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NEW ADVANCES ON PLACENTAL HYDROPS AND RELATED VILLOUS LYMPHATICS

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ABSTRACT

Fetoplacental hydrops is the final stage of several pathological conditions in which the placenta and umbilical cord become edematous and the fetus develops an anasarcatic state characterized by an excessive accumulation of extravascular fluids in at least two serous cavities of the body. It is a common histological finding of stillbirth, characterized by the appearance of markedly edematous villi, suggesting an increased interstitial fluid accumulation. The recent improved knowledge of lymphangiogenesis and the availability of monoclonal antibodies selectively labeling lymphatic endothelium lead to the hypothesis that villous edema is essentially a lymphedema from defective lymphatic function following inadequate villous blood circulation. Lymphedema is a morphologic phenotype found by our research group in a 24-case series of stillbirths from different morbid conditions such as chromosomal aberrations, congenital malformations, inherited hemoglobinopathies, and prolonged perinatal severe anoxia. Unlike long-lived organs, the placenta is devoid of innervation by the autonomic nervous system; therefore, the vascular tone regulation and the peripheral perfusion are modulated by the expression of the angiotensin converting enzyme (ACE) in the vascular endothelia. This finding may suggest to the clinician to search for a more suitable therapy in case of mother's hypertension during pregnancy.

Keywords: placental hydrops, villous lymphatics, preeclampsia, vascular endothelium, podoplanin (D2-40), angiotensin-converting enzyme (ACE), Starling forces, stillbirth

The first literary description of placental hydrops in a twin pregnancy dates back to 1609 by the work of Louise Bourgeois who regarded it as a disease of unknown etiology (1). In 1892 John William Ballantyne assumed its multifactorial etiology, suggesting that *'we are dealing not with a pathologic entity, but with a group of symptoms common to several different morbid conditions'* (2). Only in 1946, after the identification of Rhesus (Rh) factor, Edith Louise Potter distinguished immune hydrops from non-immune hydrops (3). Fetoplacental hydrops is the final stage of several pathological conditions in which the placenta and umbilical cord become edematous (*hydrops placentae*) and the fetus develops an anasarca state characterized by an excessive accumulation of extravascular fluids in at least two serous cavities of the body (*hydrops fetalis*). It may be accompanied by gravidic maternal complications such as preeclampsia, bleeding, and anemia, with possible need for emergency cesarean section. Grossly, a hydropic placenta, regardless of

the trigger causes is soft, friable, and bulky, weighing usually more than 1000 g, with a pale cutting surface. Sometimes in the hemolytic forms a jaundiced color of the umbilical cord and superficial chorionic vessels can be seen. On microscopy, the villi appear dilated with compression of the intravillous capillaries in which nucleated red blood cells (NRBCs) can be detected. The villous stroma is edematous with an increase of the cytotrophoblastic shell and of Hofbauer cells.

Epidemiology

Placental hydrops has an incidence of about 11% with a high index of fetal, perinatal, and neonatal mortality (80%) as a function of etiology, gestational age, severity, and therapeutic treatments performed (4). It correlates with a low APGAR score at birth and often intensive therapy for the neonate is needed. The APGAR score was devised by Virginia Apgar in 1953 and it is based on five tested parameters: Appearance (skin color), Pulse (heart rate), Grimace (reflex irritability), Activity (muscle tone), and Respiration (respiratory effort) each assigned a rating from 0 to 2. The maximum achievable score is 10 (5). It is checked immediately after delivery with the aim to evaluate the newborn adaptation to extra-uterine life, in terms of vitality and efficiency of the primary functions. More in detail, the examination is performed at 1, 5 and 10 minutes of the infant's life. Infants with score less than 4 are in critical conditions and they require immediate medical intervention in order to prevent longer-term neurological damage; those with a score between 4 and 6 are at risk for health in need of care, supervision, and retesting every 5 minutes; infants with score between 7 and 10 are within a healthy normal range. In the course of placental hydrops, perinatal mortality is estimated to be more than 50%, reaching 100% if genetic defects are concomitant to it (4).

The introduction of maternal prophylaxis with Rho globulins has successfully reduced the incidence of immune hydrops in Rh+ fetuses from Rh- mothers, the form most frequently found in the past (6).

Etiology

Placental hydrops is classified into immune and non-immune hydrops. Approximately 75% of cases are non-immune based (6). Immune hydrops, also known as erythroblastosis fetalis or hemolytic disease of the fetus, is caused by the transfer through the fetoplacental compartment of maternal antibodies directed against surface antigens (D, C, E, Fy, Kell, ABO) of fetal red blood cells as a result of alloimmunization (previous pregnancy, amniocentesis, abdominal trauma), with a consequent intravascular hemolytic anemia (type II hypersensitivity reaction). The bone marrow compensates for this by producing a high number of erythroblasts and by expanding into extramedullary sites, such as spleen and liver, where marked deposits of iron and consequent organ failure occur. The high-output heart failure related to anemia and hypoproteinemia from liver failure associated with an oncotic pressure decreased are responsible for the development of hydrops.

In 1989, Geoffrey Machin advanced a further sub-classification of non-immune hydrops according to the root causes updated as follows: cardiovascular abnormalities, infective conditions, hematologic disorders, genetic defects, intrathoracic lesions, nephro-intestinal diseases, and neoplastic proliferations (7-10).

Cardiovascular abnormalities

Congestive heart failure resulting from defects in electrical (tachyarrhythmias, atrioventricular blocks) or mechanical (cardiomyopathies, premature closure of the foramen ovale, septal faults, endocardial fibroelastosis, valvular insufficiencies, hypoplastic left heart, cardiac tumors) systems is the major cause of non-immune fetoplacental hydrops.

Infective conditions

Intrauterine infections caused by viruses (Parvovirus B19, Rubella, Cytomegalovirus, Herpes simplex, Hepatitis B, and Hepatitis C), bacteria (Treponema pallidum, Leptospira interrogans, Listeria monocytogenes) and protozoa (Toxoplasma gondii, Trypanosoma cruzi) can cause fetoplacental hydrops, especially in mothers not previously exposed to the infective agent.

Hematologic disorders

Non-immune anemias and related hematological disorders are responsible for placental hydrops due to high-output heart failure with increased serum levels of lactic acid, aldosterone and atrial natriuretic peptide, all factors regulating the intravascular pressure according to the Starling's model. The anemias from haemoglobinopathies are those most commonly associated with hydrops; more in detail, homozygous -thalassemia is the leading cause (86%) of fetoplacental hydrops in Southeast Asia (7). A special mention is deserved to the twin-to-twin transfusion syndrome (TTS). Superficial and deep vascular anastomoses are constantly present in all monochorionic twin pregnancies; however, in 15% of cases a massive shift of blood from a twin (donor) to another (recipient) can occur, resulting in anemia and polycythemia, respectively. This condition is known by the term twin anemia-polycythemia sequence (TAPS). It is a possible finding during monochorionic diamniotic pregnancies, and it is defined as the occurrence of anemia in the donor and polycythemia in the recipient, antenatally diagnosed by middle cerebral artery (MCA) – peak systolic velocity (PSV) >1.5 multiples of median in the donor and MCA PSV <1.0 multiples of median in the recipient, in the absence of oligohydramnios- polyhydramnios (11). Hydrops may appear in both twins, due to high-output heart failure from anemia and circulatory-overload heart failure from polycythemia. The most widely used TTTS staging system was introduced by Quintero et al, and it is based on ultrasound findings (12). The TTTS Quintero staging system includes five stages, ranging from mild disease, with discordant amniotic fluid volume, to severe disease, with demise of one or both twins. More in particular, stage IV refers to the presence of fetal hydrops as unfavorable prognostic factor (12).

Genetic defects

Placental hydrops is found in fetuses with storage syndromes such as Gaucher and Niemann-Pick, or chromosomal defect syndromes including Down (47, +21), Edwards (47, +18), Patau (47, +13), and Turner (45, X0). In this last case, the incomplete formation of the lymphatic drainage system into the thoracic duct leads to the hydropic degeneration.

Intrathoracic lesions

The intrathoracic space-occupying masses, associated with a mediastinal shift, reduce the venous return to the heart resulting in the formation of hydrops with central venous hypertension. Among these are: congenital cystic adenomatoid malformation (CCAM), chylothorax, diaphragmatic hernia, pulmonary sequestration, pleural collection, bronchogenic cyst, hamartoma and mediastinal tumors.

Nephro-intestinal diseases

The congenital nephrotic syndromes (autosomal recessive traits), in particular the Finnish type (CNF), are associated with fetoplacental hydrops for reduction of intravascular oncotic pressure. A 10-fold increase in the α-fetoprotein concentration in the amniotic fluid is suggestive for congenital nephrotic syndrome. The diseases of the gastrointestinal tract associated with hypoproteinemia (volvulus, intussusception,

perforation) are also associated with fetoplacental hydrops, due to reduction of intravascular oncotic pressure.

Neoplastic proliferations

The voluminous neoplastic masses cause fetoplacental hydrops due to high-output cardiac failure. Among these are teratomas, particularly in sacral localization, hemangiomas, rhabdomyomas, mesoblastic nephromas, multiform glioblastomas, medulloblastomas, and neuroblastomas, the last responsible for hydrops also in relation to the secretion of vasoactive catecholaminergic substances. Moreover, the advanced-stage fetal neuroblastomas are often associated with maternal preeclampsia. The cavernous lymphangioma (cystic hygroma), which usually occurs in the neck or underarm, is commonly associated with a pronounced placental hydrops and therefore presumes an abnormal placental development of the lymphatic system.

MATERIALS AND METHODS

Our research group has histologically investigated a 24-case series of stillbirth from different pathologic conditions, such as chromosomal aberrations (4 cases), congenital malformations (8 cases), inherited hemoglobinopathies (4 cases) and prolonged perinatal severe anoxia (8 cases). The placental samples from stillbirths (12 male and 12 female) were compared with 12 cases of full-term placentas from live births. All the samples were fixed in 10% neutral buffered formalin and then paraffin embedded. In addition to hematoxylin/eosin staining, immunohistochemistry for lymphatic endothelial cells (podoplanin), vascular endothelial cells (CD31), and angiotensin-converting enzyme (ACE/CD143) was performed on both pathological and healthy samples. Podoplanin is a transmembrane O-linked sialoglycoprotein (38 kDa) selectively expressed on lymphatic endothelium, as well as neoplasia of lymphoid origin including lymphangiomas and lymphangiosarcomas (13). It has been demonstrated that anti-podoplanin reacts with lymphatic and not blood vascular endothelium (14). Moreover, in neoplastic tissues, this immunostaining has been able to clearly identify the lymphatic invasion from primary cancers (15,16). CD31, also known by the acronym PECAM-1 (Platelet Endothelial Cell Adhesion Molecule), is a type 1 single-chain transmembrane protein (135 KDa) belonging to the immunoglobulin superfamily which plays an important role in adhesive interactions between adjacent endothelial cells, leukocytes, and platelets. CD31 is expressed in all continuous endothelia, including those of arteries, arterioles, veins, venules, and capillaries (17). CD31 is commonly used to demonstrate the presence of endothelial cells in histological tissue sections, favoring a correct interpretation of the degree of tumor angiogenesis and related intravascular metastasis. Malignant endothelial cells commonly retain the antigen, so CD31 immunohistochemistry is also a useful tool to identify the vascular origin of neoplasms (18). CD143 is a type 1 single-chain transmembrane metallopeptidase (171 kDa) whose cofactor is zinc and belongs to the family of carboxypeptidases. The enzyme catalyzes cleavage of decapeptide angiotensin I into the octapeptide angiotensin II by cutting the last two C-terminal aminoacids. CD143 is expressed on endothelial cells, particularly those of pulmonary and renal capillaries (19). The activation of macrophages and histiocytes induces the expression of this molecule and its main targets are angiotensin I and bradykinin acting as a blood pressure regulator. Angiotensin II is a potent hypertensive agent which controls arteriosus vasoconstriction and extravascular fluid balance through the renin-angiotensin-aldosterone system favoring water and sodium reabsorption. At the same time, ACE is able to degrade the vasodilator molecule bradykinin, thus increasing the vasoconstrictor activity of angiotensin II (19).

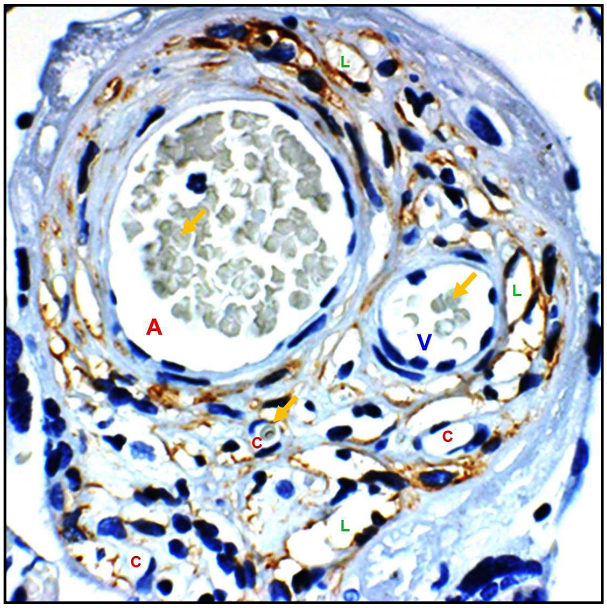

Fig. 1. A full-term placenta of healthy new-born delivered at 36th week. The staminal villous lymphatics (L) are brown-stained by the mouse monoclonal anti-podoplanin antibody. The endothelia of arteriole (A), venule (V) and hematic capillaries (C) are unstained, because they do not express podoplanin. All the hematic vessels contain red blood cells (highlighted by yellow arrows). The lymphatics do not contain erythrocytes [Chromogen: DAB; original magnification: x100].

After deparaffinization, hydration, endogenous peroxidase blocking, and heat-induced antigen retrieval, tissue sections were incubated for 30 minutes at room temperature with anti-podoplanin (clone D2-40 prediluted; Dako, Glostrup, Denmark), anti-CD31 (clone JC70 prediluted; Ventana, Tucson, AZ, USA) and anti-CD143 (clone 3C5 prediluted; Leica, Wetzlar, Germany). Biotinylated secondary antibody was applied and the staining product detected with avidin-biotin complex (ABC) against a hematoxylin counterstain. Detection of the staining reaction was achieved by an enzyme conjugated polymer complex adapted for automatic stainers from Ventana Medical Systems. Moreover, for the first time, we implemented an immunohistochemical development with double chromogen, that is 3-3' diaminobenzidine tetrahydrochloride (DAB) and new fuchsin. For each case of stillbirth two sections for immunohisto-chemistry were taken into consideration. The first section was routinely stained with only one antibody (anti-CD143) and one chromogen (DAB), while the second section was chronologically subjected to a double staining in sequence, using two different antibodies (anti-podoplanin and anti-CD31) and two different chromogens (new fuchsin and DAB), respectively. In the first step, this procedure provided detection of any lymphatic structure using specific anti-podoplanin in fuchsia color (new fuchsin). In the second step, anti-CD31 antibody was used to identify the blood vascular structures in order to avoid false lymphatic positives highlighted them in a brown color (DAB).

RESULTS

In all the control cases of full-term placentas from live births, thin-walled lymphatics were clearly demonstrated, in particular at the level of staminal villi by the employed anti-podoplanin antibody (*Fig. 1*). The staminal villus appeared to be composed

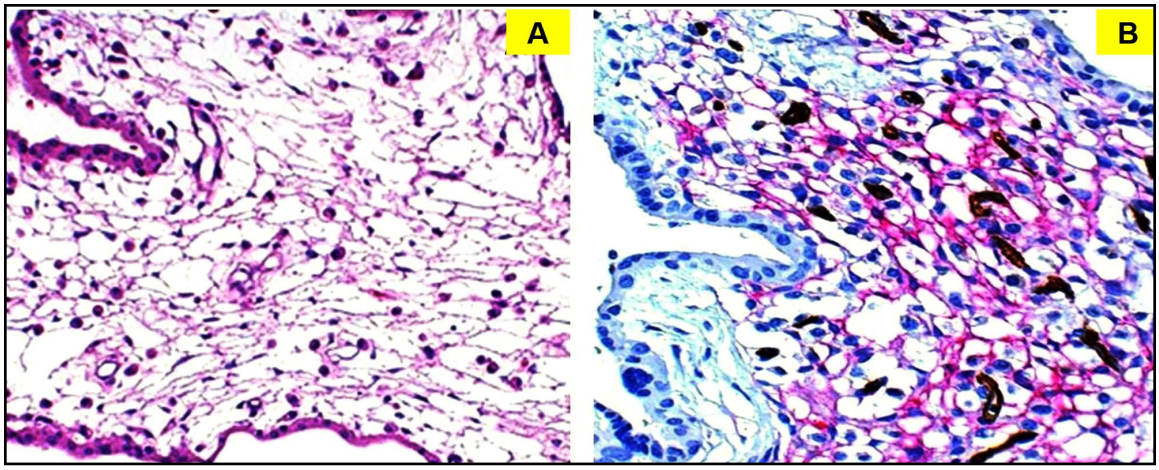

Fig. 2. A placenta of stillborn at 14th week, affected by large retro-nuchal cystic lymphangioma and thymic hypoplasia, shows marked and diffuse villous stromal edema (A, hematoxylin/eosin). A number of dilated lymphatics, devoid of red blood cells within them, are fuchsia stained by anti-podoplanin antibody, while scattered squeezed capillaries are brown stained by the mouse monoclonal anti-CD31 antibody (B), a typical marker of hematic endothelia [Chromogens: new fuchsin and DAB, respectively; original magnification: x40].

of one arteriole, one venule, and several hematic and lymphatic capillaries (*Fig. 1*). Lymphedema was a morphologic phenotype constantly found by our research group in the 24 cases of stillbirths from different pathologic conditions, that is chromosomal aberrations (4 cases), congenital malformations (8 cases), inherited hemoglobinopathies (4 cases), and prolonged perinatal severe anoxia (8 cases). In all cases a number of dilated lymphatics was related to villous edema (*Fig. 2A*). The implementation of a double chromogenic immunostaining, based on a close sequential use of anti-podoplanin and anti-CD31, has successfully allowed discernment of lymphatic from blood vessels. Moreover, it has also demonstrated compression of hematic capillaries by the adjacent hydropic lymphatics (*Fig. 2B*). ACE expression was evaluated by a semi-quantitative method based on percentage of endothelial cells which immunohistochemically showed membranous positivity for anti-CD143 assigning the following scores: 0 score = 0%, 1+ score = 1-33%, 2+ score = 34-66% and 3+ score 67-100%. In control cases, scores ranged from 1+ (6 cases) to 2+ (6 cases), while in all the aforementioned 24 cases of placental hydrops a 3+ score, and therefore an ACE overexpression, was observed in hematic endothelia and Hofbauer cells, a placental type of histiocytes (*Fig. 3*). In fact, as mentioned above (19), macrophages and histiocytes can induce the enzyme expression.

DISCUSSION

Immune and non-immune placental hydrops are a common histological finding of stillbirth, characterized by the appearance of markedly edematous villi, suggesting an increased interstitial fluid accumulation. It is a well-known finding first reported as *'presence of cavities and cisterns lined by endothelioid cells'* (20). The recent improved knowledge of lymphangiogenesis (21,22) and availability of monoclonal antibodies for immunohistochemistry, selectively labeling lymphatic endothelium (23,24), lead us to hypothesize that villous edema is essentially a lymphedema from defective lymphatic function following inadequate blood villous circulation. Our immunohistochemical results support the presence of a lymphatic

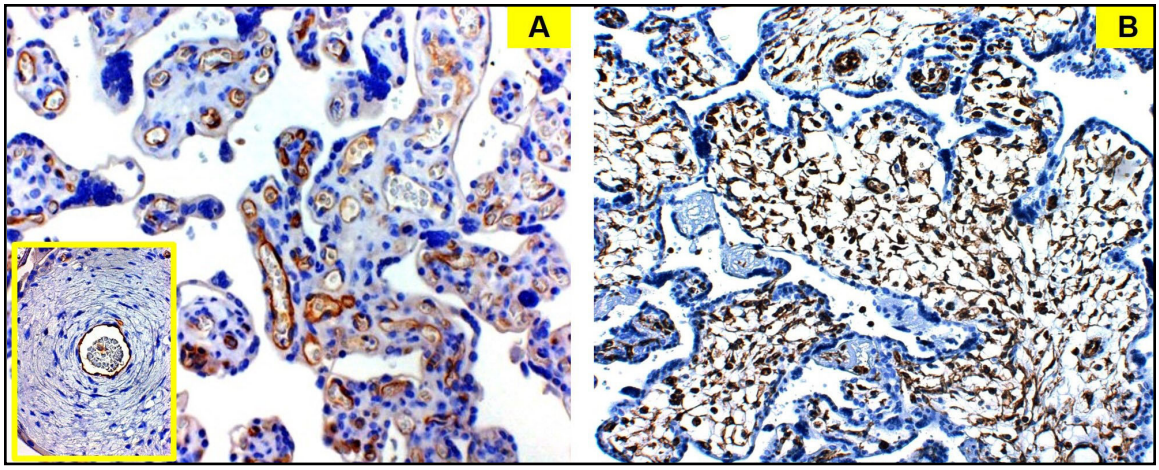

Fig. 3. A full-term placenta of healthy new-born delivered at 36th week (A): the capillaries of terminal villi and the arterial endothelium in a stem villus (insert, x100), filled with red blood cells, are brown-stained by the mouse monoclonal anti-CD143 antibody. A placenta of stillborn at 14th week, characterized by marked and diffuse hydrops (B): an ACE overexpression in hematic endothelia and in Hofbauer cells is observed by using the anti-CD143 antibody [Chromogen: DAB; original magnification: x40].

conductive network inside the placenta, as suggested by Bellini et al (25), and agree with those of Red-Horse (21), who succeeded in detecting the presence of lymphatics in human decidua by using a different marker, LYVE-1, a hyaluronic acid receptor specifically localized to lymphatic endothelia. Furthermore, Red-Horse et al (22) developed an in vivo experimental model of human placentation, together with in vitro analyses, proving that placental cytotrophoblast stimulates lymphangiogenesis. The authors considered the placenta the trigger for the development of decidual lymphatic circulation and theorized its role in maintaining fluid balance and maternal-fetal immune cell trafficking during pregnancy (22).

Unlike long-lived organs, the placental hemolymphatic unit is devoid of innervation by the autonomic nervous system; therefore, vascular tone regulation and the peripheral perfusion are modulated by ACE expression in the vascular endothelia (26-28). High levels of ACE have been associated with preeclampsia, a gestational disorder characterized by hypertension, proteinuria, and utero-placental abnormalities, such as shallow trophoblast invasion and impaired spiral artery remodeling (29-31). Moreover, angiotensin II type 1 receptor agonistic autoantibody (AT1-AA) have been detected in numerous preeclamptic women (32,33). These data, together with the evidence of an increased incidence of pregnancy-induced hypertension among mothers with pre-existing cardiovascular diseases, support a causal link between ACE activity and preeclampsia (34). On the other hand, low levels of ACE have been significantly correlated with an increased risk for intrauterine growth restriction (IUGR), preterm birth, and small birth weight (35, 36), confirming its functional importance in the adjustment of maternal-fetal nutrient exchanges. The Ballantyne syndrome, also called 'mirror syndrome,' is another gestational disorder characterized by the association of maternal preeclampsia and fetoplacental hydrops, due to a variety of obstetric problems, including immunohaematological and metabolic diseases, fetal infections, and fetal malformations (37). Although the exact etiopathogenetic mechanism of Ballantyne syndrome remains unknown, it can be

curiously found in association with TTS syndrome (38).

Moreover, our finding supports the statement by Ito et al, according to whom the feto-placental unit could induce ACE mRNA expression in response to hypoxic conditions (39), and agrees with the overview by Kovalovszki et al, who have suggested that edema fluid is an interposed barrier to gas exchange between mother and fetus (40). According to the authors, capillaries can be blocked by edematous swelling with a subsequent reduction in blood flow through the villi. This abnormality, if widespread, may reduce gas exchange and, therefore, they suggest that hypoxia could partly be prevented by preventing the development of placental villous edema (40). In the literature, data concerning the role of ACE in lymphologic abnormalities are completely missing. From our placental series, it emerges that ACE is the intrinsic regulator of the placental vascular system and, for this reason, it could have an indirect effect on surrounding lymphatics, too. Its expression likely varies depending on the intravascular pressure status. Under basal conditions, in the event of an increase of intravascular hydrostatic pressure or a decrease in the colloid-osmotic pressure, ACE expression should fall in endothelial and Hofbauer cells. On the other hand, in the case of a decrease of hydrostatic pressure or an increase in colloid-osmotic pressure, its expression should rise, following a negative feedback model. At the time when hydropic edema is established, there is a compression of the capillaries resulting in a reduction of maternal-fetal exchanges and ACE is overexpressed in the attempt to maintain the vascular intravillous perfusion perceived as inadequate to feed the fetus. If the pathological substrate, which causes placental hydrops is not removed, a further accumulation of fluid in the extravascular compartment with a subsequent increase of edema is expected due to ACE overexpression (positive feedback model). A malfunctioning hemolymphatic villous unit can explain the varied placental vascular pathology frequently observed in the second trimester of pregnancy. The idiopathic forms of placental hydrops may be the result of a dysregulation in the ACE-mediated system. The infarction events, frequently found in the placenta, could also be caused by local impairments of this regulatory system. Moreover, it may be related to the clinical presentation of recurrent preeclamptic and eclamptic episodes complicating the pregnancy. It may also suggest to the clinician to search for a more suitable therapy for the mother's hypertension during pregnancy.

The placenta is a transient, multifunctional organ connecting two different humans whose functioning is thus maintained by combined hematic and lymphatic circulations. In case of failure of both circulations, lymph-edema develops with cessation of maternal-fetal exchanges (barrier effect), a condition incompatible with the life. The maintenance of an adequate blood circulation through the villus, with related metabolic and trophic effects, is therefore essential to prevent forms of hydropic hypoxia and to ensure deliveries at term of healthy live births.

CONCLUSION

Our immunohistochemical results and the etiological evaluation of placental hydrops permit focusing its pathogenesis on the delicate balance of peripheral vascular pressure, the so-called Starling forces (25). The peripheral pressure balance of the placenta can be affected by:

- INCREASE of hydrostatic venular pressure from blood stasis, as it occurs in the course of cardiovascular abnormalities, genetic defects, intrathoracic lesions and neoplastic proliferations;
- INCREASE of hydrostatic arteriolar pressure from peripheral vasodilatation, as it occurs in the course of hematologic disorders;
- DECREASE of intravascular oncotic pressure from hypoproteinemia, as it

occurs in the course of nephro-intestinal diseases;

- DECREASE of the reflection coefficient of the capillary membrane, as it occurs in the course of infective conditions.

Some illnesses, in particular genetic defects or infectious conditions, can act with a combined pathogenic mechanism, influencing more Starling forces at the same time. The hydropic degeneration of the villi can also be considered a sign of circulatory failure during the appearance of edema the lymphatic drainage system swells as compensation to drain interstitial excess fluids, exactly as the apparatus lymphaticus peripheric absorbens (ALPA) described by Azzali (41). These lymphatics represent the sector with high absorption capacity of the lymphatic vascular system. They play a basic role in preserving tissue homeostasis and in directing interstitial capillary filtrate back to the bloodstream. The placental vasculature develops simultaneously with the maturation of the villi. The fundamental villous unit (placentone), postulated by Robert Andrew Schuhmann in 1982, consists of a villous tree with the relative intervillous space (42). The villous tree is constituted by a stem villus, immature and mature intermediate villi, and by terminal villi. A full-term placenta is composed mostly of terminal and staminal villi. Brought by the utero-placental arteries originating from deciduous plaque, the maternal blood directly reaches the center of the villous tree (hemochorial system), where nutrient exchanges take place at the level of the capillaries inside the terminal villi. The regenerated blood from the capillaries reaches the fetal organs through the umbilical vein, while the catabolite-rich blood from intervillous space is drained into the utero-placental veins. It is a logical conclusion to expect that the placental lymphatic system develops together with the blood system, placing itself between the arterial and venous vasculature and draining fluid in the high capacitance venous vessels as occurs in all human organs.

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