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ORIGINAL ARTICLE

WILEY PRENATAL DIAGNOSIS

The near disappearance of fetal hydrops in relation to current state-of-the-art management of red cell alloimmunization

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Abstract

Objective: In this study, we aim to evaluate trends in the condition of fetuses and neonates with hemolytic disease at the time of first intrauterine transfusion (IUT) and at birth, in relation to routine first-trimester antibody screening, referral guidelines, and centralization of fetal therapy.

Method: We conducted a 30-year cohort study including all women and fetuses treated with IUT for red cell alloimmunization at the Dutch national referral center for fetal therapy.

Results: Six hundred forty-five fetuses received 1852 transfusions between 1 January 1987 and 31 December 2016. After the introduction of routine first-trimester antibody screening, the hydrops rate declined from 39% to 15% (OR 0.284, 95% CI, 0.19-0.42, $P < 0.001$). In the last time cohort, only one fetus presented with severe hydrops (OR 0.482, 95% CI, 0.38-0.62, $P < 0.001$). Infants are born less often <32 weeks (OR 0.572, 95% CI, 0.39-0.83, $P = 0.004$) and with higher neonatal hemoglobin ($P < 0.001$). Neonatal hemoglobin was positively independently associated with gestational age at birth, fetal hemoglobin, and additional intraperitoneal transfusion at last IUT.

Conclusion: Severe alloimmune hydrops, a formerly often lethal condition, has practically disappeared, most likely as a result of the introduction of routine early alloantibody screening, use of national guidelines, and pooling of expertise in national reference laboratories and a referral center for fetal therapy.

1 | INTRODUCTION

Hemolytic disease of the fetus and newborn (HDFN) is a serious complication in pregnancy that has long been a major cause of perinatal mortality. The disease is caused by maternal alloimmunization to fetal red blood cell antigens, mostly concerning D, followed by K, c, and E.[1] The maternal antibodies may cross the placenta and cause fetal hemolysis and anemia, which, if untreated, may lead to fetal heart failure, hydrops, and death.

In the 1980s, important progress in the treatment of fetuses with severe HDFN was made by the introduction of intravascular intrauterine transfusion (IUT), now the cornerstone in prenatal management.[2] However, survival of especially severely hydropic fetuses remained significantly lower than that of anemic fetuses without hydrops.[3] Severe hydrops is furthermore associated with neurodevelopmental impairment on the long term.[4] Preventing hydrops is therefore of utmost importance to improve both survival and long-term outcome.

In the first 3 years after the introduction of intravascular IUT in the Netherlands (1987-1989), the majority of cases (55%) presented with hydrops.[5] A recent study from our group showed that the incidence of fetal alloimmune hydrops has declined to approximately 13%.[6]

In this study, we aimed to evaluate trends in the condition of fetuses and newborns with hemolytic disease at the time of first IUT and at birth and discuss possible contributing factors.

What's already known about this topic?

- Severe alloimmune hydrops is associated with impaired outcome on both the short term and the long term.

What does this study add?

- With routine early alloantibody screening, national guidelines, and pooling of expertise in national reference laboratories and a referral center for fetal therapy, severe alloimmune hydrops has almost disappeared.
- Survival of hydrops cases no longer differs from hemolytic disease of the fetus and newborn cases without hydrops.
- Children treated with intrauterine transfusions are now in better condition at birth.

2 | METHODS

2.1 | Study design, setting, and study population

We conducted a retrospective cohort study including all patients with red cell alloimmunization requiring IUT in the Leiden University Medical Center (LUMC) from January 1987 until January 2017. The LUMC serves as the Dutch national referral center for fetal therapy since 1965. In the Netherlands, the annual live born number was 187 000 in 1987 and 173 000 in 2016 (with a maximum of 206 000 in 2000).[7] The prevalence of a clinically relevant alloantibody (father positive for the antigen) at first-trimester screening was 0.14% to 0.33% in 2016.[8]

Patient data, IUT details, and information on pregnancy outcome of these patients were collected from our electronic prospectively filled Rhesus database. Cases were excluded if fetal or neonatal death occurred from causes other than red cell immunization. Diagnostic fetal blood samplings not followed by IUT, owing to adequate pretransfusion fetal hemoglobin, were excluded. If the diagnostic fetal blood sampling confirmed fetal anemia, but the following transfusion failed due to technical difficulties or complications, the procedure was not excluded.

Suspected severe fetal anemia requiring IUT was defined as (1) a peak systolic velocity in the fetal middle cerebral artery (MCA) of 1.5 multiples of the median for gestational age, detected by Doppler measurement, and/or (2) the presence of other signs of anemia at ultrasound examination (cardiomegaly, ascites, hydrops), or (3) amniotic fluid delta optical density measurements reaching the upper part of the Liley zone II or zone III (only in the early years of this study).

2.2 | Prevention, screening, diagnostics, and treatment

As a primary preventive measure, anti-D immunoglobulin (RhIg) is administered to all D-negative women, carrying a D-positive child, at 30 weeks' gestation (since 1998) and after birth (since 1969).[1] RhIg prophylaxis has nowadays reached a coverage of approximately 100% in the Netherlands.[9]

To prevent non-D immunizations, blood transfusions administered to women of reproductive age (<45 y) are K- (since 2004), c-, and E-matched in the Netherlands (since 2011).[10] The order of implementation of these and other preventive measures for red cell immunization in the Netherlands is summarized in Table S1.

Despite these preventive measures, alloimmunization may still occur. Since the late 1960s, D-negative women are routinely screened for the presence of alloantibodies at week 32 of pregnancy. This screening was brought forward to 30 weeks in 1998 and again forward to 27 weeks in 2011. From 1998 onwards, all pregnant women (D-negative and D-positive) are routinely screened for antibodies in the first trimester. Since 2011, the 27 weeks' screening was furthermore broadened to include c-negative women.[11]

If screened positive, the antigen status of the fetus is first assessed by testing the paternal zygosity or by cell-free DNA testing in maternal plasma (available for D, c/C, E, and K). If the fetus is assumed or determined to be antigen-positive, antibody titers and antibody-dependent cell-mediated cytotoxicity (ADCC) are measured every 2 weeks, to assess the risk of fetal hemolysis. All laboratory tests are performed at Sanquin Blood Supply in Amsterdam or the Special Institute for Blood Group Investigation in Groningen, the two national referral laboratories.[11,12]

In case of a high risk of severe hemolysis, indicated by serological testing or abnormal ultrasound findings, national obstetric guidelines advise the patient to be referred to the LUMC. The current screening and prevention protocol in the Netherlands is summarized in a recent review of de Haas et al and in a national obstetric guideline.[1,12] Patients with D antibodies are to be referred to the LUMC when titers grossly rise above 1:16 or when the ADCC is higher than 50% (>1:16 and >30% for other Rh antibodies and 1:2 and 30% for anti-K). The management of pregnancies complicated by HDFN in our center consists of weekly monitoring with MCA Doppler until anemia is suspected and intervention is needed, and information is described in more detail previously.[6,13]

2.3 | Data collection and outcome definitions

Data on maternal characteristics, primary antibody type, treatment details, the severity of fetal hemolytic disease (fetal Hb and presence of hydrops at first IUT), and perinatal outcome were collected.

In order to identify changes over time, all pregnancies were divided into time groups of 6 years each, based on the year of first IUT (1987-1992, 1993-1998, 1999-2004, 2005-2010, and 2011-2016).

Hydrops was classified as mild, if a distinct rim of ascites in the absence or presence of pericardial effusion was seen, whereas abundant ascites, in the absence or presence of pericardial and pleural effusion and skin edema, was considered as severe hydrops.[3]

Primary outcome was the presence of hydrops (total, mild, and severe) at the time of first IUT. Furthermore, we assessed the fetal (Z)Hb concentration at first IUT, which is the number of standard deviations of fetal Hb from the mean for gestational age (1 SD = 1 g/dL deviation).[14] Other outcome measures were as follows: perinatal survival, defined as surviving the first month of life or surviving hospitalization (when hospitalized >1 mo after birth), neonatal Hb, and the proportion of neonates requiring postnatal exchange transfusion(s).

Perinatal survival in consecutive time cohorts was assessed for all fetuses and for those with or without alloimmune hydrops.

2.4 | Ethical considerations

Only the caregivers knew the identity of their patients, and study data were analyzed anonymously. Therefore, the medical ethics committee of the LUMC approved this research (C15.094) and decided, according to the Medical Research Involving Human Subjects Act (WMO), that written informed consent was not needed.

2.5 | Statistical analysis

As outcome of multiple pregnancies in the same woman might be interrelated, outcomes were compared using generalized estimating equations (GEE). Within the GEE, a linear, binary logistic or ordinal logistic model was used for comparison of estimated means or odds, respectively. "Time cohort" was used as a continuous predictor to assess a linear trend in variables over time, rather than an overall difference in the variable between all time groups.

Before the use of MCA Doppler became the gold standard to set the indication for IUT around 2000, amniotic fluid delta optical density measurements were used. These are now known to be less accurate in predicting moderate to severe anemia,[15] and therefore, a sensitivity analysis was performed for our main outcome analysis, with only patients treated from 2000 onwards.

Numerical outcomes that were not normally distributed were transformed to normality (log2 transformation for titers, gestational age at birth raised to the power of 10).

If important outcomes showed a significant trend over time, we assessed possible factors associated with this outcome by multivariate regression using backward selection (Table S2). The choice for inclusion of variables was made on theoretical grounds, and a *P* value of >0.1 was used for exclusion of variables.

P values < 0.05 were considered statistically significant.

3 | RESULTS

3.1 | Characteristics of the study population

During the 30-year study period, IUTs were started in 653 fetuses in 645 pregnancies of 551 women. There were eight twin pregnancies in which both twins were treated with IUT and one more in which only one fetus required IUTs; the cotwin was D-negative. Six singleton pregnancies were excluded because fetal death occurred from causes other than red cell alloimmunization, described in more detail previously.[16] Two more cases were excluded, as fetal blood sampling did not reveal fetal anemia, and therefore, no transfusion was performed.

A total of 645 fetuses remained for analysis in 637 pregnancies of 543 women, receiving a total of 1852 transfusions. In the first time cohort, fetuses received a median of 4 (range 1-8) transfusions per pregnancy, declining to 3 (range 1-6) in the last time cohort (*P* = 0.003); 55% of fetuses treated with IUT were male, and 45% female. Table 1 shows overall baseline characteristics of these pregnancies and trends in characteristics over time.

TABLE 1 Trends in characteristics of 645 fetuses and 637 pregnancies treated with intrauterine transfusion over time

Group	Total N = 645	1987-1992 N = 92	1993-1998 N = 127	1999-2004 N = 159	2005-2010 N = 152	2011-2016 N = 115	*P*
Number of IUTs per fetus[a]	3 [1-8]	4 [1-8]	3 [1-6]	3 [1-6]	3 [1-6]	3 [1-6]	0.003
Maternal age at first IUT	32 ± 4.5	31 ± 4.2	32 ± 4.4	32 ± 4.3	32 ± 5.0	32 ± 4.4	0.125
Antibody against							0.074[b]
D	524 (81)	80 (87)	110 (87)	126 (80)	113 (74)	95 (83)	
K	83 (13)	7 (8)	11 (9)	22 (14)	29 (19)	14 (12)	
c	23 (4)	4 (4)	4 (3)	8 (5)	6 (4)	1 (1)	
Other[c]	15 (2)	1 (1)	2 (2)	3 (2)	4 (3)	5 (4)	
GA at first IUT, total	27 + 3 [16-36]	26 + 4.5 [18-34]	28 + 1 [17-35]	27 [17-36]	27 + 4.5 [16-35]	28 + 2 [16-35]	0.408[d]
D immunizations	27 + 5 [16-36]	26 + 4.5 [18-34]	28 + 1.5 [17-35]	27 + 0.5 [17-36]	28 + 6 [16-35]	28 + 3 [17-35]	
K immunizations	24 + 2 [16-35]	21 + 1 [18-30]	24 [20-31]	23 + 4 [19-31]	25 + 4 [18-35]	26 + 5 [16-33]	

Data in n (%), mean ± standard deviation, or median [range] if not normally distributed.

Abbreviation: GA, gestational age; IUT, intrauterine transfusion.

[a]If born alive.

[b]Ordinal logistic generalized estimating equations.

[c]Other includes E, e, Fya, Jka, and very infrequent antibodies.

[d]Overall GA over time.

3.2 | Trends in fetal condition at first transfusion over time

All primary and secondary outcomes are shown in Table 2. In the total 30-year cohort, at the time of first transfusion, 23% of fetuses showed signs of hydrops. Hydrops was classified as mild in 14.4% and as severe in 8.8%. The incidence of hydrops declined significantly over time, from 41% in the first time cohort (1987-1992) to 6% in the final cohort, 2011-2016 (OR for the presence of hydrops in a later time cohort 0.556, 95% CI, 0.48-0.65, $P < 0.001$). In Figure 1, the proportion of fetuses with hydrops (mild and severe) at first transfusion is shown over time. Trends in hydrops are shown separately for D and K immunizations.

The great majority of cases with hydrops were associated with D, K, or c immunization (97%). Furthermore, we noted one hydrops case with anti-e, one with anti-Fy^a, and three cases in pregnancies with antibodies against low frequent red blood cell antigens: one anti-Cw and two pregnancies of a woman with "Verdegaal" alloimmunization.

After the introduction of routine first-trimester antibody screening in 1998, the hydrops rate declined significantly, from 39% (up to 1998) to 15% (after 1998, OR 0.284, 95% CI, 0.19-0.42, $P < 0.001$); and the incidence of severe hydrops dropped from 18% to 4% (OR 0.204, 95% CI, 0.11-0.37, $P < 0.001$). From 1999 onwards, 21 out of 65 fetuses (32%) with K immunization developed hydrops despite routine screening, compared with 40/334 (12%) of fetuses affected by anti-D (OR 3.508, 95% CI, 1.82-6.78, $P < 0.001$). The median gestational age at first transfusion in K-affected pregnancies was approximately 3 weeks earlier than in D immunizations (24 wk and 6 d, range 16-35, vs 27 wk and 6 d [16-36], $P < 0.001$).

3.3 | Sensitivity analysis

A sensitivity analysis was performed with only cases included treated from the year 2000 onwards, thus excluding cases in which the decision for IUT was based on the Liley curve. For this analysis, the time cohort 1999-2004 was thus adjusted to 2000-2004, in which 32/138 (23%) of cases presented with hydrops at first IUT, significantly decreasing to 7/115 (6%) (OR for the presence of hydrops in a later time cohort 0.459, 95% CI, 0.30-0.70, $P < 0.001$).

3.4 | Substandard factors in the management of cases with severe hydrops

To identify possible substandard care factors that might have contributed to the development of hydrops, charts of the 18 pregnancies that presented with severe hydrops after the implementation of first-trimester screening were reviewed.

Eleven of these 18 patients (61%) were treated with IUT within a day after presentation with severe hydrops at our fetal therapy center, indicating a possible late referral. Detailed information was not available for one of these cases, and in two cases, hydrops resulted from patient delay (first prenatal visit at 20 and 25 wk, despite two previous affected pregnancies). In a fourth patient, first-trimester screening was negative, but severe hydrops was noted at a routine ultrasound around week 29 and appeared to result from immunization to a not previously detected private antigen (Verdegaal). The other seven patients were subject to protocol violations: delays in respectively diagnostics (N = 1, blood sent to reference laboratory a month after positive screening in regional laboratory, father not typed for antigen against which antibodies were formed) and referral from midwife to gynecologist despite positive screening (N = 3) and from gynecologist to our tertiary center although serological risk assessment prescribed referral (N = 3). Most of these cases (5/7) occurred in the first 3 years after screening implementation.

Seven out of the 18 cases were timely referred to our center but still developed severe hydrops. Management factors that may be associated with the development of severe hydrops in these cases are as follows: alternating weekly monitoring with the referring hospital, not yet optimally skilled in MCA Doppler measurements (four cases prior to 2000), postponing IUT to optimize birth timing despite amniotic fluid delta optical density measurement in "Liley 2c" zone (one case), and lack of monitoring in the time span awaiting paternal genotyping in a case with high risk of anemia (ADCC > 80%). No substandard care factors were found in the last patient.

3.5 | Trends in survival and neonatal condition over time

Trends in survival of hydropic and nonhydropic fetuses are shown in Figure 2 and Table 2. Survival of fetuses without hydrops did not increase significantly (from 49/54 [91%] in the first 6 years to 101/105 [96%]) in the last 6 years of the study (OR for survival in a later time cohort 1.343, 95% CI, 0.89-2.02, $P = 0.158$), while survival of fetuses with hydrops increased from 24/38 (63%) to 6/6 (100%, OR 2.635, 95% CI, 1.37-5.07, $P = 0.004$).

The rate of children born very prematurely (before 32-wk gestation) declined significantly over time, from 10% of all live-born children in the early years to 2% in the last time cohort (OR for very premature birth in a later time cohort 0.572, 95% CI, 0.39-0.83, $P = 0.004$). Hemoglobin at birth increased significantly over time, from 9.2 to 12.6 g/dL in the last cohort ($P < 0.001$). Multivariate regression was performed to assess factors contributing to this rise; factors included in this analysis were as follows: number of IUTs performed, type of antibody (D or K), highest antibody titer, gestational age at birth, time interval between the last IUT and birth, transfusion volume (last IUT), additional intraperitoneal transfusion (last IUT), and pretransfusion fetal hemoglobin at last IUT (Table S2). With the use of backward selection with a P-out of 0.1, gestational age at birth and additional intraperitoneal transfusion and pretransfusion fetal hemoglobin at last IUT remained and were positively independently associated with a higher hemoglobin at birth (Hb 0.187 g/dL higher with every week gestational age, $P = 0.010$, Hb 1.3 g/dL higher after intraperitoneal transfusion, $P < 0.001$, and Hb 0.62 g/dL higher/point less fetal Hb deficit, and $P < 0.001$). In the first years of the study, only 1.1% of patients received an additional intraperitoneal transfusion, increasing to 37.4% in the last time cohort.

Currently, only 17% of neonates treated with IUTs receive one or more exchange transfusions after birth, significantly declining from

TABLE 2 Outcome of 645 fetuses over time

Group	Total N = 645	1987-1992 N = 92	1993-1998 N = 127	1999-2004 N = 159	2005-2010 N = 152	2011-2016 N = 115	OR per Time Cohort (95% CI)	*P*
Hydrops	150 (23)	38 (41)	47 (37)	40 (25)	18 (12)	7 (6)	0.556 (0.48-0.65)	<0.001
Mild	93 (14)	18 (20)	28 (22)	28 (18)	13 (9)	6 (5)	0.690 (0.59-0.81)	<0.001
Severe	57 (9)	20 (22)	19 (15)	12 (8)	5 (3)	1 (1)	0.482 (0.38-0.62)	<0.001
Overall survival*	598/641 (93)	73/92 (79)	115/127 (91)	157/159 (99)	146/152 (96)	107/111 (96)	1.898 (1.37-2.37)	<0.004
Without hydrops	473/492 (96)	49/54 (91)	75/80 (94)	118/119 (99)	130/134 (97)	101/105 (96)	1.343 (0.89-2.02)	0.158
With mild hydrops	87/92 (95)	16/18 (89)	27/28 (96)	27/28 (96)	12/13 (92)	5/5 (100)	1.379 (0.52-3.67)	0.518
With severe hydrops	38/57 (67)	8/20 (40)	13/19 (68)	12/12 (100)	4/5 (80)	1/1 (100)	3.343 (1.28-8.71)	0.013
Fetal Hb at first IUT, g/dL	5.9 ± 2.4	5.0 ± 2.2	5.2 ± 2.5	5.6 ± 2.3	6.4 ± 2.2	6.8 ± 2.6	…	<0.001
ZHb at first IUT[a]	−7.2 ± 2.2	−7.9 ± 2.2	−8.0 ± 2.2	−7.4 ± 1.9	−6.7 ± 1.9	−6.2 ± 2.2	…	<0.001
GA at last transfusion	33 + 5 [16-37]	32 + 6 [18-37]	34 [18-37]	33 + 6 [26-36]	34 [16-36]	32 + 3 [21-36]	…	0.114
Time between last transfusion and birth, d	19 [0-99]	13 [0-29]	14 [0-99]	19 [0-57]	20 [0-56]	21 [0-91]	…	<0.001
GA at birth[b]	36 + 3 [28-39]	35 + 6 [29-39]	36 + 2 [30-39]	36 + 2 [28-39]	36 + 5 [31-38]	36 + 4 [29-39]	…	<0.001[c]
Births before 32-wk gestation	23 (4)	8 (10)	5 (4)	7 (4)	1 (1)	2 (2)	0.572 (0.39-0.83)	0.004
Neonates requiring exchange transfusion(s)[b]	298 (51)	68 (91)	76 (64)	94 (64)	42 (29)	18 (17)	0.420 (0.36-0.49)	<0.001
Hb at birth, g/dL	11.3 ± 2.7	9.2 ± 2.5	11.0 ± 2.5	11.2 ± 2.3	11.9 ± 2.7	12.6 ± 2.6	…	<0.001[d]

Data in n (%) or mean ± SD.

Abbreviation: GA, gestational age; IUT, intrauterine transfusion.

*Survivors/total number of fetuses with a specific hydrops category (no/mild/severe).

[a]Number of standard deviations from the mean Hb for gestational age (1 SD = 1 g/dL).

[b]If born alive.

[c]Raised to the power of 10 to achieve normal distribution.

[d]Linear generalized estimated equation adjusted for gestational age at birth.

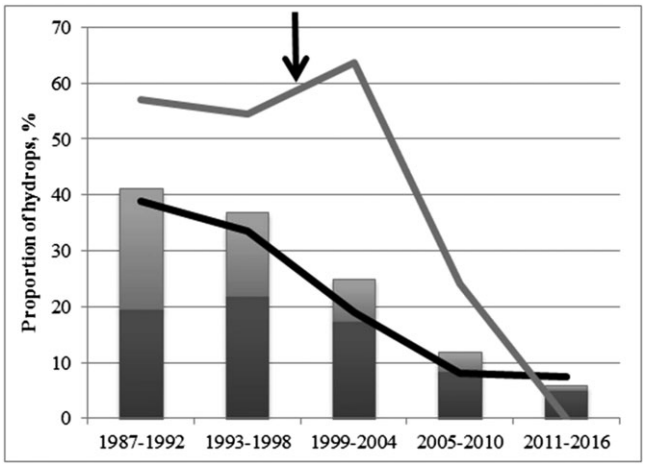

FIGURE 1 Proportion of fetuses with hydrops at time of first intrauterine transfusion. The proportion of fetuses with alloimmune hydrops at first intrauterine transfusion, out of all fetuses treated with intrauterine transfusion, is presented in columns per time cohort. Light grey means severe hydrops, and dark grey mild hydrops. The lines reflect the proportion of hydrops cases from fetuses with D (black) and K (grey) immunization. The introduction of routine first-trimester antibody screening is marked by an arrow

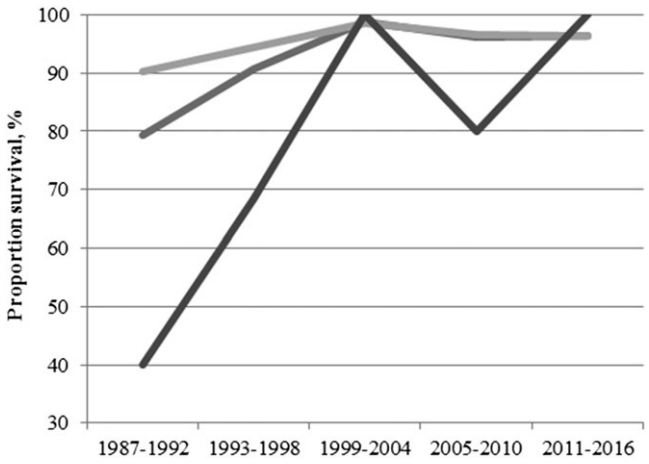

FIGURE 2 Survival rates of fetuses with and without hydrops. Lines reflect the proportion of fetuses treated with intrauterine transfusion for red cell alloimmunization that survived up to 28 d after birth (or until hospital discharge if admitted more than 28 d). The lightest grey is the total cohort, the darker grey is the fetuses without hydrops, and the darkest line reflect fetuses with mild or severe hydrops at time of first intrauterine transfusion

91% in the first cohort (OR for receiving exchange transfusion(s) in a later time cohort 0.420, 95% CI, 0.36-.49, $P < 0.001$).

4 | DISCUSSION

This 30-year cohort study describes factors that contribute to the gradual and near disappearance of severe alloimmune hydrops, a serious condition that has negatively influenced fetal survival and long-term outcome for decades. The hydrops rate at first IUT keeps declining since the introduction of routine first-trimester antibody screening in 1998. In the last years of this study, less than 1% (1/115) of fetuses suffered from severe hydrops. Survival of fetuses with and without hydrops is nowadays almost equal, rising up to or above 95%. Furthermore, fetuses are nowadays born less anemic, which was associated with additional intraperitoneal transfusion at the last IUT.

Since the introduction of early screening of all pregnant women in the Netherlands in 1998, the overall hydrops rate in fetuses requiring IUT declined from 39% to 15%, and the incidence of severe hydrops reduced more than fourfold, from 18% to 4%. This impressive currently low incidence of fetal hydrops indicates that fetuses with HDFN are nowadays identified and treated more timely. We hypothesize that this is mainly due to the implementation of national guidelines on management of alloimmunization in pregnancy, covering screening, laboratory, and clinical monitoring and protocols for timely referral to the (single) fetal therapy center. The hydrops rates we found are comparable with those reported in other Western countries with similar screening programs, which vary between 8% and 16%.[17-23] A study from Belgium also reported severe hydrops in 5% of red cell–immunized pregnancies.[18]

Although the hydrops rate in pregnancies complicated by K antibodies has declined impressively since the introduction of routine first-trimester antibody screening, the odds of developing hydrops are still 3.5 times higher than when D immunization and transfusions are performed approximately 3 weeks earlier in anti-K cases. To ensure timely referral to a tertiary center and prevent hydrops, fast determination of the fetal phenotype in K immunizations is therefore of uttermost importance.

In developing countries, where routine early antibody screening is less well organized or lacking,[24] the incidence of hydrops at time of first IUT is remarkably higher. A group from Brazil reported a 34% hydrops rate in their cohort with mainly D-immunized pregnancies, which they contributed to late referral.[25] In the largest tertiary referral center in India, the hydrops rate was 22% in patients with D immunization requiring IUT between 2011 and 2014. Equal to our findings, survival was approximately the same for hydropic and nonhydropic fetuses in this cohort (94% vs. 90%, respectively).[26]

An additional explanation for the declining incidence of hydrops is the optimization of diagnostic accuracy to predict severe fetal anemia over time. The previously used amniotic fluid delta optical density measurements[27] showed limited accuracy in the second pregnancy trimester and in K-immunized pregnancies[15,28] and were gradually replaced by measuring the peak flow velocity in the MCA by Doppler ultrasound, which is standard diagnostic care since around 2000.[29] To account for this change in diagnostic management, we performed a sensitivity analysis with only cases included from 2000 onwards. This analysis showed a similar result to the main analysis: OR for the presence of hydrops in a later time cohort 0.459, $P < 0.001$.

An important finding is that survival of fetuses with hydrops increased significantly, leading to a similar survival rate in hydropic and nonhydropic fetuses in recent years. This confirms findings by others[23,26] and is likely partly due to early detection and timely referral for treatment of fetal anemia. Thus, hydrops, if it occurs at all, is nowadays usually only mild, which is associated with favorable outcome. Furthermore, the increase in survival is most profound in cases with severe hydrops (OR 3.3). A more advanced gestational age at

birth in later years of the study and/or advancements in prenatal and especially neonatal management in the past decades may have contributed, as well as a decrease in IUT complications.[6] Centralization of fetal therapy and neonatal care in large volume centers is increasingly important for quality of care.[30]

Infants born alive after treatment with IUT(s) seem to be in better neonatal condition nowadays, being less anemic at birth (despite longer intervals between the last transfusion and birth) and needing less exchange transfusions. We found that both additional intraperitoneal transfusion, in adjunct to intravascular transfusion, and more advanced gestational age at birth were independently associated with higher neonatal hemoglobin. As we previously demonstrated, transfusions through the intrahepatic route gained popularity over the years and are associated with less procedure-related complications.[6] An additional advantage of this transfusion technique is the possibility to deposit some extra blood intraperitoneally with the aim to prolong the interval between transfusions or between the last transfusion and birth.[31]

We hypothesize that the found reduction in need for exchange transfusions is mainly caused by the gradual replacement of this invasive procedure by high-quality intensive phototherapy and the corresponding more restrictive exchange transfusion protocol in 2004.[32]

This study is conducted in a well-organized health care system for alloimmunized pregnant women. The centralization of screening and treatment gives us great insight and overview in the prevalence of alloimmunization and the details of intrauterine therapy. A limitation might be that we were not able to include fetuses with hydrops, but without IUT. It is therefore possible that some women lost their fetuses to severe hydrops before referral to our national referral center or that labor was induced before performing an IUT. We hypothesize that the subsequent underestimation of hydrops deaths will be greatest in the early years of the study, as this scenario is highly unlikely in the present screening setting.

5 | CONCLUSION

In summary, the incidence of alloimmune fetal hydrops has decreased importantly over the past decades, most likely as a result of the introduction of early screening for alloantibodies in all pregnancies, use of national guidelines, and the availability of both national reference laboratories and the pooling of knowledge and expertise in a referral center for fetal therapy. In particular, severe alloimmune hydrops, a formerly often lethal condition, has practically disappeared, not only leading to improved survival of HDFN but also diminishing risk of long-term impairment.

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CONFLICTS OF INTEREST

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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