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Hydrops Fetalis

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TABLE OF CONTENTS

SUMMARY

Hydrops fetalis is a severe, life-threatening fetal condition, characterized by abnormal fluid accumulation in two or more fetal compartments. This thesis focuses on both immune and non-immune hydrops fetalis (NIHF), with the emphasis on the latter due to its increasing prevalence and complex etiology. The primary objective of this literature review is to research and summarize knowledge on the etiology, diagnosis, and management of hydrops fetalis. Furthermore, the thesis explores opportunities for improvement of diagnostic work-ups and management of hydrops fetalis, to improve prognosis and how previously unknown causative factors could be determined. The methodology of this thesis consists of a comprehensive literature review, evaluation of recent studies on the topic and clinical guidelines published in the last decade. A structured analysis was conducted on the various etiologies of hydrops fetalis which include genetic and metabolic disorders, as well as infectious diseases, hematological anomalies, and structural malformations. The findings of the literature review underline the remaining challenges in diagnosis and management of NIHF in particular. Guidelines published by the Society for Maternal-Fetal Medicine (SMFM) in 2015 recommend karyotyping and chromosomal microarray (CMA) for diagnostics work-up, even though these methods fail to identify the cause of a major proportion of the cases. The thesis highlights the growing importance of exome sequencing (ES) and targeted gene panels, which significantly improve the diagnostic yield. Additionally, they are more cost-effective compared to other methods, offer potential early interventions and improve the patient counselling. Clinically, management of NIHF must be individual and take into consideration gestational age, etiology, maternal and fetal condition, therapeutic possibilities, and prognosis. In conclusion, the thesis emphasizes the urgent need for improved diagnostic protocols and the incorporation of ES and targeted gene panels to better understand the multifaceted and complex etiology of hydrops fetalis. More research of the genetic and pathophysiological basis of hydrops fetalis could lead to improved prenatal counselling, diagnosis, therapy and ultimately to a better prognosis. Moreover, clinical guidelines should be updated to include the advances in knowledge emerging from a decade of research and clinical studies.

KEYWORDS

Hydrops Fetalis, Nonimmune Hydrops Fetalis, Intrauterine Transfusion, TORCH Infections, Parvovirus B19

ABBREVIATIONS

AF - atrial flutter
AVB - atrioventricular block
B19V - Parvovirus B19
BBB - blood brain barrier
BMI - body mass index
CC - congenital chylothorax
CMA - chromosomal microarray analysis
CMV - cytomegalovirus
CMV - hyperimmunoglobulin CMV HIG
CNVs - copy number variants
CPAM - congenital pulmonary airway malformation
CTR - cardiothoracic ratio
ES - exome sequencing
ERT - enzyme replacement therapy
ET - exchange transfusion
EXIT - extra-uterine intrapartum treatment
FcRn - Fc receptor
FSA - fetal structural anomaly
HDFN - hemolytic disease of the fetus and newborn
HSCT - hematopoietic stem cell transplantation
IEM - inborn errors of metabolism
IURT - intrauterine replacement therapy
IUTs - intrauterine transfusions
IUFD - intrauterine fetal death
IVIG - intravenous immunoglobulin
JHR - Jarisch-Herxheimer reaction
LSDs - lysosomal storage disorders
MCA - middle cerebral artery
MCA-PSV - middle cerebral artery peak systolic velocity
MCH - mean corpuscular hemoglobin
MCV - mean corpuscular volume
MPS VII - Mucopolysaccharidosis VII
MVP - maximum vertical pocket
NIHF - non-immune hydrops fetalis
PAC - premature atrial contraction
PCR - polymerase chain reaction
QALYs - quality-adjusted life years
QF-PCR - quantitative fluorescence polymerase chain reaction
RBCs - red blood cells
SASD - sialidosis and sialic acid storage disease
SMFM - Society for Maternal-Fetal Medicine
SVT - supraventricular tachycardia
TOP - termination of pregnancy
TsB - total serum bilirubin concentration
TTTS - Twin-to-Twin-Transfusion Syndrome
WGS - whole genome sequencing

INTRODUCTION

Hydrops fetalis is a life-threatening disease, characterized by abnormal interstitial fluid accumulation in two or more fetal compartments including pleura, pericardium, peritoneum, and subcutaneous tissues. It is classified according to the etiology which is either immune or nonimmune. Immune etiology of hydrops fetalis is characterized by erythrocyte destruction and fetal anemia due to Rh-incompatibility. The prevalence of immune etiology of hydrops fetalis has decreased significantly due to the now widespread use of anti-D immunoglobulin prophylaxis. Consequently, nonimmune hydrops fetalis (NIHF) now accounts for estimated 76-87% of cases.(1) NIHF has numerous etiologies, including cardiovascular, idiopathic, chromosomal, hematological, infectious or metabolic disorders. (2) Despite medical advances in neonatal intensive care and in ultrasonography, the underlying etiology remains unidentified in many cases and is classified as idiopathic by most studies. (3) The prognosis of NIHF is poor, possible outcomes ranging from preterm birth to intrauterine fetal demise, stillbirth, neonatal morbidity, and mortality. (4) Nonimmune fetal hydrops nowadays still poses a big medical burden and threat to fetuses and neonates. It is important to improve preterm diagnostics and establishment of the underlying etiology to perform the correct treatment and to decrease neonatal morbidity and mortality.
This work aims to perform a comprehensive literature review on the latest studies discussing the etiology, epidemiology, signs and symptoms, diagnostics, and treatment of hydrops fetalis. A focus will be put on possibilities to improve prognosis, diagnostics, preterm and postnatal treatment as well as how to reduce morbidity and mortality.

LITERATURE REVIEW

Etiology and Epidemiology

Hydrops Fetalis can be categorized as immune or nonimmune. Immune Hydrops Fetalis is defined as severe fetal anemia caused by rhesus alloimmunization of red blood cells, called hemolytic disease of the fetus and newborn (HDFN). It is also known as Erythroblastosis fetalis. It is defined as a hemolytic disorder which primarily occurs in Rhesus positive (Rh+) fetuses and newborns born to Rhesus negative (Rh-) mothers. Usually, the mother starts producing anti-D antibodies after the birth of the first child which inherited the paternal D-antigen that the mother lacks. The D antigen is the most immunogenic of the Rh antigens. During pregnancy and birth or due to fetomaternal hemorrhage (FMH), mixing of maternal and fetal blood occurs and maternal production of IgM anti-D antibodies start. IgM antibodies cannot cross the placental barrier, but isotype switching

initiates the production of IgG antibodies which subsequently are able to cross. (5). Generally, FMH occurs when there is a disruption in the placental barrier which enables blood from the fetal circulation to enter the maternal circulation. This may be caused by various causes, including intrauterine fetal demise and trauma (6), prenatal invasive diagnostic procedures, external cephalic version and conditions that cause placental abnormalities like pre-eclampsia or chorioangiomas (7). On the other hand, small volumes of fetal red blood cells (RBCs) cross into the maternal circulation in almost all pregnancies without significant clinical effects. This is due to the bidirectional passage of nucleated cells and red blood corpuscles through the placenta (8). This occurs without history of trauma or evidence of placental abruption, which is why it can be described at spontaneous. Spontaneous FMHs are typically low in volume and not hemodynamically significant, but they can still induce maternal alloimmunization. FMH becomes symptomatic only when blood loss reaches a volume of 20% of the fetoplacental blood volume. Such FMH could be described as massive but could still be classified spontaneous. The underlying causes and pathophysiology of massive spontaneous FMH is not fully understood. However, outcomes can be unfavourable and include severe fetal anemia, hydrops, encephalopathy and stillbirth (8). The incidence of massive spontaneous FMH is estimated to be 0.3 to 1 in 1,000 births (7). Another cause that could trigger the process of antibody formation is a blood transfusion the mother had received earlier (9). Maternal antibodies can also be directed against the KEL1 blood group antigen, often referred to as Kell antigen, which is present on the fetal RBCs. It is the second most important antibody to cause HDFN and has a severe disease outcome in more than 50% of cases. The mother develops anti-K antibodies due to the same principle she could develop anti-D antibodies, during a preceding pregnancy or due to a RBC transfusion (10). This process is also referred to as maternal alloimmunization. Introduction of the Rh- immunoprophylaxis in 1968 has led to a significant decrease of the incidence of HDFN. Before the introduction, HDFN occurred in 1% of pregnancies and had a death rate of 50%, whereas the incidence decreased to 0.5% after its introduction. In 1970, Rh D immunoprophylaxis was introduced which further decreased the incidence of HDFN to 0.1% (5). Since the widespread use of rhesus (D) immunoglobulin in mothers at risk, NIHF nowadays accounts for almost 90 % of hydrops fetalis cases with a prevalence of 1 in 1700-3000 pregnancies (11). NIHF is a non-specific symptom of an underlying disease which might occur in different trimesters of pregnancy. Possible etiologies include hematological and cardiovascular abnormalities, chromosomal anomalies, thoracic abnormalities, inborn errors of metabolism, congenital infections, lymphatic dysplasia, twin-to-twin transfusion syndrome, thoracic abnormalities, placental abnormalities, fetal tumors, and idiopathic causes. The frequency of each possible underlying disorder varies greatly depending on in which trimester NIHF is diagnosed. In

women who were diagnosed at up to 13+6 weeks, chromosomal abnormalities were the cause in 69.8 %, according to a study by Sileo et al (11). Guo et al. found similar results with chromosomal abnormalities being the cause in 65 % of cases of diagnosed NIHF in the first trimester.(4) The two main types of chromosomal abnormalities are numerical and structural aberrations. A failure of chromosome divisions causes numerical aberrations like extra chromosomes or the absence of one or more chromosome. The most common numerical aberrations include triploidy, trisomy and aneuploidies. The predominant chromosomal abnormality in NIHF is aneuploidy, particularly Down syndrome, Turner syndrome and Edwards syndrome (4). Reischer et al. concluded in their study that Turner Syndrome was the most common etiology in chromosomal aberration, followed by Trisomy 18 and Trisomy 21 (12). According to Sileo et al., in the second trimester, chromosomal abnormalities were the cause in 20.8 % of cases and in 4.1 % of cases in the third trimester (11). The higher prevalence of chromosomal abnormalities as the underlying etiology before the 22nd week was also supported by Turkoglu Unal (1). Based on the study of Reischer et al., the median gestational age at diagnosis of fetuses with aneuploidies or other chromosomal aberrations was 13.3 weeks (12). As the pregnancy progresses, the proportion of chromosomal abnormalities as the etiological factor of NIHF decreases.

Another important etiological factor, especially in pregnancies which exceed 13+6 weeks, are cardiovascular abnormalities. Based on the study carried out by Sileo et al., cardiovascular abnormalities were the etiology of NIHF in 12.7 % of cases at 13 + 6 weeks and less, in 8.5 % of cases between the 14th and 24th + 6 week, and in 23 % of cases at more than 25 weeks (11). Anomalies that can lead to NIHF include rhythm disturbances, structural heart defects, cardiomyopathies, myocarditis, and cardiac tumors. Kosinski et al., found that congestive heart failure or fetal cardiac arrhythmias, including supraventricular tachycardia or congenital heart block are the dominating underlying mechanisms of hydrops in cardiac diseases (2). Hu et al. conducted a study of pregnant women hospitalized with fetal arrhythmias and they concluded that 16.6 % of cases were complicated by fetal hydrops. According to the authors of this study, supraventricular tachycardia (SVT) and atrial flutter (AF) are the most common signs in cases when NIHF is caused by fetal arrhythmias. The mean gestational age of diagnosis was 27.68 ± 3.79 weeks of gestation (13). In the study of Sileo et al., arrhythmias were the most common cardiovascular etiology of NIHF in 64.7 % of cases (11). On the other hand, the study by Reischer et al., describes that cardiovascular causes of NIHF are mainly congenital heart defects, then arrhythmias and cardiomyopathies. They detected a cardiovascular etiology in 12.5 % of cases in their cohort study (12). According to the Society for Maternal-Fetal Medicine (SMFM), the most common congenital heart defects in NIHF are right heart defects (14). The normal fetal heart rate ranges between 110

and 160 bpm. Arrythmias can be classified into three different categories: tachyarrhythmia, bradyarrhythmia or irregular arrhythmia (13). Generally, supraventricular tachycardia is the most common cardiac rhythm abnormality, accounting for 90% of all fetal tachyarrhythmias (15). Additionally, if the arrhythmia was present for more than 50% of the time during echocardiographic examination, it is classified as sustained. If the arrhythmia was present less than 50% of the time, it is classified as intermittent. Sustained fetal arrhythmias can lead to hemodynamic changes, which can result in NIHF, but also in cardiac dysfunction, fetal stress or death (13). Hu et al. performed a retrospective observational study which encompassed perinatal outcomes of intrauterine fetal arrhythmias over a 10-year span. If NIHF was present in the included pregnancies, SVT and AF were the most common signs. An estimated 35% to 60% of fetal SVT cases are accompanied by nonimmune fetal edema and the fetal mortality is as high as 17%. In their study, Hu et al. found that 28.57% of cases presented with atrioventricular block (AVB), 23.8% were complicated with SVT and 14.3% with premature atrial contraction (PAC) (13).

Another possible etiology of NIHF is congenital infection. The most important congenital infection to note is the parvovirus B19 infection. The parvovirus B19 is a small, non-enveloped DNA virus from the Parvoviridae family. The through respiratory droplets. The virus mostly affects children, but adults are at risk for infection as well. About 30 % of pregnant women lack immunity to the virus at the start of the pregnancy. While the parvovirus B19 is not teratogenic itself, it can lead to a series of complications during pregnancy, including fetal hydrops. In about 30-50 % of cases maternal-fetal transmission occurs, and the likelihood of developing severe fetal anemia or hydrops is around 3-4%. Infection is most dangerous during the early gestational age: between 9 and 20 weeks of gestation, the risk of fetal loss rises to approximately 6-7%. Fetal infection with parvovirus B19 generally leads to suppression of erythropoiesis and subsequently, to severe anemia and high-output cardiac failure. If the fetal Hb is below 5g/dl, hydrops occurs. Parvovirus B19 infection can also cause fetal hydrops by causing endothelial damage and thus increasing capillary leakage or by causing fetal myocarditis and cardiomyopathy directly (16). Another infection important to mention is early congenital syphilis, which results from the transplacental transmission of Treponema pallidum. The risk of fetal infection increases with gestational age whereas the risk of vertical transmission is highest during the early weeks of pregnancy and decreases towards the end of pregnancy (17). Untreated syphilis can cause fetal heart failure, an increase in central venous pressure and fluid accumulation, which eventually leads to fetal hydrops (18). Maternal infection with the cytomegalovirus (CMV) can lead to fetal anemia and subsequently, to fetal hydrops, even though it is a rare manifestation (19). The study by Sileo et al. identified an infectious agent in 26 of the included pregnancies which were complicated by NIHF. The results support that Parovirus B19

is the most common infectious agent causing NIHF, as it was identified in 69.2% of these pregnancies. Cytomegalovirus was the second most common with 15.4% of cases, followed by Toxoplasma gondii in 7.7% of cases (11).

Infective agent	n *(%)*
Syphilis	1 (3.8)
Varicella	1 (3.8)
Cytomegalovirus	4 (15.4)
Toxoplasma	2 (7.7)
Parvovirus B19	18 (69.2)

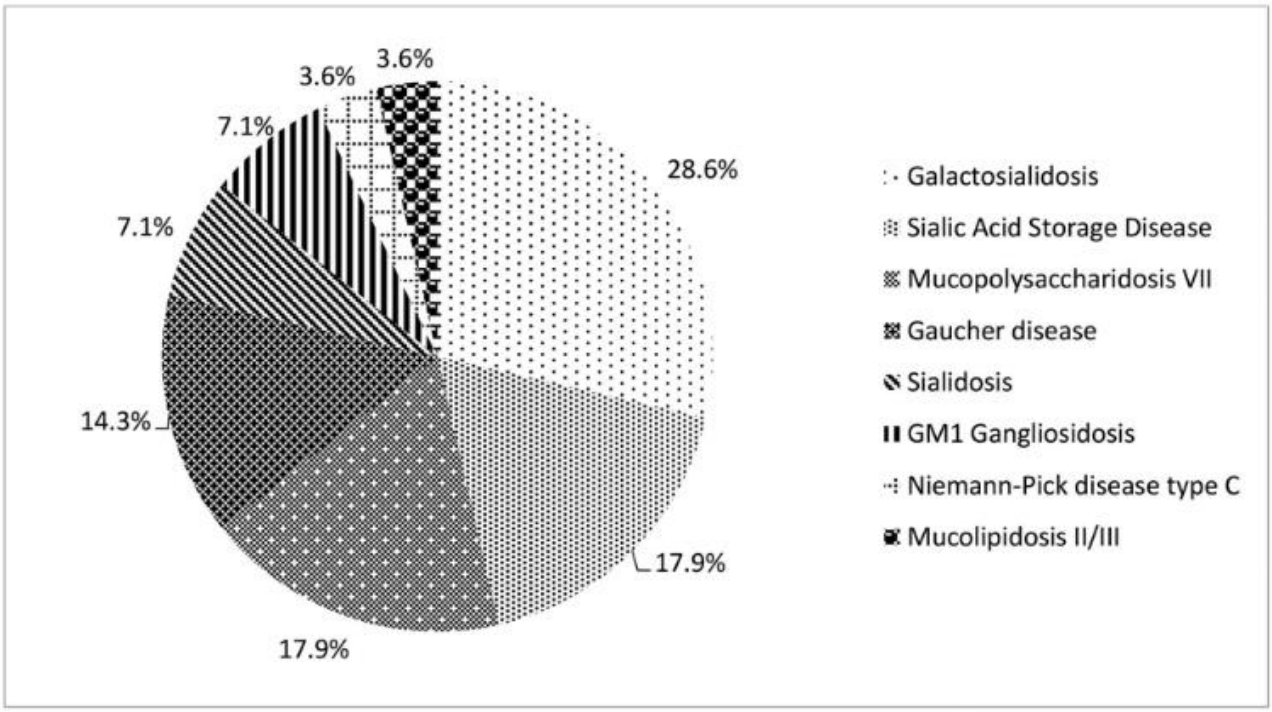
Table 1: Etiologic infectious agents identified in 26 fetuses with non-immune fetal hydrops during the second and third trimesters of pregnancy

Source: : Sileo FG, Kulkarni A, Branescu I, Homfray T, Dempsey E, Mansour S, et al. Non-immune fetal hydrops: etiology and outcome according to gestational age at diagnosis. Ultrasound Obstet Gynecol. 2020 Sep;56(3):416–21

Monogenic syndromes contribute for 5% to 10% of NIHF cases. A monogenic syndrome is defined as a disorder caused by gene mutations, which is characterized by a set of symptoms. There is a large number of syndromes which are related to NIHF, like the Noonan syndrome, the Miller-Dicker syndrome, the Neu-Laxova syndrome or Generalized lymphatic dysplasia (2). Noonan syndrome is one of the RASopathies and an autosomal dominant congenital genetic disorder. Its prevalence is estimated to be 1 in 1000-2500 births and it is characterized by distinctive facial features, cardiac defects, and other comorbidities. Noonan syndrome is a clinically and genetically heterogenous condition with various genotypes. It increases the risk of hydrops fetalis and cystic hygroma prenatally (20). Furthermore, Turner syndrome (45,X) and Down syndrome (trisomy 21) account for about 13% of NIHF cases, according to the SMFM clinical guidelines. The authors of these guidelines also report that NIHF has been associated with aneuploidies like trisomies 13 and 18 as well as triploidy (14).

Other important monogenic syndromes which can cause NIHF are inborn errors of metabolism (IEM). According to Herzeg et al., IEM are even estimated to account for up to 15% of NIHF and should especially be thought of in cases of idiopathic NIHF. Lysosomal storage disorders (LSDs) are the most common IEM and responsible for 15% to 29% of idiopathic NIHF. LSDs are a group of rare inherited disorders caused by a deficiency or malfunction of lysosomal enzymes, which are needed for the break down of complex molecules in the cell. This break down is necessary to maintain normal cellular function. The deficiency leads to a buildup of substrate within lysosomes, which leads to progressive cellular damage, impaired organ function, and eventually to severe and

often fatal clinical manifestations (21). The spectrum of LSDs is extensive. In a Study by Al-Kouatly et al., Galactosialidosis was the most common LSD to cause NIHF as it was diagnosed in 28.6% of cases. The second and third most diagnosed LSDs in combination with NIHF were sialidosis and sialic acid storage disease (SASD) and Mucopolysaccharidosis VII (MPS VII), being present in 17.9% and 17.9%, respectively. However, according to Grant et al., the type of LSD that is the cause for NIHF most often is MPS VII with a rate of NIHF in MPS VII of 30% to 40% (22). This is also described by Herzeg et al., who report that MPS VII is the most common of the LSDs to cause NIHF, followed by Gaucher disease and GM1-gangliosidosis (21).



Picture 1: Spectrum of LSD in NIHF according to Al-Kouatly et al.

Source: Al-Kouatly HB, Felder L, Makhamreh MM, Kass SL, Vora NL, Berghella V, et al. Lysosomal storage disease spectrum in nonimmune hydrops fetalis: a retrospective case control study. Prenat Diagn. 2020 May;40(6):738–45.

Another well-established etiology of NIHF is the spectrum of hematological diseases. The most common monogenic disease worldwide is α -thalassemia, affecting approximately 5% of the population. Its prevalence is highest in Southeast Asia, China, India, Africa, and the Middle East. Due to immigration, the incidence rises in the Western United States. In California, 1 in 10 000 newborns have a clinically significant α-thalassemia and α^0-thalassemia occurs in 0.2 per 100 000 state births (24). The hematological diseases can be subdivided into α^0-thalassemia or Hb Bart's hydrops fetalis and α^+- thalassemia (4). Bart's hydrops fetalis is caused by inactivation or deletion

of all four α-globin genes, preventing the production of α-globin chains. As a result, the γ-globin chain, which is primarily formed before birth, forms Hb Bart's (γ4) instead. However, Hb Bart's is a nonfunctional hemoglobin that cannot carry oxygen effectively. According to Mendelian inheritance, when both parents carry the same α^0-thalassemia genotype, there is a 25% chance that their offspring will inherit Hb Bart's hydrops fetalis syndrome (25).

Diamond-Blackfan anemia (DBA) is a rare inherited bone marrow failure syndrome which can manifest with hydrops fetalis. The disorder is characterized by a congenital erythroid hypoplasia. This erythroid defect leads to erythroblastopenia in the bone marrow, which leads to a moderate to severe macrocytic aregenerative anemia. Hydrops fetalis is a characteristic of DBA which is most likely underestimated (26).

Twin-to-twin transfusion syndrome (TTTS) is a serious complication in 10-15% of monochorionic twin pregnancies and can cause NIHF. Placental vascular anastomoses between the two circulations of the fetuses lead to a circulatory disequilibrium. Hypovolemia is present in the donor fetus and the recipient fetus is hypervolemic with circulatory overload. Discordance in amniotic fluid and bladder filling are observable on ultrasound: Increased preload and stretch on the heart chambers of the donor fetus releases atrial natriuretic peptide and brain natriuretic peptide, stimulating diuresis and causing polyhydramnios. The volume overload can cause myocardial hypertrophy, impaired diastolic function, and valvular regurgitation. Eventually, cardiac failure can lead to hydrops fetalis, which is a late manifestation (27).

Another condition which can cause NIHF is Chylothorax. It is defined as a buildup of lymphatic fluid in the pleural cavity. Congenital chylothorax (CC) is the leading cause of pleural effusion in the perinatal period. CC can be identified either prenatally or postnatally and is considered a rare condition. Its estimated prevalence is approximately 1 in 15,000 pregnancies for fetal chylothorax and between 1 in 10,000 and 1 in 24,000 live births for neonatal chylothorax. The condition has a male-to-female ratio of 2:1 and may present unilaterally or bilaterally. The underlying causes of CC include intrathoracic structural anomalies that obstruct lymphatic drainage or primary lymphatic disorders. These disorders can occur in isolation or in association with genetic conditions like trisomy 21, Noonan Syndrome or Turner syndrome. However, in majority of cases, the exact cause remains unknown.

Clinically, CC can have serious consequences. Prenatally, the mass effect can lead to pulmonary hypoplasia, reduced cardiac preload, heart failure, and NIHF (28).

Another fetal thoracic abnormality that can cause NIHF is congenital pulmonary airway malformation (CPAM). Large lesions or effusion can shift the mediastinum which might impair

venous return and cardiac output. This and an additional esophageal compression can cause polyhydramnios. In about 5% of cases, fetal hydrops occurs (14).

Skeletal dysplasias have also been associated with NIHF, including achondroplasia, achondrogenesis osteogenesis imperfecta, osteopetrosis, thanatophoric dysplasia, short-rib polydactyly syndrome, and asphyxiating thoracic dysplasia. The underlying mechanism how these diseases cause fetal hydrops is not known (14). According to a study conducted by Ergani et al., skeletal anomalies accounted for 9.5% of cases (29).

Neoplastic diseases or fetal tumors can also cause cardiac failure which in return can cause NIHF. The tumors and diseases include lymphangiomas, hemangiomas, sacrococcygeal, mediastinal and pharyngeal teratomas, and neuroblastomas (14).

Other less common etiologies include urogenital and gastrointestinal abnormalities (12).

In a vast number of cases of NIHF, the underlying etiology is either only suspected and not confirmed eventually, or it is unknown. According to Ergani et al., the etiology was unknown in 46% and suspected in 9.2% of cases included in their study (29). Guo et al. found that because of the limited resolution of karyotyping and CMA, the underlying cause of 35.7% of the NIHF cases included in their study could not be determined (4). Additionally, single-gene variants responsible for NIHF fall outside the diagnostic resolution capabilities of CMA and karyotyping. Quinn et al. identified 131 genes with strong evidence of association with NIHF and 46 genes with emerging evidence, encompassing a range of multisystem syndromes, cardiac disorders, hematologic disorders, and metabolic disorders. Many of these genes would not be detected by currently used sequencing panels, which is why it is important to update panel offerings and include genes with strong evidence (30).

Clinical Presentation and Diagnosis

As per definition, hydrops fetalis presents with an abnormal accumulation of fluid in at least two different fetal body cavities or in one body cavity accompanied by skin edema of more than 5mm. The cavities include the pleura, the peritoneum, and the pericardium. The diagnostic tool of choice to identify these fluid accumulations is ultrasound. According to a study conducted by Turkoglu Unal et al., subcutaneous skin edema of more than 5mm and ascites were the most common ultrasound findings, both being present in 81% of included cases. Pleural effusion was detected in 42.9% of cases whereas pericardial effusion presented in 4.8% of cases. (1). Reischer et al. describe that skin edema was the most common manifestation in their study as well, being present in 85% of fetuses. But contrary to Turkoglu Unal et al., only 43% of fetuses had ascites in this study. Generally, 52.1% of cases presented with abnormal fluid accumulation in two compartments,

31.9% in three and 14.4% of cases in four compartments. Additionally, Reischer et al. found that cervical hygroma presented in 70% of fetuses and was furthermore associated with chromosomal aberrations. 68.7% of these fetuses had an abnormal karyogram and were particularly associated with monosomy X, trisomy 18, and trisomy 21. On the other hand, they predominantly observed ascites and pericardial effusions in euploid fetuses, with 66% and 85% of cases, respectively. There was no significant difference in chromosomal findings depending on if pleural effusions presented or not (12). Supporting the findings of the two beforementioned studies, Hasija et al. concluded that 73% of cases included in their study presented with effusion in two or more cavities (31). Pleural effusion, pericardial effusion, abdominal ascites, subcutaneous edema, increase in placental thickness, and polyhydramnios are significant findings in the prenatal examinations (1). Sparks et al. additionally include increased thickness of nuchal translucency with 3.5mm or more and cystic hygroma in the possible ultrasonographic findings. Furthermore, they question the traditional definition of fetal hydrops, that at least two bodily compartments must be affected by abnormal accumulations of fluid. Fetal hydrops can manifest with abnormal fluid collection in only one compartment, especially if the underlying etiology is genetic, and the types of fluid accumulations can change throughout the pregnancy (32). Other possible findings in ultrasound are pneumothorax, chylothorax and hepatosplenomegaly. Additionally, some of the features found in NIHF may lead to complications which then might be visible on ultrasound images as well. For example, large ascites can compress the bowel or cause pulmonary hypoplasia. Pleural effusion could be bilateral or unilateral and may also lead to pulmonary hypoplasia and associated respiratory or circulatory complications. Furthermore, NIHF is often associated with polyhydramnios, which can be diagnoses by ultrasound as well.

After identification of hydrops fetalis on ultrasound, further diagnostic ultrasound imaging is indicated. This includes fetal echocardiography to visualize possible underlying etiologies of NIHF such as cardiac abnormalities. Additional valuable diagnostic imaging tools are umbilical artery pulsatility index and end-diastolic flow, as well as middle cerebral artery (MCA) Doppler assessment. The latter will be discussed in more detail further on in this work (33).

After the diagnosis of fetal hydrops is made by the physician, a diagnostic work-up is recommended to find the underlying etiology. As a first step, the mother’s obstetric and family history is taken. The treating doctor should ask about previous miscarriages or fetal losses and if the mother has been diagnosed with NIHF before. According to Sparks et al, 8% of women included in their study have had a previous pregnancy with NIHF (32). Ergani et al. describe that 16.7% of included patients have had a history of NIHF in previous pregnancies (29). Another important aspect to consider is whether consanguinity is present. Consanguinity was often reported to be higher in

parents of fetuses presenting with NIHF than the mean average of consanguinity in the country. In the study by Ergani et al., consanguinity was 16.7% (29). Hasija et al. found even higher percentages of consanguinity in their study, with it being present in 52% of NIHF cases (31). Ergani et al. believe that consanguinity is an important factor in the development of NIHF and should therefore be questioned (29). Turkoglu Unal et al. as well support the hypothesis that reducing consanguineous marriages will reduce the incidence of NIHF since it will lower the rate of autosomal recessive genetic diseases. Furthermore, in their study consanguinity presented in 47.6% of cases in the study group (1). Another clue to a possible genetic underlying condition is to ask if any developmental delay, genetic conditions have been observed or diagnosed in other children or family members. Childhood or premature adult deaths may also be included in the anamnesis. The treating doctor should asses the risk of fetal viral infections and which pathogen is most likely the causative agent (34).

Another condition that can be initially diagnosed by ultrasound, but which is much rarer, is TTTS. In the recipient twin, polyhydramnios should be identified by ultrasound. It is characterized by a maximum vertical pocket (MVP) of amniotic fluid exceeding 8 cm. In the donor twin, oligohydramnios should be visualized. It is characterized by an MVP of less than 2 cm. These measurements exceed the 95th percentile and drop below the 5th percentile, respectively, for the corresponding gestational age. Measurements should be taken in area without fetal parts and without the umbilical cord with the patient in a dorsal supine position. To asses severity of TTTS, the Quintero staging, shown in Table 2, is widely accepted. It involves evaluation of bladder filling, conducting Doppler assessments of the umbilical artery, umbilical vein, and ductus venosus, and checking if of fetal hydrops or fetal death present (27).

Since hydrops fetalis has a broad spectrum of possible underlying etiologies, it can be helpful to narrow down the possibilities. Depending on which trimester of the pregnancy the mother currently is in, some diagnoses become more likely than others. For example, it can safely be assumed that hydrops fetalis which presents in the first trimester is of non-immune etiology, since red cell antibodies do not pass the placental barrier until the midtrimester. If diagnosis of NIHF is made before 14 weeks of gestation, according to Khairudin et al., more than two thirds of the cases are due to chromosomal abnormalities, followed by cardiac anomalies which account for 12.7% of cases. 8% of cases at this gestational age are due to extracardiac structural anomalies and due to infections.

Stage	Recipient	Donor
1	MVP >8 cm	MVP <2 cm
2	Visible bladder	No bladder filling
3	UA A/REDV, DV absent/reversed a-wave, UV pulsations in either twin	
4	Hydrops of either twin	
5	Single or double fetal demise	

Table 2: Staging criteria for TTTS

A/REDV: absent/reversed end diastolic velocity; UA: umbilical artery; UV: umbilical vein; DV: ductus venosus; MVP: maximum vertical pocket;

Source: Miller JL. Twin to twin transfusion syndrome. Transl Pediatr. 2021 May;10(5):1518–29

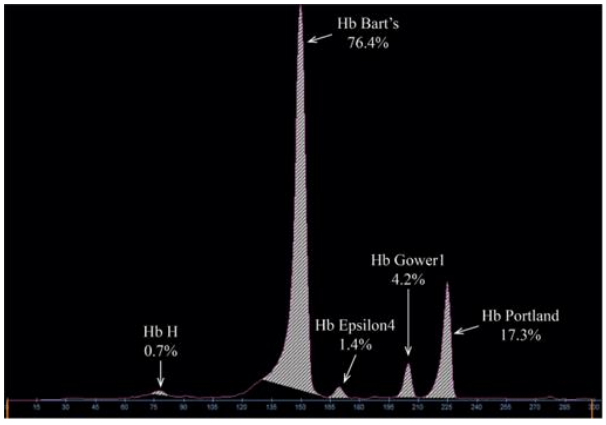
If hydrops fetalis is diagnosed in the second or third trimester, it should be managed as a fetal emergency. The first step is to determine whether the hydrops fetalis immune-anemic, non-immune-anemic, or non-immune non-anemic. A Doppler ultrasonography of the MCA should be performed urgently, and if the MCA peak systolic velocity exceeds 1.5 multiples of median, the fetus is considered anemic. Further down the diagnostic pathway the reason for the anemia must be identified (34). One reason for fetal anemia is HDFN. Like described before, fetal anemia can lead to hydrops fetalis and to fetal death, if left untreated. The ongoing hemolysis in HDFN furthermore causes hyperbilirubinemia which can lead to kernicterus, which is a severe cerebral condition (9). Another symptom and complication of HDFN is hydrops fetalis. De Winter et al. conducted a literature review which showed that hydrops fetalis was the most common clinical outcome that was reported across studies. Among pregnancies with Rh(D)-mediated HDFN that were treated with intrauterine transfusion (IUT), the rate of hydrops fetalis was 14.9%. Among pregnancies with K-mediated HDFN treated with IUT, the rate of hydrops fetalis was even higher with 39.2% (9). For mothers who are Rhesus negative, an antibody screen should be performed. If this screen is negative, sensitization during the pregnancy should be prevented. Exposure of the mother's blood to the fetal blood can have different reasons, including miscarriage, vaginal bleeding, amniocentesis, placental abruption, and abdominal trauma. If the antibody screen is positive initially, the titer should be checked every 4 weeks and if it remains lower than 1:16, pregnancy can be managed expectantly. If it exceeds 1:16, serial amniocentesis should be started from 16 to 20 weeks of gestation, as titers this high have a higher association with hydrops fetalis. Fetal cells can then be collected and analyzed; the Rh antigen can be checked to determine if the fetus is Rh positive. If the test comes back positive, the fetal MCA must be checked with Doppler ultrasound. Like mentioned

earlier, increased velocity measurements indicate fetal anemia which should lead to more invasive testing and to potential treatment (35).

Another possible cause for fetal anemia is FMH. FMH presents with unspecific symptoms, which include abnormal fetal heartrate, decreased fetal movements and hydrops fetalis. All these symptoms are unspecific. If FMH is suspected, measuring the MCA-PSV belongs to the standard work-up to evaluate fetal anemia. To detect fetal cells in the maternal blood, the Kleihauer-Betke test is widely used, but other methods like flow cytometry and liquid chromatography can be considered as well (36). For this test, maternal blood smears are treated with an acid solution. Fetal hemoglobin is acid-resistant and therefore remains unaffected, whereas HbA is removed. The smear is then stained using Shephar's method which leaves the fetal RBCs rose-pink in color and the maternal RBCs appear ghost-like due to the absence of staining (6).

TORCH serology should be evaluated to screen for gestational infections. TORCH is an acronym which is used to describe a group of perinatal infections that pose a significant risk of perinatal morbidity and mortality. These infections include toxoplasmosis, rubella, cytomegalovirus, and herpes simplex virus. Other important infections which are not included in the acronym, but belong to the serology test panel include parvovirus B19, syphilis, and varizella zoster virus (37). Out of these infections, parvovirus B19 plays the most important role in the etiology of NIHF. It commonly causes fetal anemia. Kagan et al. summarize patterns of fluid accumulation in fetuses with NIHF and parvovirus B19 compared to other etiologies. Among fetuses with parvovirus B19 and NIHF, 67% presented with both ascites and pericardial effusion, whereas skin edema and pleural effusion were observed less frequently, occurring in 57% and 45% of cases, respectively. 11% of fetuses presented with cystic hygroma. According to Kagan et al., ascites is usually the first symptom of NIHF associated with parvovirus B19 and pleural effusions mainfest only later. Before the onset of ascites, the bowel appears hyperechogenic and hepatomegaly may develop. The myocardium may show as hyperechogenic and development of cardiomegaly and tricuspid or bilateral atrioventricular valve regurgitation may occur. Placentomegaly and polyhydramnios are both clues for parvovirus B19 infection and for hydrops fetalis in general. Like mentioned before, another diagnostic clue for parvovirus B19 could be fetal anemia. Again, the measurement of the peak velocity in the middle cerebral artery should be utilized to determine if anemia is present or not. Generally, fetal hydrops usually develops 2-6 weeks after infection of the mother, but it can manifest until up to 10 weeks after infection as well. In case fetal anemia is suspected and the examiner performs fetal blood sampling, a polymerase chain reaction (PCR) test for Parvovirus B19 in the blood should be performed, and the fetus should be monitored by ultrasound to look for development of fetal hydrops (16).

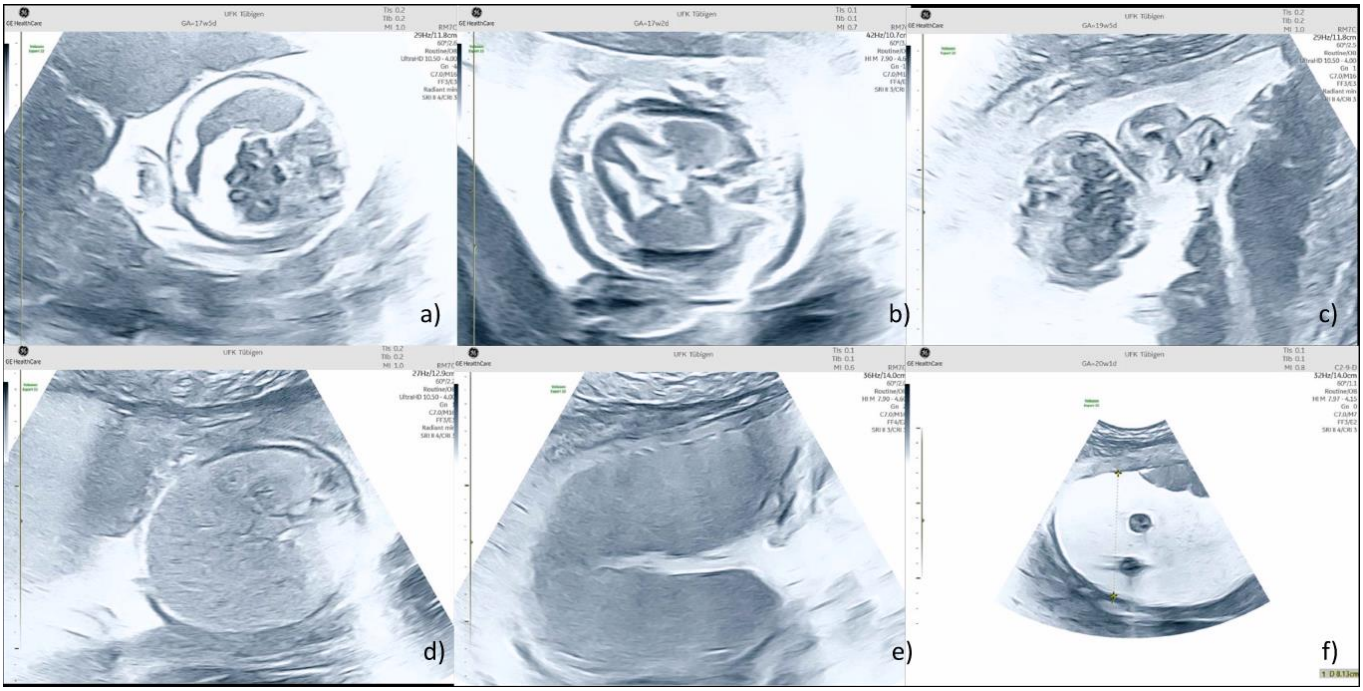
Hb Bart's hydrops fetalis syndrome represents the most severe mainfestation of α-thalassemia and is often fatal, leading to stillbirth or hydrops fetalis before. If both parents carry the same α^0-thalassemia genotype, there is a 25% chance of having offspring with this condition. Because Hb Bart's hydrops fetalis results in neonatal death, prenatal testing is usually offered to pregnant women to prevent continuing affected pregnancies. Prenatal diagnosis has been proven to markedly reduce the number of severe thalassemia births. Screening for α-thalassemia through blood tests is essential for both prevention and treatment. Deletions in the α-globin genes result to notable effects, including low mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and Hb A2 levels, and depending on the number of nonfunctional α-globin genes. Currently, hemoglobin electrophoresis is a key method for detecting hemoglobin disorders and measuring Hb Bart's levels in umbilical cord blood. As shown in Picture 2, Hb Bart's hydrops fetalis syndrome has a distinct pattern of embryonic hemoglobin fractions that sets it apart from other forms of α-thalassemia. When Hb Bart's hydrops fetalis is present, embryonic hemoglobins can be detected during exclusively the second or third trimester of pregnancy. Because of this, Hb Bart's hydrops fetalis syndrome almost always leads to stillbirth or neonatal death. Additionally, the condition poses serious health risks to the mother, including complications that can be life-threatening. Early diagnosis is crucial. Research has shown that ultrasound is a reliable and highly effective tool for detecting affected fetuses, even as early as the late first or early second trimester. In a study conducted by Zhong et al., abnormal ultrasound findings played a key role in early detection. Signs such as thickening of the placenta, an increased cardiothoracic ratio (CTR), and elevated middle cerebral artery peak systolic velocity (MCA-PSV) were strong indicators of fetal Hb Bart's disease, even in the late first or early second trimester.



Picture 2: Hb analysis cord blood, showing the distinct pattern of embryonic hemoglobin fractions in Hb Bart's hydrops fetalis

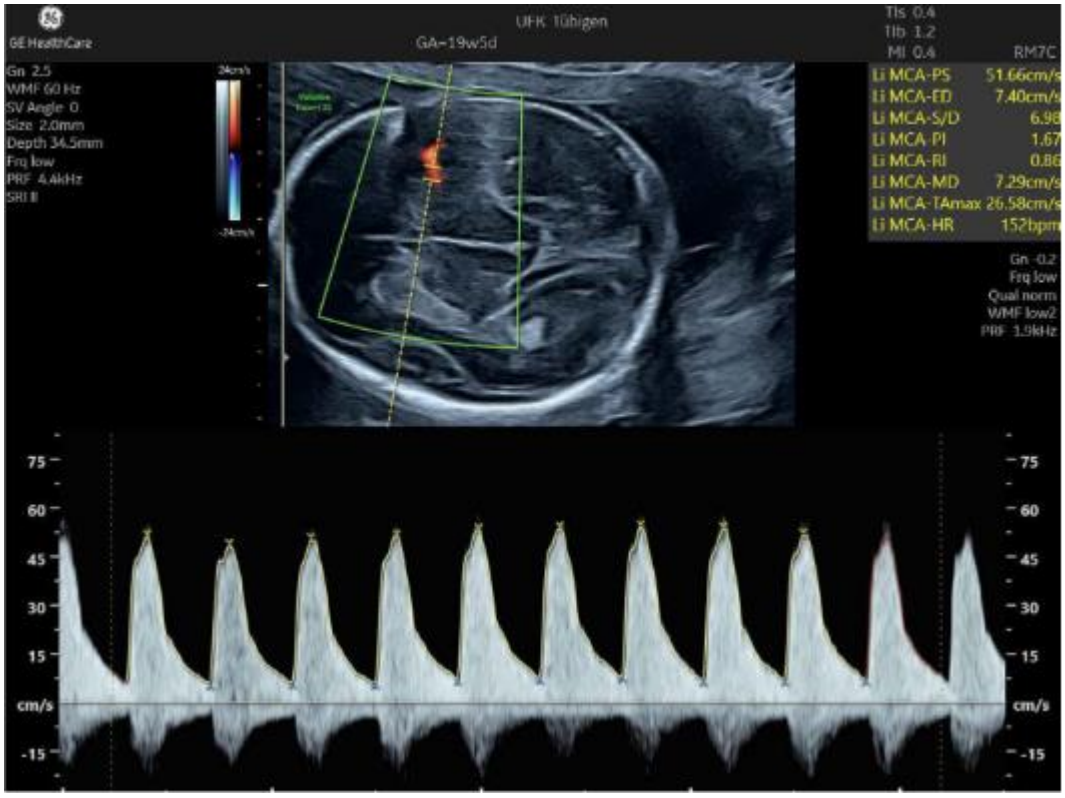
Source: Zhong Z, Chen D, Guan Z, Zhong G, Wu Z, Chen J, et al. A novel case of Hb Bart's hydrops fetalis following prenatal diagnosis: Case report from Huizhou, China. Pract Lab Med. 2024 Nov;42:e00438.

According to the Society for Maternal-Fetal Medicine guidelines, the next first-line diagnostic genetic test is either karyotyping or CMA. CMA involves isolation genomic DNA isolation from both the test sample and from a control sample. The technology was developed to detect chromosomal imbalances. Standard karyotyping may fail to identify abnormalities if the deleted or duplicated fragment is below a certain size threshold. Small aberrations that are smaller than 5Mb are called copy number variants (CNVs) and are detected via prenatal CMA (2). To undertake these tests, fetal DNA must be obtained prenatally. These samples can be taken from chorionic villi, amniotic fluid, or umbilical cord blood. Postnatal samples can be tested as well and are taken from fetal tissues (38). In the study by Sparks et al., the most prevalent source for fetal and infant DNA was amniocentesis with 57%, followed by chorionic villus sampling with 21%, 13% were obtained from placental tissue, 3% from a buccal-swab sample, 2% from fetal blood sampling, 2% from umbilical-cord blood and lastly, 1% was obtained from pleural fluid (32). However, CMA and karyotype only detect a genetic etiology in 25% of cases, according to Avram et al (39). Mone et al. even conclude that in up to 50% of NIHF cases, the underlying etiology remains unclear with the use of CMA and karyotyping (40). Single-gene disorders which can causes NIHF and the phenotype are thereby missed.



Picture 3: Ultrasonographic findings in fetuses with NIHF and parvovirus B19 infection

Source: Kagan KO, Hoopmann M, Geipel A, Sonek J, Enders M. Prenatal parvovirus B19 infection. Arch Gynecol Obstet. 2024 Jul 29;310(5):2363–71.



Picture 4: Increased peak velocity measurements in the MCA in an anemic fetus, peak velocity is 52 cm/s (16)

Source: Kagan KO, Hoopmann M, Geipel A, Sonek J, Enders M. Prenatal parvovirus B19 infection. Arch Gynecol Obstet. 2024 Jul 29;310(5):2363–71.

Targeted gene panels target genes associated with RASopathis, inborn errors of metabolism or other disorders which can cause NIHF. Several commercial laboratories offer such targeted gene panels, but the number and types of genes assessed vary greatly between different panels (39). Other tests include biochemical testing or gene panels directly testing for disorders which are associated with NIHF, such as Noonan-Syndrome. Some of these commercial panels can simultaneously evaluate 130 genes which are associated with NIHF. But since there is such an abundance of possible genetic etiologies for NIHF, these panels are far from comprehensive (41).

To pinpoint a vaster variety of genes which could cause NIHF, exome sequencing (ES) is recommended to be the next step in diagnosis. ES analyses the protein-coding regions of genes throughout the genome more broadly. On the other hand, targeted gene panels are usually cheaper than ES which, at the first impression, seems like an advantage. However, there are phenotypes in NIHF which have an extensive differential diagnosis and the diagnostic approach should be as broad as possible. To establish an accurate prenatal diagnosis is at utmost importance considering that different diagnoses have different impacts on risk of morbidity and mortality with NIHF. With a definite diagnosis, counselling of the parents and decision-making regarding the pregnancy is

improved. It influences if the pregnancy will be terminated or continued, how prenatal monitoring and observation is conducted, what route of delivery will be chosen and what degree of resuscitation after birth might be necessary. Avram et al. conducted a study focused on the cost effectiveness of exome sequencing versus targeted gene panels for the prenatal diagnosis of NIHF. They included patients diagnosed with NIHF at different weeks of gestation. All of them underwent karyotyping and /or CMA which were nondiagnostic and all of them were tested negative for viral infections and alloimmunization. The authors compared different panels including the RASopathy panel, the metabolic panel and tested all these panels followed by ES. Eventually they compared them to ES alone without any preceding panel test. The study encompassed a theoretical cohort of 470 pregnancies at <18 weeks of gestation diagnosed with NIHF who underwent next generation sequencing (NGS). Results showed that ES alone was the least expensive strategy and with the highest quality-adjusted life years (QALYs). Furthermore, strategies with ES had fewer adverse perinatal outcomes like stillbirth, neonatal death and mild to severe neurodevelopmental phenotypes. In addition, the study included 399 patients between 18 and 22 weeks of gestation and again, ES alone was the strategy with the lowest costs and the highest QALYs. Like in the beforementioned group, ES was associated with fewer stillbirths, neonatal deaths and mild to severe or profound neurodevelopmental phenotypes. Lastly, 430 patients who were diagnosed with NIHF at >22 weeks of gestation were included in the study. In this group as well, ES alone was the most cost-effective strategy with the lowest cost and the highest QALYs. ES led to more frequent termination of pregnancy (TOP) in the groups who were diagnosed before the 22nd week of gestation, but it was equally cost-effective in pregnancies >22 weeks of gestation when TOP was not an option anymore. This likely reflects that ES enables a more efficient way to diagnosis which reduces extensive postnatal evaluation which would mean a large amount of imaging studies, laboratory tests, and organ-specific examinations. This reduces not only cost but also time to wait for clinicians and especially parents to know their child's diagnosis, possible treatment options and outcomes. Additionally, establishing a definite diagnosis prenatally helps to gain knowledge about how to focus further treatment and to prepare delivery at what kind of institution. For example, after the diagnosis of Diamond-Blackfan anemia, focused ultrasound imaging can be utilized to evaluate for additional fetal anomalies. If mucopolysaccharidosis type VII is diagnosed, enzyme replacement therapy can be planned directly postnatally in an appropriate institution (39). Generally, inborn errors of metabolism can cause irreversible damage after birth which can be prevented by prenatal diagnosis and subsequent initiation of treatment without delay. Furthermore, knowing the exact diagnosis, its treatment options and probable outcomes can help parents decide whether to continue the pregnancy or to decide for TOP. It can also help to determine a recurrence risk for further

pregnancies. Finally, a diagnosis helps parents to understand their child's condition and may explain that severe outcomes were due to a genetic event. The guidelines of SMFM for the diagnostic work-up of NIHF have been introduced in 2015 and regardless of rapid advancements in the clinical genetic sequencing technologies, have not been updated since. The guidelines recommend karyotype and CMA as first line in diagnosis, as well as fetal echocardiography, viral PCR with an option to focus on single gene etiologies if the patient presents with an appropriate history and the doctor has a clinical suspicion (30). Mone et al. conducted an extended PAGE study cohort to assess the diagnostic yield of exome sequencing over karyotyping or CMA in prenatally diagnosed NIHF. They included 850 cases in which a fetal structural anomaly (FSA) was detected on ultrasound prenatally. All these cases underwent ES. Of the 28% cases which met the diagnostic criteria for NIHF, 50% presented with an additional FSA. In 96.4% of cases, CMA was the initial genetic test, the remainder underwent karyotyping. Overall, ES provided an additional diagnostic yield in 25% of all NIHF cases, in 21.4% of cases with isolated NIHF and 28.6% of cases with NIHF associated with an additional FSA. The severity of NIHF was not associated with a significant effect on the incremental yield. These findings support that ES or whole-genome sequencing (WGS) should be favoured over a stepwise approach in the diagnostic evaluation of NIHF. However, because of the high incidence of aneuploidy in NIHF, the importance of karyotyping and quantitative fluorescence polymerase chain reaction (QF-PCR) should be respected. CMA on the other hand only provides limited additional information over karyotyping. WGS can detect structural variants and aneuploidy, which is why the authors of this study suggest to favour WGS as a second-line test after QF-PCR. Additionally, the list of novel genes associated with NIHF is expanding continuously and prenatal NGS will help to discover and understand how the prenatal phenotypes develop (40). Kouatly et al. summarized that many studies are already aimed at discovering new genes which are causative for NIHF. These studies included cases with a high probability of genetic etiologies, like for example consanguineous families. Furthermore, Kouatly et al. found that one third of cases included in their study could have been diagnosed by narrower testing like biochemical testing or by RASopathy panels, but ES provided a diagnostic yield over 50% of cases which were undiagnosed initially. Additionally, ES provided a possible diagnosis in 22.7% of previously undiagnosed cases (41). All the listed studies prove that ES has a significant impact on diagnosing genetic disorders which can cause NIHF and it helps to discover and understand new associated genes and their phenotype. According to the study by Mone et al., ES should be the second line test in the diagnostic work-up of NIHF, following karyotyping or QF-PCR and replacing CMA.

As mentioned before in this thesis, DBA can be diagnosed by genetic testing. Furthermore, the disorder can manifest in several clinical presentations. 50% of DBA-affected patients are affected by congenital anomalies, mostly in the cephalic region and in the extremities, specifically thumbs. Additionally, they present with short stature and cardiac and urogenital tract abnormalities. For diagnosis, specific criteria must be met, which are shown in Figure 1 (26).

Diagnosis criteria	Age less than 1 year
	Macrocytic anemia with no other significant cytopenias
	Reticulocytopenia
	Normal marrow cellularity with a paucity of erythroid precursors
Supporting criteria	
Major	Gene mutation described in "classical" DBA
	Positive family history
Minor	Elevated eADA activity
	Congenital anomalies described in "classical" DBA
	Elevated HbF
	No evidence of another inherited bone marrow failure syndrome

Figure 1: Diagnosis criteria of DBA from the international consensus conference
Source: Da Costa LM, Marie I, Leblanc TM. Diamond-Blackfan anemia. Hematol Am Soc Hematol Educ Program. 2021 Dec 10;2021(1):353–60.

A specific set of diseases that can be diagnosed by ES and CMA are LSDs. They belong to the group of IEM. ES can come in handy because LSDs are estimated to account for 30% of previously unexplained NIHF cases. LSDs exhibit diverse phenotypic presentations depending on the specific metabolic deficiency and the type of mutations involved. In fetuses, clinical manifestations may include hepatosplenomegaly and skeletal anomalies, whereas neonates may also present with coarse facial features, spasticity, ataxia, seizures, developmental delays, as well as ocular and facial abnormalities. According to Al-Kouatly et al. the most common clinical finding was hepatomegaly, which was present in 76.2% of patients with LSD. Splenomegaly and hepatosplenomegaly were present in 60% and in 50% of cases, respectively. The data on the timing of these findings in the included cases were inconclusive in this study. While most individuals with LSDs are diagnosed postnatally once clinical symptoms appear, some cases present in utero as NIHF (23). Prenatal diagnosis of LSDs can also be performed through genetic or enzymatic tests. For these tests, samples from the chorionic villus or amniotic fluid are utilized. Early intervention is linked to better outcomes, making prenatal detection essential for the potential positive outcome of intrauterine enzyme replacement therapy (IUERT). Due to their rarity, a lot of LSDs are not implemented in carrier screening panels. Routine prenatal screening primarily relies on ultrasound examination.

While certain LSDs may manifest as NIHF in pregnancy, the majority do not exhibit detectable abnormalities on prenatal ultrasound. CMA can be useful for diagnostics of LSDs, however, many are triggered by point mutations, small deletions, or insertions at single gene level. These could be detected by ES.

A rare condition with many possible underlying etiologies which can cause NIHF is CC. It can be identified during either the fetal or neonatal period. In the fetal stage, the buildup of pleural effusion raises intrathoracic pressure, which reduces fetal swallowing of amniotic fluid, potentially leading to polyhydramnios and preterm labor. Additionally, fetal chylothorax increases the risk of mortality and complications due to lymphatic fluid accumulation in the pleural space. This can impair lung development, disrupt pulmonary and cardiovascular function, and cause further complications due to the loss of essential lymphatic components (42). Prenatal diagnosis of CC is important to prepare delivery and immediate treatment.

An important aspect doctors should consider as well is the maternal status. Women should be asked about discomfort and symptoms of preeclampsia and they should be encouraged to monitor their own well-being closely. Symptoms which resemble and could also indicate preeclampsia include new onset of elevated blood pressure, proteinuria, and peripheral edema. In patients with hydrops fetalis, these symptoms can not only indicate preeclampsia but also Maternal Mirror Syndrome (34). This is a rare complication that is not extensively described in literature and studies. It is also called Ballantyne syndrome. Clinically, it is characterized by maternal, fetal and placental edema (43). Placental edema is defined as a placental thickness of more than 4 cm up to 20 weeks and more than 6 cm after 21 weeks of gestation. Maternal edema include peripheral pitting edema, facial, sacral and pulmonary edema and ascites (44). It occurs in approximately 0.02% of pregnancies during the second and third trimester (43). A study by Mogharbel et al. examined 10 pregancies with Maternal Mirror Syndrome out of 276 cases of fetal hydrops. This makes a 3.6% incidence in this cohort (44). The symptoms have a significant overlap with the symptoms of preeclampsia, which is why Maternal Mirror Syndrome is most likely often overlooked. Additionally, third trimester maternal symptoms rather lead to fast delivery than to extensive diagnostic evaluation. Clinical symptoms of mirror syndrome include edema, elevated liver enzymes, increase in weight, hypertension, anemia, headache, and visual disturbances. Many different fetal conditions that cause fetal hydrops can induce Maternal Mirror Syndrome, including isoimmunization, TTTS, hemoglobinopathies, fetal cardiomyopathies, viral infections, supraventricular tachycardia, chylothorax, and placental chorioangioma (43). Mogharbel et al. concluded that in their cohort study, most cases of Maternal Mirror Syndrome were associated with structural abnormalities of the fetus and in 80% of cases, non-immune or non-infectious causes for

fetal hydrops were observed. Maternal Mirror Syndrome is associated with elevated body mass index (BMI), multiparity, hemodilution, hypoalbuminemia, anemia, and hyperuricemia. Median gestational age at diagnosis was 22 weeks and 3 days (44). Gavin et al. report a median gestational age of 24+5 weeks at presentation of symptoms (43).

Generally, it is possible to diagnose fetal hydrops before 12 weeks of gestation, but it is usually diagnosed later in the pregnancy. A study by Ergani et al. found that median age of diagnosis in the included hydrops groups was 18 weeks. Hydrops fetalis that was diagnosed in the first trimester was subsequently confirmed in the second trimester by detailed ultrasonography. However, the study's authors did not find a statistically significant difference between NIHF patients identified before 12 weeks of gestation and those identified after the end of the first trimester regarding hydrops history, fetal anomalies, and consanguineous parentage. In the study by Kosinski et al., the median gestational age at diagnosis was 24 weeks. (2). In the study by Sileo et al., median gestational age at diagnosis was 20 weeks (11). Huang et al. conclude in their study that the median gestational age at diagnosis is 30.5 weeks if no fetal intervention takes place. The results of these studies show that NIHF is mostly diagnosed in the second and third trimester.

Another maternal characteristic that is worth mentioning is age. Advanced maternal age can have a significant influence on maternal and perinatal outcomes and can increase the likelihood of pregnancy complications. These complications include disorders that also play a role in the development of NIHF, like chromosomal abnormalities and congenital anomalies. Advanced maternal age generally applies to women aged 35 years or more (45). In the study by Sielo et al., which included 273 singleton and six twin pregnancies, the median maternal age was 31 years old (11). Reischer et al. describe that the average maternal age in their study was 31.5 years (12), in the study by Sparks et al. the median maternal age was 32 years old (32). While these findings would imply that on average, women with diagnosed NIHF are below the advanced maternal age, Hasija et al. describe in their study a median maternal age of 35 years (31).

Therapy

After diagnosis of hydrops fetalis, careful counselling of the patients to determine the next step is crucial. Hydrops fetalis has a serious impact on the outcome of the pregnancy and on the life of the unborn child.

If diagnosis is made at early gestational age, NIHF is more often associated with genetic abnormalities, severe cardiovascular conditions, and poor outcomes overall and therefore TOP should be offered and discussed. This might even be decided without further diagnostic testing if this meets the woman's wishes. Testing is still possible postnatally if fetal or placental tissue can be

obtained, but it should be explained to the parents that it might not be possible to gain DNA of sufficient quality (34). If the diagnosis of NIHF is made in the second and third trimester, TOP may still be feasible, depending on the country's laws. The United Kingdom for example offers termination of pregnancy under Ground E of the Abortion Act which demands that there must be a significant risk that the child would suffer from such severe physical or mental abnormalities that it, if it was born, would be seriously handicapped (34).

After diagnosis, fetal treatment options should be discussed and offered, if available, to improve fetal physiology and perinatal outcome. To initiate fetal treatment, the underlying etiology should be reversible, the woman should be committed to the pregnancy and informed about procedural risks, benefits, and alternatives. Additionally, a multidisciplinary team should be compiled (34).

If HDFN is diagnosed, the PSV in the fetal MCA should be measured. If it exceeds a MoM value of 1.5, treatment with IUT should be initiated (46). Group 0 negative blood should be cross-matched with the maternal blood and then transfused (5). In a study by Drozdowska-Szymczak et al., 16.8% of included fetuses with Hemolytic Disease of the Fetus and Newborn required IUT. Maximum of required IUTs for one fetus in this study was 11. 13.5% of children required a top-up transfusion with the maximum being three required top-up transfusions. In other, similar studies, the number was much higher, with 68-83% of newborns requiring top-up transfusions. De Winter et al. report that the average gestational age at the time of first IUT is 27 weeks, which could potentially be postponed by administration of intravenous immunoglobulin (IVIG) or plasmapheresis, although evidence on this is scarce (9). IUT carries several risks, including preterm birth, meaning before 37 weeks' gestation, prelabor rupture of membranes and fetal death. A study by Moise et al. researched if the drug Nipocalimab can delay or prevent fetal anemia and subsequent IUT. Nipocalimab is a neonatal Fc receptor (FcRn) blocker with an affinity for this receptor that us 1000 times higher than that of IVIG, which partly works in a similar way. FcRn is the only transporter for placental IgG, it maintains circulating maternal serum IgG concentrations. The study by Moise et al. treated patients with Nipocalimab who were at high risk for recurrent early-onset severe HDFN. The authors report that in 54% of pregnancies, live birth occurred at 32 weeks' gestation or later without IUT. A substantial decrease in maternal IgG concentrations of 85% below baseline was observed. Nipocalimab does not affect non-IgG immunoglobulins, IgG production or key immune-cell functions (47).

Due to the breakdown of red blood cells during hemolysis, bilirubin is released in increased amounts. During pregnancy, bilirubin is excreted through the placenta and therefore does not harm the fetus. Postnatally on the other hand, the neonate's liver is not able to excrete these increased amounts of bilirubin due to its immature enzyme system. Total serum bilirubin concentration (TsB)

increase is the result, which can cause kernicterus and cerebral palsy. To reduce the risk of such severe consequences, each neonate with HDFN must be monitored carefully. Phototherapy is most commonly utilized to treat hyperbilirubinemia. Drozdowska-Szymczak et al. report that the duration of phototherapy treatment was found to be shorter in neonates who received IUTs. Another possible treatment for hyperbilirubinemia is exchange transfusion (ET). ET carries a lot of risks, such as hyperkalemia, thrombocvtopenia, hypocalcemia, leukopenia, and sepsis. Other possible complications include cardiac arrhythmias and arrest, apnea and necrotizing enterocolitis. The risk of neonatal death after ET is 8%. This is why indications for ET should be carefully assessed. The TsB concentration, gestational age at birth, presence of neurological symptoms should be taken into account (46). Generally, the aim should be to prevent HDFN overall. To achieve this, IVIG should be given to pregnant Rh- women who have not been sensitized (5).

FMH may be another cause of fetal anemia. Management depends on the gestational age at diagnosis. Diagnosis of FMH at 34 to 36 weeks of gestation justifies immediate birth. Before 32 weeks of gestation, correction of anemia should be contemplated. Beyond 32 weeks of gestation, the decision to perform and IUT must weigh the potential risks of the procedure against those associated with prematurity or the need for neonatal transfusion. IUT is a well-established procedure for treatment of fetal anemia before 32 weeks gestation as well, but it remains unclear how to properly monitor these pregnancies following the first IUT to assess recurrence rate (36).

Another important causes of NIHF that can also induce fetal anemia include the TORCH infections. In this thesis, the parvovirus B19, syphilis and cytomegalovirus will be discussed in greater detail.

Prenatal Parvovirus B19 (B19V) infection is associated with a risk of development of anemia and subsequent development of NIHF. This is why, after a confirmed B19V diagnosis, the fetus must be closely monitored for fetal anemia. The aim is to prevent hydrops fetalis. NIHF caused by B19V infection is associated with many risks and adverse outcomes for the fetus. According to a literature review by Kagan et al., the risk for IUFD is 5.5% and 28.9% if hydrops fetalis is absent or present, respectively. The risk for neonatal demise was approximately 5% in fetuses without hydrops fetalis and almost 50% in fetuses with hydrops fetalis. Abnormal findings on brain imaging and abnormal development was found in 10% of cases when hydrops fetalis was present and in no cases if hydrops fetalis was absent (16).

If anemia develops, the only available therapy is to utilize an IUT. Certain criteria for the blood used in IUTs must be met. These include that the blood is Rhesus D negative, has a hematocrit of around 80-85% and is ionized (16). Kosan et al. conducted a study in which they investigated 103 fetuses that received IUTs due to severe anemia caused by parvovirus infection. The vast majority with 89.8% of transfusions in this study were done with intravascular route. The intraperitoneal and

intracardiac routes of transfusion were utilized much less with 7% and 3.2% of cases, respectively (48). An anterior placenta and easy access to the umbilical vein at the placental cord insertion are ideal conditions for an IUT, whereas it becomes more challenging with a posterior placenta or use of a free umbilical cord loop (16). If IUTs are performed before the 20th gestational week, the peritoneal route is chosen (16), which is why the rate of peritoneal transfusion was significantly higher before gestational week 20+0 in this study. All intracardiac transfusion led to intrauterine fetal death (IUFD) and 5 out of 6 cases were performed as a rescue approach in hydropic fetuses. The mean hemoglobin concentration before the first IUT was 5.0 g/dl. Generally, IUTs become necessary at earlier gestational ages in anemia due to B19V infection than in anemia due to red cell alloimmunization. Additionally, more fetuses are severely anemic and hydropic at the time of diagnosis compared to fetuses affected by alloimmunization. Hydrops was more commonly observed in fetuses who received an IUT at a gestational age of 20 weeks or later (48).
Another important complication that often affects anemic fetuses with B19V infection is thrombocytopenia (16). In the study by Kosian et al., thrombocytopenia was observed in 68% of fetuses, 22.7% of which even had severe thrombocytopenia. In hydropic fetuses, thrombocyte count was significantly lower than in non-hydropic fetuses (48). Post-exposure prophylaxis with IVIG for B19V is not recommended (16). It is sometimes used in cases of congenital B19V infection but the possible treatment of fetal anemia with IVIG targeted against B19V still has to be researched more (49).

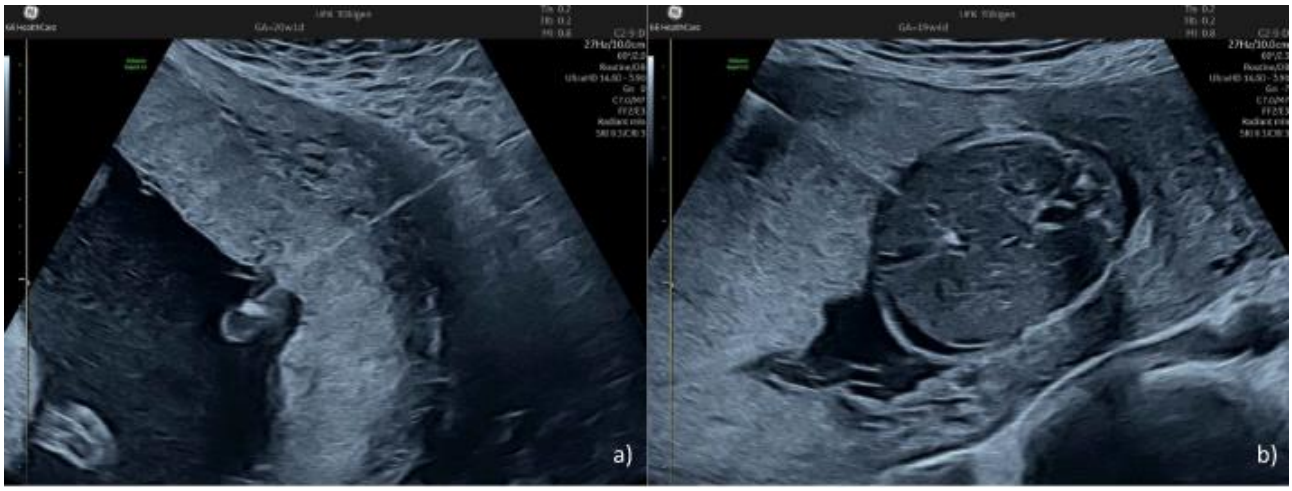
Characteristics	<20+0 weeks	≥20+0 weeks	*p* value
Hydrops (%, *n*)	38.6% (22)	47.8% (22)	0.42
Mean hemoglobin before 1st IUT, g/dl	5.0 (0.3–10.7)	4.94 (1.1–12.1)	0.66
Survivor (%, *n*)	80.7% (46)	89.1% (41)	0.28
Non-survivor (%, *n*)	19.3% (11)	10.9% (5)	0.28
Number of IUTs (%, *n*)			0.39
1	50.9% (29)	36.9% (17)	
2	29.8% (17)	47.8% (22)	
3	12.3% (7)	10.9% (5)	
4	7% (4)	2.2% (1)	
5	0	0	
6	0	2.2% (1)	

p value < 0.05 is considered significant

Table 3: Shows the characteristics of fetuses with B19V infection who were either transfused <20+0 or ≥20+0 weeks of gestation

Source: Kosian P, Hellmund A, Geipel A, Bald R, Geist OM, Böckenhoff P, et al. Intrauterine transfusion in 103 fetuses with severe anemia caused by parvovirus infection. A multicenter retrospective study. Arch Gynecol Obstet. 2022 Aug 2;308(1):117–25.

Another infection included in the TORCH serology that can cause NIHF is syphilis. If abnormal findings on ultrasound are present, it is more commonly associated with poor outcomes and neonatal treatment failure (18). If syphilis is diagnosed, parenteral benzathine penicillin G should be administered intramuscularly (50). Since there is no satisfactory alternative to penicillin, patients with a penicillin allergy should be desensitized before start of treatment. Generally, maternal treatment to prevent congenital syphilis is successful in 98% of cases (17). Doctors should keep possible complications of penicillin treatment in mind. Besides allergies, Jarisch-Herxheimer reaction (JHR) can present in both the mother and the fetus.

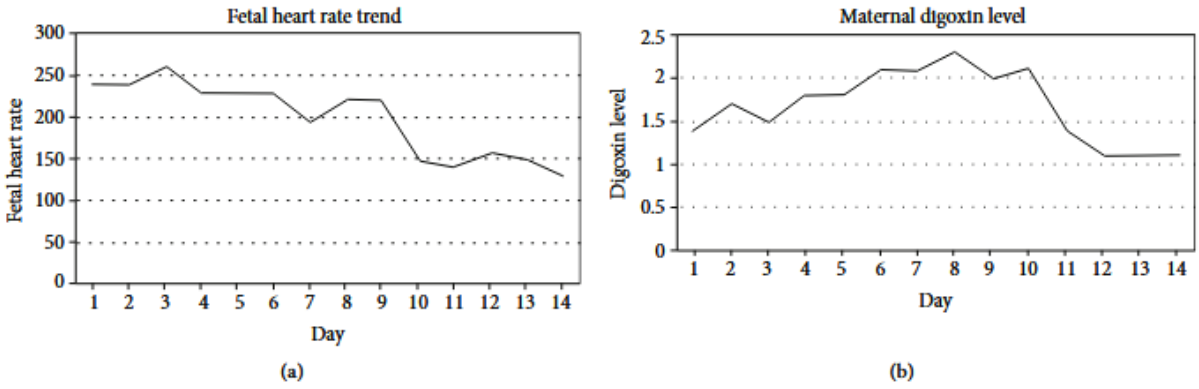


Picture 5: Intrauterine transfusion administered via the umbilical vein at the placental cord insertion site (a) and through the intrahepatic portion of the umbilical vein (b).
Source: Source: Kagan KO, Hoopmann M, Geipel A, Sonek J, Enders M. Prenatal parvovirus B19 infection. Arch Gynecol Obstet. 2024 Jul 29;310(5):2363–71.

In adults, this reaction usually induces intensified skin rashes, shaking chills and a rise in temperature. It occurs in up to 40% of pregnant patients who are being treated for syphilis. In the fetus JHR shows differently. Decreased fetal movement, transient late fetal heart rate decelerations and uterine contractions were initially observed after the first dose of penicillin, but not after the second dose (18).
The most common TORCH infection worldwide is cytomegalovirus (CMV), and it can cause NIHF, too. Guidelines for treatment of CMV however are lacking, as well as efficient vaccines. CMV hyperimmunoglobulin (CMV HIG) is discussed in some studies and might be useful in prevention and minimization of vertical transmission of CMV (51). However, until now CMV HIG is not indicated for treatment. It is generally recommended for pregnant women to follow do's and don'ts practices to reduce CMV infection, since there is no universal treatment recommendation. These practices include increased personal hygiene during pregnancy, like thorough hand washing

after being in contact with children's laundry, diapers, toys, pacifiers, or toothbrushes, since it is believed that children younger than 3 years shed CMV in their urine and saliva. Sharing food, drinks, utensils should be avoided (52).

Another treatable etiology of NIHF are fetal arrhythmias. The most common fetal arrythmia is SVT, which carries a substantial risk of fetal hydrops and fetal death if not treated (15). The goal of treatment is to slow the fetal heart rate to ensure sufficient cardiac output. The treatment options depend on gestational age, severity, associated congenital abnormalities, and maternal wishes. After transplacental treatment, daily electrocardiograms, and cardiac monitoring for the well-being of the mother are performed. In 65% to 95% of cases, fetuses without NIHF undergo cardioversion within 2 to 7 days after start of treatment (53). Several drugs, such as digoxin, flecainide, sotalol, and amiodarone, can be utilized for treatment. Unfortunately, the transplacental transfer of most antiarrhythmic drugs is decreased in the presence of fetal hydrops which makes it difficult to obtain sufficient drug levels. The leading first-line drug Digoxin has limited effect in NIHF. Flecainide, sotalol and amiodarone cross the placenta more efficiently. Amiodarone can also be used together with digoxin or flecainide to achieve cardioversion of fetal SVT or ventricular dysfunction. Due to its long half-life, amiodarone may accumulate in fetal compartments, but prompt and persistent control of a fetal arrhythmia is crucial to prevent neurological complications such as ischemia (15).



Picture 6: Fetal intramuscular injection of digoxin occurred on Day 9, and fetal heart rate normalized on Day 10 at 150 bpm (a). Maternal digoxin level. Day 1 represents maternal digoxin levels prior to the third dose of oral digoxin, dosed at eight-hour intervals. Levels returned to the therapeutic range on Day 11 (b) (53)

Source: Source: Munoz JL, Lewis AL, Song J, Ramsey PS. Fetal Intervention for Refractory Supraventricular Tachycardia Complicated by Hydrops Fetalis. Case Rep Obstet Gynecol. 2022;2022:5148250.

If Hb Bart's is diagnosed, therapeutic options were limited until recently. This is why parents often chose pregnancy termination. However, IUTs are now commonly used to treat various fetal anemias, including Hb Bart's. While the initial IUT treats severe anemia, subsequent transfusions are typically administered every three weeks to maintain appropriate hemoglobin levels. The majority of fetal centers initiate IUTs at 18 weeks of gestation, using protocols comparable to those for isoimmunzation that are associated with low rates of complication. Since Hb Bart's is usually diagnosed after 18 weeks gestation, the standard approach involves intravenous infusion through the umbilical vein. O-negative blood with a high hematocrit is transfused to correct anemia. Overloading the fetus with excess volume should be avoided. Several reports suggest that this fetal therapy improves perinatal and neonatal outcomes by reversing fetal growth restriction, anemia, and hydrops. Additionally, preterm births are reduced, whereas higher Apgar scores and shorter neonatal ventilation times can be achieved, compared to untreated cases. Furthermore, case series indicate long-term benefits for growth and development. After birth, infants with Hb Bart's continue to depend on further transfusions, following a protocol similar to that for β-thalassemia major (BTM). As survival rates improve, parents can now consider fetal therapy as an option during nondirective prenatal counseling (24).

In a fetus diagnosed with DBA, there are mainly two treatment options. Either, blood transfusion can be started to maintain the Hb of more than 90 g/L with iron chelation therapy started when ferritin level is more than 500 μg/L, or corticosteroid treatment. Treatment is generally aimed at neonates and infants. Corticosteroid therapy must not be started before the first year of life to support the maximal growth and to prevent short stature (26). Hydrops fetalis is described in literature as an unusual presentation and that DBA could explain unclear anemia during fetal life. Progress in molecular diagnostics could lead to a more thorough understanding of the matter (54). Physicians should also keep IEM and LSDs in their list of differential diagnoses for NIHF. As there is a spectrum of LSDs which can cause NIHF, treatment differs in detail, depending on the underlying diagnosis. One possibility of treatment is hematopoietic stem cell transplantation (HSCT). For example, it can be utilized for the treatment of several MPS, including MPS VII, which is one of the most common LSDs to cause NIHF. In MPS VII, HSCT has had inconsistent outcomes in the past and is associated with substantial risks, including rejection and life-threatening complications. However, recent studies suggest that symptom reversal and clinical improvement may be achievable. Due to these mixed outcomes, the decision to pursue HSCT for MPS VII patients should be carefully assessed on an individual basis. Enzyme replacement therapy (ERT) is a widely recognized postnatal treatment approach for many LSDs. Patients are treated with a recombinant form of the missing or deficient enzyme. When given systemically, ERT binds to

Mannose-6-Phosphate receptors or Mannose receptors. depending on the specific disease. Then, it is transported to lysosomes where it aids in restoring normal function. This process is called as "cross-correction,". Despite its promising benefits, ERT has several limitations, like its inability to cross the blood-brain barrier (BBB) and reach other protected sites including the bone and cornea. There is also a risk of inducing neutralizing anti-drug antibodies, which can reduce the treatment's effectiveness. Lastly, the treatment has a limited ability to address disease complications that arise during the prenatal period. IUERT, a novel approach, has the potential to overcome the challenges associated with postnatal ERT by benefitting of several distinctive aspects of the intrauterine environment. For example, the fetal BBB is more permissive and their tolerogenic immune system. Furthermore, IUERT offers the opportunity to intervene before irreversible damage occurs, potentially enabling phenotypic rescue. Additionally, because the enzyme dosage is calculated specifically for the fetus, IUERT offers the advantage of lower initial drug doses at reduced costs and an improved drug-target ratio. To be eligible for prenatal molecular therapies, such as IUERT, several essential criteria must be met. First, the expected benefits of the treatment should surpass its risks relative to the standard of care and a precise prenatal molecular diagnosis of the condition must be available. Also, given that the therapy is administered before clinical symptoms appear, there should be a well-established genotype-phenotype correlation, with a severe presentation based on prior cases or family history. Lastly, a multidisciplinary team with expertise in all aspects of the disease and its treatment, including immune tolerance induction, IUERT administration, laboratory monitoring, and long-term follow-up, should be involved. For now, IUERT is a promising approach for the management of LSDs, but several challenges, such as the need for improvement of antenatal diagnosis, still need to be addressed (21).

If diagnosis of CC is made prenatally, treatment can be initiated. The primary goal of prenatal treatment is to reduce fetal intrathoracic fluid accumulation, promoting lung maturation and supporting cardiac output. Prenatal procedures to achieve this include thoracocentesis, placement of pleuro-peritoneal shunts, and pleurodesis. After birth, management focuses on facilitating lung expansion while allowing the impaired lymphatic system to remodel. Nutritional therapy with medium-chain triglyceride-enriched formulas and pharmacological treatment with somatostatin or its analogue, octreotide, have been shown to effectively reduce lymphatic leakage. This therapy improves clinical outcomes in patients with congenital chylothorax. To reduce the duration of potential hypoxia during delivery, extra-uterine intrapartum treatment (EXIT) has been used in cases of prenatally diagnosed severe CC. EXIT is a neonatal intervention performed while the fetal-placental circulation remains intact. In a study by Tai et al., prenatal intervention was performed in 39.3% of pregnancies and the intrapartum treatment EXIT in 46.4%. If treatment was postnatal,

chest tube drainage and mechanical ventilation were applied in 86% and 89.3% of cases, respectively. Octreotide was used in only 14.3% of cases. Unfortunately, due to the rarity of the condition and limited conclusive evidence, there is no established consensus on the optimal prenatal or postnatal management of CC (28).

CPAM can be treated with needle drainage or thoracoamniotic shunt placement, if the lesion is macrocystic. Predominantly solid or microcystic lesions can efficiently be treated by either corticosteroid therapy or in utero resection, the former being recommended as first-line by the SMFM clinical guidelines (14).

Treatment modality	***N* = 28**
Prenatal intervention (%)	11 (39.3%)
Pleural-amniotic shunt insertion	6
In utero thoracocentesis	4
Amniotic fluid reduction	1
Intra-partum treatment (EXIT)	13 (46.4%)
Thoracocentesis only	10
Endotracheal intubation + Thoracocentesis*	3
Post-natal management	
Chest tube drainage	24 (86.0%)
Mechanical ventilation	25 (89.3%)
High frequency oscillator	8
Conventional ventilator	17
Octreotide	4 (14.3%)
Starting day of age, median (range)	6 (2–11)
Maximum dose used (mcg/kg/h), median (range)	3.5 (2–8)
Duration of treatment (days), median (range)	17 (14–22)

Table 4: Prenatal, intra-partum, and post-natal interventions in a study by Tai et al.
Source: Tai HL, Mok TYD, Chao AS, Chu SM, Lien R. Staged Management of Congenital Chylothorax With Hydrops Fetalis: An Insight Into EXIT Related Procedures. Front Pediatr. 2021;9:633051.

Treatment for neoplastic disease or fetal tumors is more complicated. The SMFM clinical guidelines discuss that open fetal surgery lead to a survival in 55% of cases in a systematic review, whereas minimally invasive therapy resulted in survival in 30% of cases (14).

TTTS is a rare etiology which, without treatment, has a very poor prognosis. The only intervention which aims at curing TTTS is the fetoscopic laser ablation. It closes the intertwin vascular anastomoses between the twins which presents an improved survival rate to both fetuses. Typically, this procedure is performed between 16 to 26 weeks of gestation. Local anesthesia and intravenous sedation are administered as required, epidural or with general anesthesia occasionally. The physicians should use preoperative ultrasound mapping of the placental cord insertions, the intertwin membrane and estimation of fetal size discordance for the orientation of the vascular

equator. With ultrasound guidance, the physician enters the recipient twin's sac with a fetoscope and identifies the intertwin membrane and vascular equator under direct visualization. With a 400 to 600 μm laser fibre which is advanced through the fetoscope, the vessels at the site of anastomosis are coagulated. Before removing the fetoscope, amnioreduction is performed to restore a normal fluid pocket around the recipient twin. This so-called ''Solomon technique'' lead to double twin survival rates of up to 65%. Following the immediate postoperative phase, close ultrasound monitoring is essential to assess the resolution of TTTS. According to Miller et al., this usually occurs during the first two weeks after the laser surgery. A course of betamethasone is recommended whereas the timing can be determined based on the patient's clinical situation. With an uncomplicated postoperative course, pregnancy can be continued until 34 to 36 weeks gestation. Alternative treatment options for TTTS include amnioreduction, selective fetal reduction, or pregnancy termination. Amnioreduction may be considered when referral to a laser center is not feasible or as a temporary measure, especially in later gestation. Selective fetal reduction is generally performed in cases where one twin has associated fetal anomalies or when survival is unlikely following TTTS treatment. Additionally, some parents may choose to terminate the pregnancy before viability if the perioperative risks and potential outcomes are considered unacceptable (27).

As discussed previously, Maternal Mirror Syndrome is a rare condition which further complicates fetal hydrops. Diagnosis is made difficult by a lack of uniform diagnostic criteria, the overlap of symptoms with the presentation of severe preeclampsia and a limited representation in literature. However, if diagnosed, the goal of treatment of Maternal Mirror Syndrome is usually to reverse the hydrops and placental edema and potentially reversing the maternal symptoms as well. This should delay the need for premature delivery of the fetus. Extensive studies on the potential improvement in outcomes after treatment have yet to be carried out. Generally, the symptoms and the underlying pathology causing fetal hydrops must be treated. Sichitiu et al. conducted a study in which 52.4% of included patients were treated with diuretics, 19% with antihypertensives and 28.6% with a combination of both. In this study, ten cases received a fetal shunt insertion, six underwent fetoscopic laser ablation of placental anastomoses for TTTS, four received IUTs for fetal anemia, and one underwent both radiofrequency ablation of the feeding vessels and IUT for a sacrococcygeal teratoma. Therapeutic fetal intervention led to the resolution of the syndrome in 38% of cases, allowing for the prolongation of pregnancy. However, preterm delivery still occurred in 76% of cases (55).

Generally, after diagnostic work-up of hydrops fetalis, parents should be counselled about treatment options and about the possibility of termination of pregnancy (TOP). Chromosomal aberrations,

which are one of the most common underlying causes of NIHF (12), and genetic syndromes can be serious and potentially lethal. Prognosis and comfort care must be discussed if TOP is not pursued (56). In the study conducted by Reischer et al., 50.7% of patients chose TOP and it was significantly related to the underlying etiology, with genetic aberrations being diagnosed in most of these cases (12). In a study conducted by Morey-Olivé et al., TOP was performed in 76.8% of pregnancies. In case the women had received an antenatal diagnosis of genetic abnormalities, rates of elective termination of pregnancy were notably higher than in women who did not receive it (57). Pregnancies with this etiology also presented with the highest rates of miscarriages. Prognosis too is poor in case the parents opted for continuation of pregnancy. In 28.7% of cases, miscarriage occurred, and 18.5% of patients experienced IUFD after 20 weeks of gestation. Of the 52.8% of fetuses that were live born, 22.7% died within the first week of life. Adverse pregnancy outcomes were also associated with nuchal thickness of more than 2.5mm, lower gestational age at diagnosis and with the number of body compartments affected by fluid accumulation. Prognosis was worse with increasing number of affected body compartments (12). Sileo et al. examined the outcomes of pregnancies in regards to the gestational age at diagnosis of NIHF. They included 273 fetuses with NIHF and divided the pregnancies into three groups. Group A included pregnancies up 13+6 weeks, Group B included pregnancies from 14 to 24+6 weeks, and Group C included pregnancies from 25 weeks onward. Group B and C were divided at 24-25 weeks because termination of pregnancy (TOP) was still available until 23 weeks of gestation. In 44.3% of included cases, TOP was performed. In Group A, 79.7% of pregnancies were terminated, 45.3% and 11% of pregnancies were terminated in Group B and in Group C, respectively. This means that in total 152 women or 55.7% of women decided to continue their pregnancy, of which 31.6% underwent fetal intervention. Fetuses who underwent intervention more frequently presented with congenital infection and fetal anemia. The mean gestational age at diagnosis was 27.1 and 32.8 at birth in cases when fetal intervention was initiated. In cases in which no fetal therapy was started, mean gestational age at diagnosis was 23.4 and at birth 29.4. The rate of resolved hydrops did not differ significantly between the pregnancies which underwent fetal intervention and those that did not. But the risk of perinatal death decreased significantly: the pregnancies which underwent fetal therapy had a risk of 31.3% compared to a risk of 60.6% in pregnancies that did not undergo fetal intervention (11). Taking the findings of this study into account, it is important to explain the risk of miscarriage and neonatal death to the parents before deciding for a treatment option or for TOP. Table 1 summarizes the findings of Sileo et al. The probability of live birth increases with gestational age, but one has to

keep in mind that a part of intrauterine death (IUD) is due to TOP (11).Management of NIHF in the delivery room might require intubation, positive pressure ventilation, cardiac compression, need for surfactant treatment and other interventional procedures like thoracocentesis as an example. Whether or not the neonate requires these interventions or not can be a predictive value as well. According to Turkoglu Unal et al., the need for intubation was higher in non-survivors, as well as the need for resuscitation at birth and surfactant treatment. On the other hand, positive pressure ventilation was more often utilized in survivors. In total, paracentesis was the most commonly applied interventional procedure in this study, followed by thoracocentesis, thoracic tube placement and blood exchange. Pericardiocentesis was not performed in any of the neonates. Thoracocentesis and paracentesis were more commonly applied in non-survivors whereas thoracic tube placement

Outcome	*≤ 13 + 6 weeks (n = 15)*	*14 to 24 + 6 weeks (n = 64)*	*≥ 25 weeks (n = 73)*	P
Live birth	3 (20)	26 (40.6)	45 (61.6)	0.003
Neonatal death	2 (13.3)	6 (9.4)	16 (21.9)	0.12
IUD or miscarriage	10 (66.7)	32 (50)	12 (16.4)	< 0.001

Data are given as *n* (%). *Patients who opted for termination of pregnancy were not included in analysis (*n* = 121). IUD, intra-uterine demise.

and blood exchange were more common in survivors (1).

Table 5: Outcome of 152 included pregnancies with non-immune fetal hydrops, according to gestational age at initial diagnosis

Source: Sileo FG, Kulkarni A, Branescu I, Homfray T, Dempsey E, Mansour S, et al. Non-immune fetal hydrops: etiology and outcome according to gestational age at diagnosis. Ultrasound Obstet Gynecol. 2020 Sep;56(3):416–21.

METHODS

This literature review aimed at summarizing current knowledge on the etiology, diagnosis, and treatment of hydrops fetalis, with a focus on NIHF. The review was conducted using peer-reviewed scientific articles, clinical guidelines, and relevant case studies. A comprehensive search was carried out using databases including PubMed, ScienceDirect, and Google Scholar. The literature search was focused on publications from 2020 to 2024 to ensure inclusion of the most recent findings. Search terms included “hydrops fetalis”, “nonimmune hydrops fetalis”, “fetal anemia”, “parvovirus B19”, “fetal arrhythmias”, “lysosomal storage disorders”, “Maternal Mirror

Syndrome", "Fetomaternal Hemorrhage" and "outcomes nonimmune hydrops fetalis". Articles were included if they met the criteria which included that the article was published in English, focused on human subjects and related to the prenatal diagnosis, etiology, treatment, or outcomes of hydrops fetalis. Studies were excluded if they were non-peer reviewed, lacked full text, were published before 2015 or focused on postnatal outcomes and therapy only, without relevance to prenatal diagnosis or intervention.

DISCUSSION

In February of 2015, the Society for Maternal-Fetal Medicine (SMFM) published the 7th Clinical Guideline for nonimmune hydrops fetalis. As discussed earlier in this thesis, the next steps after identifying a hydropic fetus on ultrasound is to find the underlying etiology, establish the suitable treatment, if available, and to determine optimal timing for delivery. As shown in Picture 7, the guidelines provide several steps to aid in this process. Ruling out genetic disorders with recurrence risk in the future and identifying treatable conditions is of particular importance. Ultrasound as the first device of diagnostics is useful to determine if structural abnormalities, cardiac arrhythmias, or TTTS are present. The fetus, umbilical cord, and placenta must be assessed for anomalies, and the amniotic fluid volume must be evaluated. A fetal echocardiogram further aids in the diagnostics of cardiac anomalies, since they are one of the most common causes of NIHF. If no structural anomalies are identified, alloimmunzation should be ruled out next, according to the guidelines. This is done by assessment of maternal blood type and Rh(D) antigen status, as well as by an indirect Coombs test to search for circulating red blood cell antibodies. The next step should be to perform middle cerebral artery Doppler studies to evaluate if fetal anemia is present. Regardless of the identification of sonographic anomalies, the guidelines recommend to offer fetal karyotyping, fluorescence in situ hybridization, and/or CMA. These tests can be conducted via amniocentesis or fetal blood sampling. The latter is useful for direct analysis of fetal hematocrit and hemoglobin in case anemia is suspected. If no other etiology has been identified in a structurally normal fetus, the guidelines recommend invasive testing for LSDs and PCR studies for parvovirus, toxoplasmosis, and CMV infection. To rule out genetic abnormalities, the guidelines advise to take a thorough family history and to assess for consanguinity. SMFM Clinical Guidelines strongly recommend to only undertake preterm delivery for obstetric indications because prematurity likely worsens the prognosis. Given the lack of evidence, studies, and because of the wide spectrum of underlying etiologies of NIHF, treatment and care should be individualized. Generally, a delivery by 37 to 38 weeks of gestation should be considered. Delivery should be prompt in case of mirror syndrome.

The development or worsening of NIHF can be an indication for delivery at a gestational age of approximately 34 weeks (14).

The SFMFM Clinical Guidelines recommend diagnostic genetic testing with karyotype and/or CMA. Targeted gene panels which are offered by several commercial laboratories and ES are not mentioned in the recommendations. According to Avram et al., identification of genetic etiologies with CMA and/or karyotyping is successful only in 25% of cases, leaving many cases undiagnosed. Targeted gene panels are cheaper compared to ES and can be helpful if the phenotypic features are already indicative of certain genetic disorders. However, NIHF and fetal effusions are common final manifestations of a wide spectrum of genetic conditions like chromosomal abnormalities and single-gene disorders that impact multiple organ systems. The prenatal phenotypic presentation of many genetic syndromes is poorly understood, and may evolve over time. This makes it difficult to narrow down the broad differential diagnosis prenatally and to pinpoint a genetic etiology by standard testing. Additionally, numerous genetic syndromes and novel variants have just recently been linked to NIHF and fetal effusions. These genetic syndromes had not been previously known to present with these phenotypic features and therefore are not included in most targeted gene panels. As previously described, ES are cost-efficient despite being more expensive in comparison to CMA and karyotype (39). Therefore, updating the clinical guidelines and including ES would help to identify underlying etiologies which would otherwise stay undetected. Knowing the exact underlying disorder helps to counsel the affected families, to plan treatment, pregnancy and delivery and to discuss the possibility of TOP keeping mind the prognosis of the associated genetic condition. Furthermore, more knowledge about possible etiologies would be gained and the pathophysiology can be studied more thoroughly. Generally, literature and studies on NIHF are still scarce and sometimes contradicting. Research would not only uncover more genetic conditions which can manifest as NIHF and improve understanding of the pathophysiology but would also improve prenatal counseling.

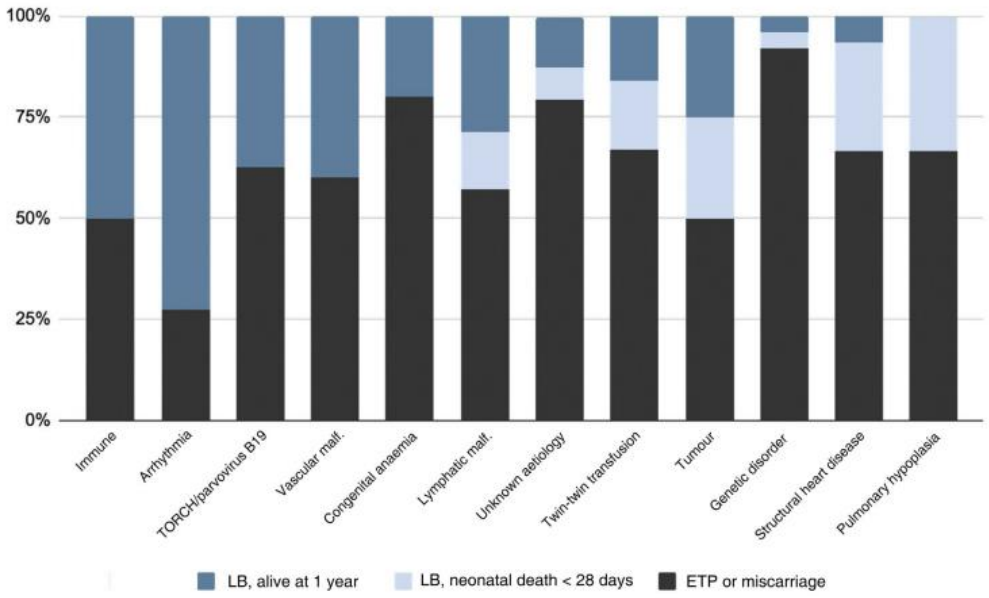
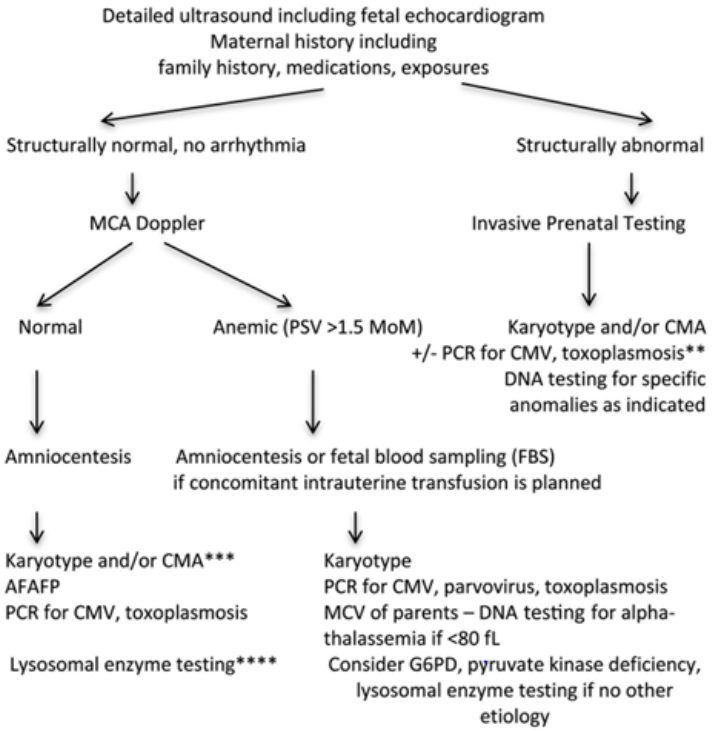


Table 6: Obstetric and neonatal outcomes by aetiology of hydrops fetalis.

LB, live birth; malf., malformation (57)

Source: Morey-Olivé M, Marín Córdoba C, Camba Longueira F, Rodó Rodríguez C, Arévalo Martínez S, Maíz N, et al. Neonates with a prenatal diagnosis of hydrops fetalis: A 10-year experience in a tertiary care center. An Pediatría Engl Ed. 2024 Feb;100(2):115–22.



Picture 7: Workup of nonimmune hydrops fetalis according to SMFM

Source: Norton ME, Chauhan SP, Dashe JS. Society for Maternal-Fetal Medicine (SMFM) Clinical Guideline #7: nonimmune hydrops fetalis. Am J Obstet Gynecol. 2015 Feb;212(2):127–39.

CONCLUSIONS

Hydrops fetalis remains a complex and life-threatening fetal condition. With the decline in incidence of immune-related cases due to effective Rh immunoprophylaxis, NIHF now accounts for the majority of cases. The underlying etiology of NIHF is complex and not yet fully researched. Diagnostic tools like ES and targeted gene panels should be included in the official guidelines to uncover more possible causative genetic etiologies. A more thorough understanding of the causes of NIHF would improve prenatal counselling, choosing the appropriate therapeutic management and would help to plan birth at the best possible moment to improve fetal and maternal outcome. The underlying mechanism of conditions like fetomaternal hemorrhage and Maternal Mirror Syndrome are not yet understood and the literature inconclusive. More research must be implemented to reduce the perinatal fetal mortality and to understand the complex and broad pathophysiology of the different causes of NIHF.

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