copley Medical Center

G. Date Nominated: 11/16/2016

H. Identified Through: *British Medical Journal*

I. PURLs Editor Reviewing Nominated Potential PURL: Corey Lyon

J. Nomination Decision Date: 11/23/2016

K. Potential PURL Review Form (PPRF) Type: Cohort Study

L. Assigned Potential PURL Reviewer: Corey Lyon

M. Reviewer Affiliation: University of Colorado

A. Abstract: OBJECTIVE:

To determine the accuracy of non-invasive fetal testing for the RHD gene in week 27 of pregnancy as part of an antenatal screening programme to restrict anti-D immunoglobulin use to women carrying a child positive for RHD DESIGN: Prospectively monitoring of fetal RHD testing accuracy compared with serological cord blood typing on introduction of the test. Fetal RHD testing was performed with a duplex real time quantitative polymerase chain reaction, with cell-free fetal DNA isolated from 1 mL of maternal plasma The study period was between 4 July 2011 and 7 October 2012. The proportion of women participating in screening was determined.

SETTING:

Nationwide screening programme, the Netherlands. Tests are performed in a centralised setting.

PARTICIPANTS:

25 789 RhD negative pregnant women.

MAIN OUTCOME MEASURES:

Sensitivity, specificity, false negative rate, and false positive rate of fetal RHD testing compared with serological cord blood typing; proportion of technical failures; and compliance to the screening programme.

RESULTS:

A fetal RHD test result and serological cord blood result were available for 25 789 pregnancies. Sensitivity for detection of fetal RHD was 99.94% (95% confidence interval 99.89% to 99.97%) and specificity was 97.74% (97.43% to 98.02%). Nine false negative results for fetal RHD

testing were registered (0.03%, 95% confidence interval 0.01% to 0.06%). In two cases these were due to technical failures. False positive fetal RHD testing results were registered for 225 samples (0.87%, 0.76% to 0.99%). Weak RhD expression was shown in 22 of these cases, justifying anti-D immunoglobulin use. The negative and positive predictive values were 99.91% (95% confidence interval 99.82% to 99.95%) and 98.60% (98.40% to 98.77%), respectively. More than 98% of the women participated in the screening programme.

CONCLUSIONS:

Fetal RHD testing in week 27 of pregnancy as part of a national antenatal screening programme is highly reliable and can be used to target both antenatal and postnatal anti-D immunoglobulin use.

B. Pending PURL Review Date: 9/14/2017

SECTION 2: Critical Appraisal of Validity
[to be completed by the Potential PURL Reviewer]

- A. The study address an appropriate and clearly focused question. Well covered
Comments: Yes, to determine the accuracy of non-invasive fetal testing for the RHD gene in week 27 of pregnancy to restrict Rhogam use to those carrying at a RHD positive child.
- B. The two groups being studied are selected from source populations that are comparable in all respects other than the factor under investigation. Not applicable N/A
Comments: The same group received both the intervention (fetal cell free RHD testing) and the comparison (cord blood sampling post-partum). 80% of the cell free group also gave cord blood samples but there was a statistically significant difference in the # of cord blood samples received based on RHD positivity or negativity on cell free testing (40.6% without a cord blood vs. 37.8% with a cord blood sample had a RHD negative test. $P < 0.0001$). Despite this, only those who received both tests were used in the final analysis which was very large and likely unaffected by this.
- C. The study indicates how many of the people asked to take part in it in each of the groups being studied. Well covered
Comments: Well-covered. Yes, since the screening program was nationwide, the researchers tracked and concluded there was a 98% participation rate.
- D. The likelihood that some eligible subjects might have the outcome at the time of enrollment is assessed and taken into account in the analysis. Not applicable
Comments: N/A based on study question. Study question is evaluating how accurately a test performs in determining eligible subjects have a particular outcome.
- E. What percentage of individuals or clusters recruited into each arm of the study dropped out before the study was completed? Adequately addressed. Study was a prospective analysis in which all enrollees received testing, not an RCT with arms. However, 20% of patients who received the cell free RHD testing did not receive cord blood serology. Only the 80% that received both the cell free RHD testing and cord blood sampling could be included in the final analysis.

- F. Comparison is made between full participants and those lost to follow up, by exposure status. Poorly addressed
Comments: See E for further details.
- G. The outcomes are clearly defined. Well covered
Comments: Yes. Main outcomes are sensitivity, specificity, false negative rate, and false positive rate of fetal RHD testing compared with serological cord blood typing; proportion of technical failures; and compliance to the screening program. The authors also report on an indepth analysis that was done on the 9 false negatives and 225 false positives.
- H. The assessment of outcome is made blind to exposure status. Adequately addressed
Comments: Results of cord blood typing were determined without lab technician/researcher knowledge of RHD status. Patients and providers were aware of fetal RHD results at the time of potential cord blood sampling, which likely contributed to the 20% who did not receive cord blood typing.
- I. Where blinding was not possible, there is some recognition that knowledge of exposure status could have influenced the assessment of outcome. Adequately addressed
Comments: This is acknowledged in the results section. The remaining sample size of 25,789 samples who receive both types of testing remains sufficiently large for analysis.
- J. What are the key findings of the study? N=25,789 results. Sensitivity for fetal RHD = 99.94% (95% CI 99.89-99.97%), Specificity for fetal RHD = 97.74% (95% CI 97.43-98.02-99.97%). There were 9 false negative results for fetal RHD testing (e.g. RHD+ fetus to RH-mother in which false negative result could result in failure to give rhogam and risk of alloimmunization). There were 225 false positive results of fetal RHD tests. However, weak RhD expression was seen in 22 cases, meaning the cord blood sampling was actually the false negative in these cases. The NPV for fetal RHD screening was 99.91% (95% CI 99.82-99.95%) and the PPV was 98.60% (95% CI 98.40-98.77%).
- K. How was the study funded? Any conflicts of interest? Any reason to believe that the results may be influenced by other interests? The study was done as a national screening program in the Netherlands and no funding was received.

SECTION 3: Review of Secondary Literature
[to be completed by the Potential PURL Reviewer]
[to be revised by the Pending PURL Reviewer as needed]

Citation Instructions: For up-to-date citations, use style modified from http://www.uptodate.com/home/help/faq/using_UTD/index.html#cite & AMA style. Always use Basow DS on editor & current year as publication year.

Example: Auth I. Title of article. {insert author name if given, & search terms or title.} In: Basow DS, ed. UpToDate [database online]. Waltham, Mass: UpToDate; 2009. Available at: <http://www.uptodate.com>. {Insert date modified if given.} Accessed February 12, 2009. [whatever date PPRF reviewer did their search.]

For DynaMed, use the following style:
Depression: treatment {insert search terms or title}. In: DynaMed [database online]. Available at <http://www.DynamicMedical.com>. Last updated February 4, 2009. {Insert date modified if given.} Accessed June 5, 2009. {search date}

A. DynaMed excerpts

noninvasive fetal RhD genotyping using maternal blood may be accurate ([level 2 \[mid-level\] evidence](#))

based on systematic review with limited reporting of quality assessment
systematic review of 37 studies and 44 protocols using serum, plasma, or fetal cells from 3,261 maternal blood samples for RhD genotyping of fetal DNA
gestational ages at time of sampling varied between studies (ranging 8-42 weeks gestation)
overall prevalence of Rh alloimmunization 25.4%
pooled diagnostic performance of fetal RhD genotyping from maternal blood in analysis of 31 studies and 37 protocols with 3,078 Rh-negative pregnant women

- sensitivity 95.4% (95% CI 92.5%-98.9%)
- specificity 98.6% (95% CI 96.7%-99.9%)
- positive predictive value 99% (95% CI 98.4%-99.9%)
- negative predictive value 92.1% (95% CI 81.8%-97.9%)

Reference - [Am J Obstet Gynecol 2006 Oct;195\(4\):1163](#), commentary can be found in [Am J Obstet Gynecol 2007 Jul;197\(1\):116](#)

B. DynaMed citation/ Title. Author. In: DynaMed [database online]. Available at: access date www.DynamicMedical.com Last Updated:Accessed

Hemolytic disease of the fetus and newborn (HDFN). Ihor Bilyk. DynaMed [database online]. Available at: 13 Sept 17. www.DyanamedMedical.com. Last updated: 15 Aug 2017.

C. Bottom line recommendation or summary of evidence from DynaMed (1-2 sentences)

States fetal RHD genotyping from maternal blood may be accurate based on one systematic review of 37 studies with limited reporting on quality assessment and wide range of gestational age at sampling time (8-42 weeks gestation) written in 2006. Reports fetal RHD testing to have a sensitivity of 95.4%, specificity of 98.6%, PPV of 99%, an NPV of 92.1%.

D. UpToDate excerpts

“Cell free DNA testing — Noninvasive assessment of fetal *RHD* using cfDNA is widely available in the United Kingdom and Europe. It is also available in the United States, but may not be covered by insurance.

The fetal *RHD* genotype is determined by testing a sample of maternal plasma after 10 weeks of gestation. If the fetus is *RHD*-negative and the mother has no additional red cell antibodies, it is not at risk for hemolytic disease of the fetus and newborn (HDFN) and further maternal or fetal monitoring for HDFN is unnecessary. If the fetus is *RHD*-positive, then maternal indirect Coombs titers (ie, indirect antiglobulin test) are obtained serially until a critical titer is reached. (See ['Follow maternal anti-D titers in at risk fetuses until the critical titer is reached'](#) below.)

Fetal cfDNA can be detected in the maternal circulation as early as 38 days of gestation. It comprises 10 to 15 percent of the total cfDNA in the maternal circulation during the late first and early second trimesters, increases with advancing gestation, and disappears soon after delivery. Fetal *RHD* status is determined by evaluation of cfDNA sequences in maternal plasma using a reverse transcriptase PCR. A 2016 meta-analysis of studies of cfDNA for *RHD* determination reported sensitivity of 99.3 percent (95% CI 98.2-99.7) and specificity of 98.4 percent (95% CI 96.4-99.3) in the first and second trimesters; real-time quantitative PCR sensitivity was higher than conventional PCR [4].

Assays for the *RHD* exon 4; exons 5 and 7; exons 4, 5, and 7; or exons 4, 5, 7, and 10 have been recommended, and should be done after approximately 10 weeks of gestation so there will be adequate fetal cfDNA [3,5-9]. Detection of these *RHD* exons in maternal plasma indicates fetal cfDNA is present and the fetus is *RHD*-positive. If *RHD* exons are absent, the fetus is *RHD*-negative as long as it can be proven that fetal cfDNA and not maternal cfDNA was tested (algorithm 2). Identification of Y chromosome gene sequences (*SRY*) in the plasma sample confirms the presence of fetal cfDNA and validates the test results. If the fetus is female, single nucleotide polymorphisms (SNPs) that are not common to the general population can be used to confirm fetal cfDNA in the sample [10]. Maternal SNPs are identified in the white cells (maternal) of the buffy coat. A significant difference in the types of SNPs between the buffy coat and the plasma (at least six different SNPs) indicates paternally-acquired SNPs in the plasma, thereby confirming the presence of fetal cfDNA and validating the test result. The hypermethylated *RASSF1A* promoter has also been reported as a universal fetal marker to confirm the presence of fetal DNA [11-13].

False positive results have been attributed to phenotypically Rh(D)-negative mothers who carry the *RHD* pseudogene or another *RHD* gene variation and pass this gene on to their fetus [14]. The *RHD* pseudogene has been described in 69 percent of South African blacks and 24 percent of African Americans [6]. In this situation, all 10 exons of the *RHD* gene are present; however, translation of the gene into a messenger RNA product does not occur because of a stop codon in the intron between exons 3 and 4. Therefore, no Rh(D) protein is synthesized, and the patient is serologically Rh(D)-negative. The *RHD* pseudogene can often be detected by using primers targeting exons 4 and 10 of the *RHD* gene. Such false positives, if not detected, could result in unnecessary invasive interventions. If *RHD* pseudogenes are suspected, the cfDNA result will return as "indeterminate"; testing fetal DNA in amniocytes is then recommended. In these cases, maternal and paternal blood samples should accompany the amniotic fluid for laboratory analysis. As with any cfDNA test, a vanishing twin or graft-derived cfDNA in women with organ transplantation are other potential sources of false-positive results [15,16].

False negative results would be more serious as appropriate monitoring and interventions might be withheld. More than one D region (eg, exons 7 and 10, intron 4) should be examined to ensure that negative results reflect true *RHD* negativity and not the presence of D variants. False negatives can also be due to a low level of fetal cfDNA in a maternal sample because it was drawn too early in gestation (<8 weeks of gestation) or to insensitive laboratory techniques [17]. We consider the false negative rate of cfDNA sufficiently low that we do not obtain serial titers when cfDNA indicates an *RHD*-negative fetus. False negatives will be identified soon after

delivery as all newborns routinely undergo direct Coombs testing through cord blood sample analysis at birth.

- E. UpToDate citation/ Always use Basow DS as editor & current year as publication year. Access date Title. Author. In: UpToDate [database online]. Available at: <http://www.uptodate.com>. Last updated: Accessed

Editor: Basow DS (Section editor is Charles J Lockwood). Accessed: 13 Sept 2017. Author: Kenneth J Moise Jr. In: UpToDate [database online]. Available at: <http://www.uptodate.com>. Last updated 29 Aug 2017.

- F. Bottom line recommendation or summary of evidence from UpToDate (1-2 sentences)
Up to date provides an algorithm that suggests testing cell free RHD in managing Rh- pregnant patients in whom paternal testing is RHD positive and found to be heterozygous for D or in which paternity is uncertain or paternal blood samples cannot be obtained. That being said, they comment that cell free RHD testing may not be covered by insurance in the U.S. “A 2016 meta-analysis of studies of cfDNA for *RHD* determination reported sensitivity of 99.3 percent (95% CI 98.2-99.7) and specificity of 98.4 percent (95% CI 96.4-99.3) in the first and second trimesters; real-time quantitative PCR sensitivity was higher than conventional PCR [4].”

- G. Other excerpts (USPSTF; other guidelines; etc.)

- H. Citations for other excerpts

- I. Bottom line recommendation or summary of evidence from Other Sources (1-2 sentences)

Evidence cited in uptodate and Dynamed support that cell free DNA may be accurate but that systems barriers such as insurance coverage may be barriers to use.

SECTION 4: Conclusions

[to be completed by the Potential PURL Reviewer]

[to be revised by the Pending PURL Reviewer as needed]

- A. **Validity:** Are the findings scientifically valid? 2 Compared to current gold standard (cord blood sampling), results from fetal RHD not available to those resulting cord blood samples, large sample size.
- B. If **A** was coded 4, 5, 6, or 7, please describe the potential bias and how it could affect the study results. Specifically, what is the likely direction in which potential sources of internal bias might affect the results?
- C. **Relevance:** Is the topic relevant to the practice of family medicine and primary care practice, including outpatient, inpatient, obstetrics, emergency and long-term care? Are the patients being

studied sufficiently similar to patients cared for in family medicine and primary care in the US such that results can be generalized?

3 Topic is very relevant to family medicine practice of routine obstetrics. This study was performed on a large sample of patients in the Netherlands and ethnicity distribution was not similar to that of the U.S. and prevalence of various RHD alleles are stated to vary by ethnic group.

D. If **C** was coded 4, 5, 6, or 7, please provide an explanation.

E. **Practice changing potential:** If the findings of the study are both valid and relevant, are they not a currently widely accepted recommendation among family physicians and primary care clinicians for whom the recommendation is relevant to their patient care? Or are the findings likely to be a meaningful variation regarding awareness and acceptance of the recommendation?

3

F. If **E** was coded as 1, 2, 3, or 4, please describe the potential new practice recommendation. Please be specific about what should be done, the target patient population and the expected benefit.

Ultimately, the decision to replace universal treatment of Rh- pregnant patients with Rhogam in favor of targeting only those with fetal RHD positive cell free maternal DNA depends on cost analysis of the two programs and insurance coverage of the testing. In its current state, insurance barriers and lack of universal screening recommendations prevent this from changing practice. A 2013 analysis of the cost of fetal RHD testing would be \$682/pregnancy vs. \$351/pregnancy in the current approach (Hawk AF. Costs and clinical outcomes of noninvasive fetal RhD typing for targeting prophylaxis. *Obstet Gynecol* 2013; 122:579-85). However, increased use of cell-free DNA testing and economies of scale may have shifted costs over time.

G. **Applicability to a Family Medical Care Setting:**

Is the change in practice recommendation something that could be done in a medical care setting by a family physician (office, hospital, nursing home, etc.), such as a prescribing a medication, vitamin or herbal remedy; performing or ordering a diagnostic test; performing or referring for a procedure; advising, education or counseling a patient; or creating a system for implementing an intervention? 1 (definitely could be done in a medical care setting) It's a blood draw.

H. If **G** was coded as a 4, 5, 6, or 7, please explain.

I. **Immediacy of Implementation:**

Are there major barriers to immediate implementation? Would the cost or the potential for reimbursement prohibit implementation in most family medicine practices? Are there regulatory issues that prohibit implementation? Is the service, device, drug, or other essentials available on the market? 3 Reimbursement. Lack of guideline recommendations to replace universal Rhogam use in Rh- mothers.

J. If **I** was coded 4, 5, 6, or 7, please explain why.

K. Clinically meaningful outcomes or patient oriented outcomes:

Do the expected benefits outweigh the expected harms? Are the outcomes patient oriented (as opposed to disease oriented)? Are the measured outcomes, if true, clinically meaningful from a patient perspective? Could reduce the number of Rh- women requiring rhogam administration.
3

L. If **K** was coded 4, 5, 6, or 7 please explain why.

M. In your opinion, is this a pending PURL? 3

1. Valid: Strong internal scientific validity; the findings appear to be true.
2. Relevant: Relevant to the practice of family medicine.
3. Practice Changing: There is a specific identifiable new practice recommendation that is applicable to what family physicians do in medical care settings and seems different than current practice.
4. Applicability in medical setting.
5. Immediacy of implementation

N. Comments on your response for question M.