# IMMUNOHISTOCHEMICAL STUDIES IN A HYDROPTIC FETUS WITH PULMONARY LYMPHANGIECTASIA AND TRISOMY 21

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*ABSTRACT*

*This case report presents a hydroptic trisomy 21 fetus affected by lymphatic dysplasia with no other malformations. Our studies using CD31, CD34, smooth muscle actin, desmin, and D2-40 antibodies immunohistochemistry confirm the diagnosis of severe pulmonary lymphangiectasia associated with lymphangiectasia in the mediastinum and small bowel.*

**Keywords:** pulmonary lymphangiectasia, trisomy 21, hydrops fetalis

Hydrops fetalis (HF) is the specific term for a disorder characterized by generalized soft tissue edema and various degrees of cavity effusions in fetuses and affected newborns. It is estimated that 50% of all cases diagnosed in utero result in fetal death and that 50% of all live-born infants with non-immune hydrops die soon after birth. Prematurity, pulmonary hypoplasia, chromosome disorders, structural malformations, and any form of pleural effusions eventually leading to hydrops are all associated with poor prognosis (1-3).

We report the autopsy findings of a hydroptic fetus affected by trisomy 21 who presented with pulmonary lymphangiectasia and lymphatic vessel dysplasia in the small bowel and mediastinum with no other malformations. To the best of our knowledge, this is the second report of HF in trisomy 21 caused by generalized lymphatic dysplasia with no other anomalies.

## MATERIALS AND METHODS

### Case Report

A 43-year old pregnant woman underwent ultrasound fetal examination in the 16th week of gestation, which revealed hydrops fetalis. Previous ultrasound studies had been normal, and in particular, heart malformations and genitourinary malformations had been ruled out. Family history was negative. When hydrops was diagnosed, fetal heart echography and Doppler blood flow assessment were normal. Maternal evaluation, including blood type, Rh antibody screening, Kleihauer-Betke stain, TORCHES-CLAP titer (TOxoplasma gondii; Rubella virus; Cytomegalovirus; Herpes simplex virus; Enterovirus; Syphilis; Chickenpox [varicella-zoster] virus; Lyme disease [borrelia burgdoferi]; AIDS; Parvovirus B19), metabolic studies, and hemoglobin electrophoresis were all normal. Fetal TORCHES-CLAP was negative and fetal karyotype demonstrated trisomy 21 (47,XY,+21) (G-bands). The mother terminated the pregnancy, and permission was given to carry out post-mortem examination of the fetus and placenta.

## Methods

Autopsy of the fetus was performed following an established protocol (4). Specimens from each organ were fixed in formalin for 12 hours, paraffin embedded, and 3-4 micron sections were prepared and stained with hematoxylin and eosin. Lung tissue, dermal tissue of the neck, peri-thymic interstitial tissue, peri-sternal mediastinal tissue, and small bowel tissue showing dilatation of lymphatic vessels were then evaluated by immunohistochemical techniques. The following monoclonal antibodies were used: CD31, a platelet endothelial cell adhesion molecule-1 (PECAM-1) that has proven to be highly specific for vascular endothelial cells (Dako, Glostrup, Denmark, 1A10) (1); CD34, the human hematopoietic progenitor cell antigen, recognized by several monoclonal antibodies including QBEnd10. It is a 110-kd protein that is expressed by embryonic cells of the hematopoietic system, including endothelial cells (Dako, Glostrup, Denmark) (2); smooth muscle actin for identifying smooth muscle cells of the bronchial and bronchiolar wall as well as walls of vessels, myofibroblasts, and myoepithelial cells (Ventana, Arizona, USA, 1A4) (3); desmin, an intermediate filament protein (53 kD) found in smooth muscle cell walls (Ventana, Arizona, USA, NCL-DE-R-11) (4); and D2-40, an antibody that recognizes the transmembrane immuno-protein podoplanin (Signet, England, D2-40) (5). Recent investigations have shown that podoplanin is selectively expressed in the lymphatic endothelium (6) but not in the blood capillary wall and specifically reacts with an O-linked sialoglycoprotein (MW 40K) that is found in the lymphatic endothelium.

All immunohistochemical staining was performed using an automatic stainer (Benchmark XT, Ventana, Arizona), except for D2-40 (does not require pretreatment) which was diluted to 1:120 and incubated for 60 minutes at room temperature. Endogenous biotin was blocked for D2-40, CD31, CD34, desmin, and smooth muscle actin. For all antibodies, endogenous peroxidase was inhibited by incubation with 3% hydrogen peroxide solution in water followed by dipping in pH 8 EDTA-borate. Primary antibodies were followed by a biotin-conjugated secondary antibody and subsequently an avidin/streptavidin-enzyme conjugate for colorimetric visualization by light microscopy (7). Each step of the automated incubation process lasts between 4 and 32 minutes, includes washes between steps, and is processed at 37°C (except for smooth muscle actin, which does not require heating during incubation) (8).

## RESULTS

### Autopsy Findings

External examination revealed a severely hydropic male fetus consistent with trisomy 21. The fetus was 17.5 cm in length and weighed 230 g. Head, thoracic, and abdominal circumferences were 17 cm, 15 cm, and 16.5 cm, respectively.

### Internal Findings

The brain was of normal shape and volume. Ventricles were normal, and no malformations were seen in the cerebral cortex, basal ganglia, mesencephalon, reticular ganglionic mass with cranial nerve nuclei, cerebellar cortex, and spinal cord. The meninges, meningeal vessels, and hypophysis were all normal. No congenital cardiac abnormalities were observed. Foramen ovale and ductus arteriosus were patent. Endocardium and pericardium were of normal consistency and no fluid was present in the pericardial cavity. Abdominal examination revealed no macroscopic anomalies of the liver, spleen, pancreas, gastrointestinal tract, and genitourinary tract, and no fluid was found in the abdominal cavity. There were no other pertinent gross findings.
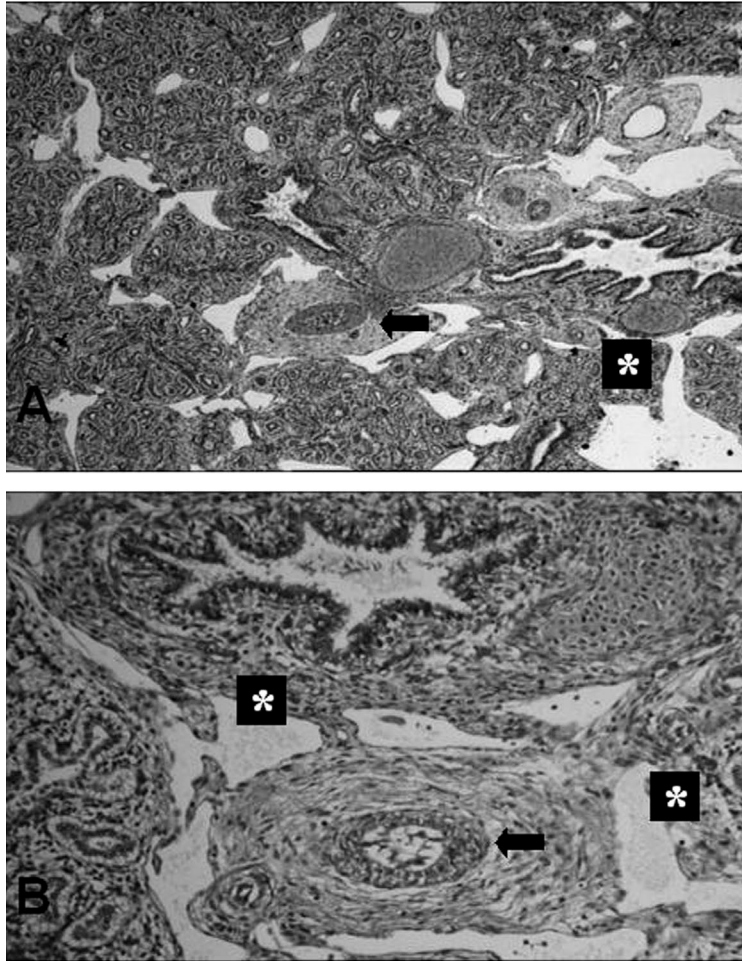
*Fig. 1 (A,B). Microscopic features of the lung showing lymphatic cystic dilatation [hematoxylin–eosin stain; original magnification 2.5X (panel A) 10 X (panel B)]. *=lymphatics; arrows= blood vessels.*

The lungs appeared to be of normal shape and volume. A network of dilated lymphatics was evident in the visceral pleura, which appeared spongy upon cutting and no fluid was found in the pleural cavity. No mass or fibrous processes constrained the mediastinal organs or lungs.

*Histologic Findings (see Figs. 1-4)*

Under microscopic examination, specimens from the neck and upper thorax showed that pulmonary histostructure was appropriate for gestational age (9). The pseudoglandular structure in all lobes of both lungs was separated by thickened interlobular connective tissue that was slightly edematous. Numerous lymphatics in the thickened pleura, interlobular areas, bronchovascular sheaths, and perialveolar spaces were dilated and ranged from 1 to 4 mm in size *(Fig. 1)*. The tunica media of the pulmonary arteries was mildly thickened. Dilated lymphatic vessels were also present in the small bowel interstitial tissue, in the peri-thymic interstitial tissue, and in the peri-sternal mediastinal dermal tissue *(Fig. 2)*. Immunohistochemistry confirmed their lymphatic origin (CD31 and
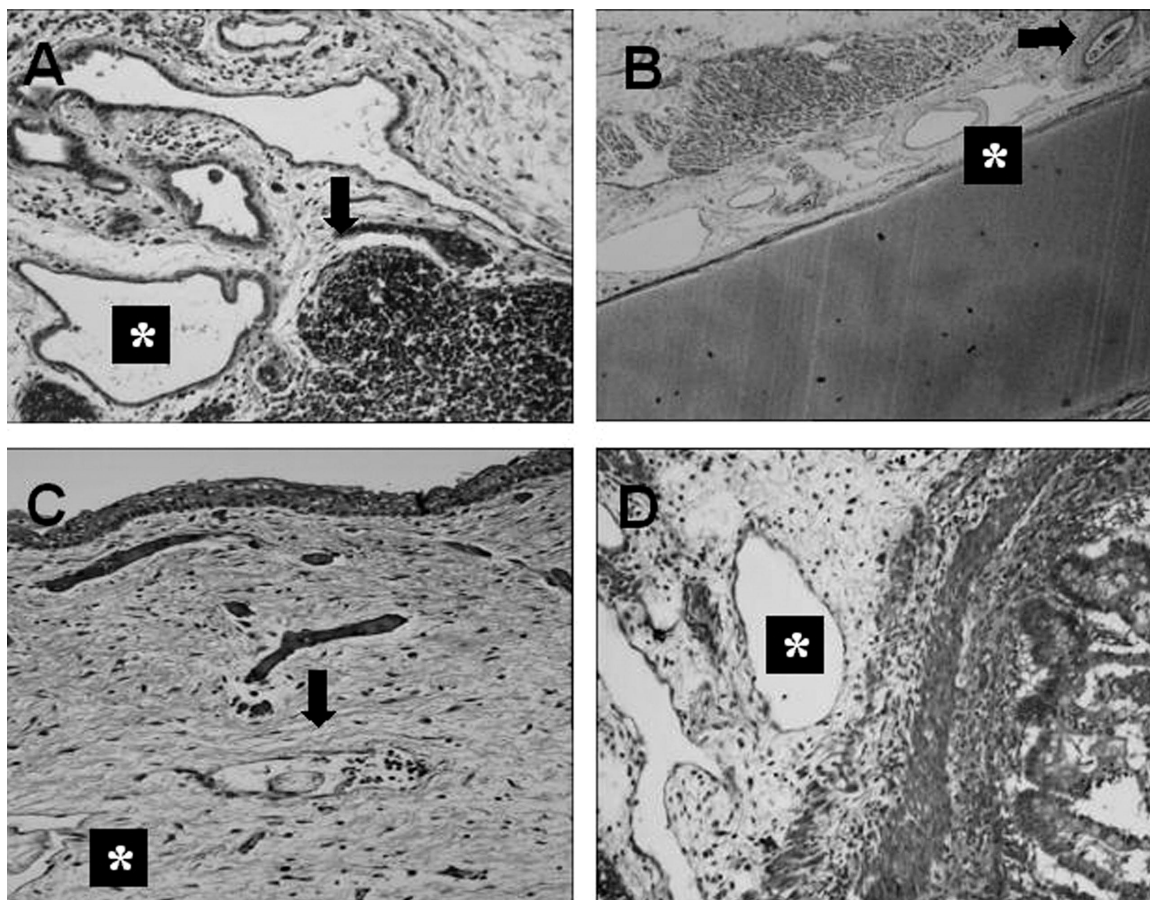
*Fig. 2 (A-D). High number of distended lymphatics are present in the peri-thymic interstitial tissue (panel A), in the peri-sternal mediastinic tissue (panel B), in the dermal tissue of the neck (panel C) and in the small bowel (panel D) [hematoxylin–eosin stain; original magnification 10X (A) 2.5X (B) 2.5 X (C) 10X (D)]. \*=lymphatics; arrows=blood vessels.*

D2-40 positive, CD34 negative) *(Fig. 3).* Lymphatic vessels of the kidneys, heart, spleen, and pancreas were normal. No other significant abnormalities were revealed by the histologic examination.

### *Examination of the Placenta*

Macroscopic examination of the placenta showed that it was single with a retro-membranous hemorrhage of 2 cm in diameter. The umbilical cord was 12 cm long. The oval shaped chorionic disk was 11.5 cm and 8.5 in diameter and weighed 126 g. The amnio-chorionic vessels were dispersed. Histologic study of the placenta showed that the morphology was compatible with the second trimester of gestation, with diffuse hydrops of the villi and dilatation of the terminal villi capillaries *(Fig. 4).* No signs of inflammation were present in the membranes or in the umbilical cord.

### *DISCUSSION*

Our findings confirm a trisomy-21 male fetus affected by hydrops fetalis and severe pulmonary lymphangiectasia with
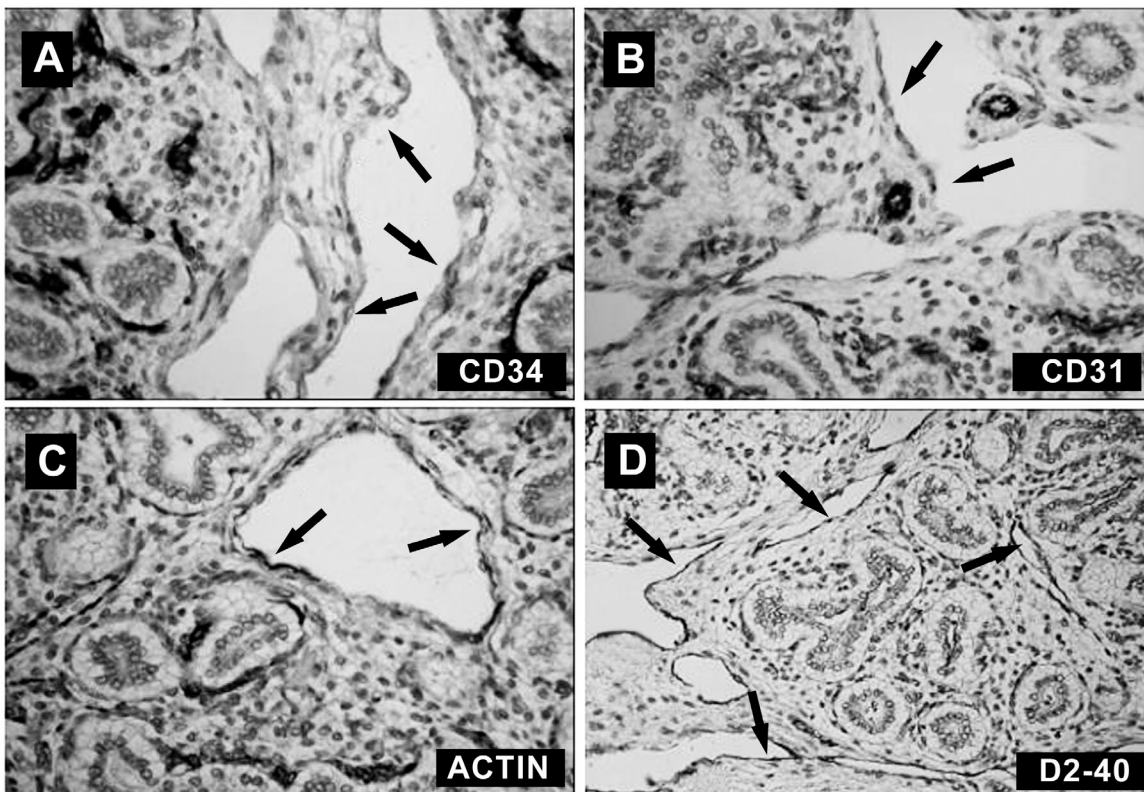
*Fig. 3 (A-D). Lung immunohistochemical studies demonstrate CD34 negative (A), and both CD31 (B) and D2-40 (D) positive lymphatic endothelium. Smooth muscle actin was also seen in rare elongated smooth muscle cells of vessel wall (C). [Original magnification 16X (A-C); 10X (D)].*

lymphangiectasia of the small bowel and mediastinum. It is of interest that macroscopic and histologic findings were otherwise normal, and specifically that the lymphatic vessels of the kidneys, heart, spleen, and pancreas were normal.

The term "Hydrops Fetalis" refers to the end stage of a variety of conditions leading to a pathologic increase in interstitial and total fetal body water content. It primarily appears in soft fetal tissue and serous cavities. The umbilical cord and placenta may also be edematous with placental thickening.

The distinction between immune and non immune hydrops fetalis was made by Potter in her classic article published in 1943 (10). The advent of routine Rhesus immunoprophylaxis drastically reduced the occurrence of immune hydrops fetalis. Cardiovascular,

chromosomal, syndromic, and infectious conditions are the most frequently identified causes of non-immune hydrops (1,11-20). Non-immune hydrops is the end stage of a variety of disorders and is reached through three primary mechanisms: congestive heart failure; decreased plasma osmotic pressure and increased capillary permeability; and congenital lymphatic dysplasia (2,21).

Cardiac impairment, which is usually due to congenital heart malformations that may lead to congestive heart failure, could also be the cause of hydrops fetalis in trisomy 21 affected subjects. It is noteworthy that in our case no cardiac malformations were detected. Trisomy 21 was diagnosed in the present case. Hydrops fetalis has been associated with the occurrence of trisomy 21, as well as with other chromosomal defects
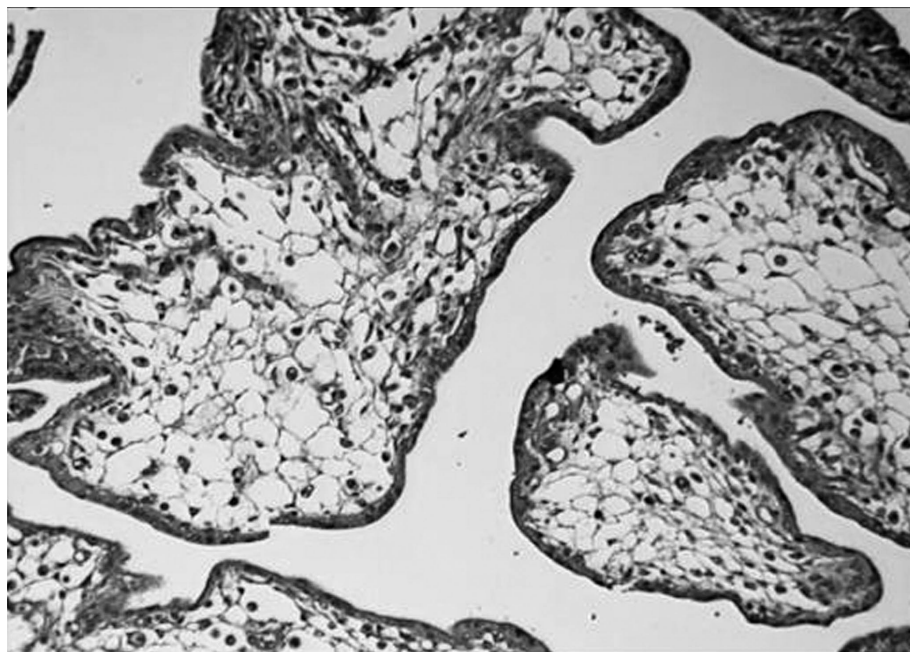
*Fig. 4. Chorionic villi presenting with diffuse hydrops [hematoxylin–eosin stain; original magnification 10X].*

(1), and aneuploidy has been associated with lymphatic phenotype disturbance, in particular with nuchal edema (22).

Congenital pulmonary lymphangiectasis (PL) is a rare developmental disorder involving the lung, and it is characterized by pulmonary subpleural, interlobar, perivascular, and peribronchial lymphatic dysplasia. Congenital PL may be associated with non-immune hydrops fetalis and with congenital chylothorax (1,23). The incidence as well as the etiology of PL are not known (24), and the condition carries a grave prognosis with a mortality rate ranging from 50% to 98% (1). The number of cases of PL may actually be much higher than what has been reported in the literature, most likely due to the fact that lymphangiectasia can only be identified at autopsy, and we can assume that most patients who had PL did not undergo post-mortem examination. On the basis of improved characterization of the clinical presentation and recent noteworthy progress in intensive neonatal care, PL is currently classified into two major categories defined as primary and secondary PL (24,25). Primary PL (as in the present case) may be caused by a congenital defect in the primary development of the lung, or may represent the localized expression of more generalized lymphatic involvement. When it is part of generalized lymphatic dysplasia, PL presents with dilated pulmonary lymphatics as part of a generalized form of lymphangiectasia.

Post-mortem lung examination may be difficult and sometimes not very informative. Lung removal during autopsy causes the lymphatics to collapse, thus preventing the highlighting of the network of intercommunicating channels and making it very difficult to study fetal histology in PL (26). The pathological findings in PL patients may change a great deal over time and may span from initial recognition of minimal evidence of lymphatic dilatation, possibly related to a technical artifact (cross-clamping of the lung), to proof of severe lymphangiectasia (27).

It can be difficult to distinguish lymphatic dysplasia from lymphangiomatosis by histological examination. Pathological

features of lymphangiomatosis include a proliferation of complex anastomosing lymphatic channels that markedly expand the typical lymphatic routes within the lungs and mediastinum. Unlike what is observed in pulmonary lymphangiectasis, lymphangiomatosis displays a prominence of collagen and spindle-shaped cells surrounding the endothelial lined channels. In addition, there is a greater number of dilated lymphatic channels, while there is no increase in the amount of lymphatics in PL. Pleural effusions are common in lymphangiomatosis (28).

In our case, severe dilatation of the intrapulmonary lymphatics was evident, and it was associated with the presence of dilated lymphatic vessels in the small bowel interstitial tissue, in the peri-thymic interstitial tissue, and in the peri-sternal mediastinal tissue, while lymphatic vessels of the kidneys, heart, spleen, and pancreas were normal. The dilated vessels were characterized by a thin wall, rare or absent smooth muscle, and slightly dilated lumen. Furthermore, the lymphatic vessels were lined with flattened endothelial cells. Immunohistochemical studies allowed us to distinguish lymphatic dysplasia from lymphangiomatosis, clearly showing severe involvement of lymphatics which were positive for CD31 and lymphatic-specific D2-40 and did not express CD34, a specific marker of blood endothelial cells (29), in the mediastinum and small bowel.

In summary, although it is well known that hydrops fetalis is associated with aneuploidy and visceral (mostly cardiac) malformations, to the best of our knowledge, besides the original description by Ochiai et al (30), this is the second description of the simultaneous occurrence of trisomy 21 and NIHF due to generalized lymphatic dysplasia, with no other anomalies. We suggest that detailed immunohistochemical histologic analysis should be included in the evaluation of all cases of fetal hydrops in the search for possible abnormal lymphatic phenotypes.

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