Effects of ischaemia and hypoxia on the development of the nervous system in acardiac foetus

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The twin-reversed arterial perfusion (TRAP) sequence and development of an acardius are rare and severe complications in monozygotic twin pregnancy. Haemodynamic disturbances in placental perfusion via abnormal vascular anastomoses allow inter-twin transfusion to occur. Because of blood perfusion, one of the twins is poorly oxygenated and contains metabolic waste products. Retrograde placental perfusion leads to the formation of a non-viable malformed acardiac foetus.

We studied the effects of haemodynamic disturbances in acardiac foetus on the development of the nervous system. The acardius was a product of a 32-weeks pregnancy. Caesarean section yielded a skin covered ovoid mass (size, 10 x 8 cm; weight, 220 g). The dissection of the acardiac twin showed a skin with hair and appendages, rudimentary lower limbs, vertebral column and brain mass. The rudimentary brain tissue was considerably disorganised structurally. We distinguished two main morphological forms of various appearances. In the centre, we observed a scarcely vascularised mass of tissue containing mature and immature neurones, glial cells and randomly distributed fibres. The mass of tissue appeared poorly differentiated, although there were some arrangements reminiscent of cerebral structures. Clusters of neurones provided a slight suggestion of nuclear or fibre structure. The cerebellar cortex was the only well recognisable structure. In the other fragment of the tissue, we found a slit cavity with ependymal outline and well-developed choroid plexus, which seemed to represent the 3rd ventricle. The scarcely vascularised disorganised tissue was surrounded by the highly vascularised one. It included many thin-walled sinusoid vessels. In some places, they were so concentrated that they resembled cavernous haemangioma. The spinal cord appeared comparatively well organised with a slightly dilated central canal. The morphological picture of the rudimentary brain tissue was similar to the picture of the cerebrovasculosa area.

The effect of ischaemia in the presented case is the anomalous formation of the cerebral structures. The morphological features imply that the failure occurred after neurulation and before the prosencephalic began to grow. The failure of neural tube formation occurred on the 22nd–25th day of gestation. The malformed formation of the nervous system might be caused by impaired induction due to altered gene expression or to the interference of exogenous agents that interrupt normal development. The haemodynamic abnormal placental circulation, which induced lack of oxygen supply and nutritional deficiency, implies the morphological pattern of the anomaly.

key words: acardiac foetus, rudimentary cerebral tissue, triplet pregnancy
INTRODUCTION

During placental development, in the case of the monozygotic twin placenta, many variations in the patterns of vascular appropriation of placental parenchyma by the circulation of each twin are possible. In effect, the twins share and compete for placental territory. In monozygotic twins, the chorion may be common to the twins, and in such cases there are vascular anastomoses between the portions serving each twin. Vascular anastomoses within the placenta allow inter-twin transfusion to occur, which is a normal event in most cases [2, 17]. However, imbalance in this flow may lead to the abnormal clinical sequels. Reverse transfusion describes the most bizarre form of inter-twin transfusion by arterial-to-arterial vascular anastomoses-acardiac twinning. Retrograde perfusion of one of the twins with deoxygenated blood leads to the formation of a non-viable acardiac foetus and a donor twin-a pump twin-struggling to maintain the cardiac output required to perfuse both twins. Consequently, the pump twin provides the acardius with blood, which is poorly oxygenated and contaminated with metabolic waste products. The abnormal inverted direction of the placental circulation and the lowered blood pressure of the acardiac twin alter the foetal haemodynamics and determine the pathologic development of the body. Acardiac anomaly spectrum, besides the absence of heart, is associated with variable grades of developmental disturbances. Thus, no two cases are similar [9, 20]. There are great variations in gross appearance and pathologic features of acardiac foetuses. In acardius anceps 50 to 75% of cases have no cranial vault or brain. In 25 to 50% the cranial vault is present and it is either open or intact, and in 0 to 25% there is a partial cranial vault [3]. In the literature, there are only a few descriptions of the central nervous system in the acardiac foetuses [9, 21, 26, 29].

The twin-reversed arterial perfusion (TRAP) sequence and development of an acardius are severe and rare complications in monozygotic twin pregnancy; triplet pregnancy with the acardiac is even much rare [1, 5–7, 15, 27].

Recently, we had the opportunity to examine a case of acardiac foetus with rudimentary brain tissue in the course of triplet pregnancy. We focused our attention on the effects of haemodynamic disturbances due to abnormal placental circulation of the TRAP syndrome and abnormal cardiovascular system of the acardiac foetus, resulting in ischaemia and hypoxia, on the development of the foetal nervous system.

CLINICO-PATHOLOGICAL DATA

The mother, a 30-year-old, had an unremarkable personal and familial history. She already had a healthy girl of 5 years. It was her second pregnancy, without complications up to the 28th week of gestation. After 28 weeks of pregnancy, maternal hypertension and proteinuria were observed. The gestation was diagnosed as a twin pregnancy in the 30th week of amenorrhea by ultrasound examination. The labour began spontaneously after about 32 weeks of gestation. The first child was girl, her weight was 1140 g, length 38 cm, and the baby had Apgar’s score of 8 after 1 minute. The first twin went home in good condition after staying at the neonatal unit.

The second child was born by caesarean section because of neglected transverse presentation. The female infant weighed 640 g and her length was 30 cm. The baby had Apgar’s score of 1 after 1 minute and because of asphyxia she received assisted respiration. She died on the 20th postnatal day of acute cardiac insufficiency, respiratory insufficiency and septicaemia. Intrauterine hypotrophy, perinatal hypoxia and multiorganic insufficiency were determined. Pathological examination showed interstitial pneumonia, haemorrhagic necrosis of the adrenal glands and passive hyperaemia of internal organs. Macroscopic examination of the central nervous system disclosed no abnormalities of the cerebral gyri. No pathological changes were observed.

Caesarean section also yielded a skin covered ovoid mass (size, 10 × 8 cm; weight, 220 g). It was floating in the remaining fluids, attached to the placenta by a very thin umbilical cord. The umbilical cord had a single umbilical artery and a marginal insertion. The mass was consistent with malformed foetus with a cephalic extremity, rudimentary lower limbs and oedematous body mass (Fig. 1). The malformed foetus was partly hairy on the most superior part of the trunk. Dissection of the foetus showed a skin with hair and appendages, vertebral column with spinal cord and poorly developed skull with rudimentary brain tissue. It neither resembled the normal anatomical brain nor had any evidence of bilateral symmetry. An extremely small, solid mass of tissue was located at the cranial base. Most of the body mass was fatty tissue in which in the omphalocele stomach and intestine were embedded. The deficiencies of heart and other internal organs (lungs, liver, spleen, genito-urinary organs) were determined.

Pathological examination of the placenta revealed dichorionic-triamniotic placenta weighing 380 g. There were arterio-arterial anastomoses between umbilical
cord cords of the second twin and the acardius. The placenta from the first twin showed maternal floor infarction. There were excessive fibrin deposits in the decidua basalis. No cytogenetic analysis was done.

**MATERIAL AND METHODS**

The study of the nervous tissue was performed on the material fixed in 4% formalin and embedded in paraffin. The rudimentary brain of the acardiac foetus was cut into four pieces. The representative slides from the anterior, middle and posterior portions of the rudimentary brain and spinal cord were taken. The serial sections were stained with hematoxylin and cresyl violet. In order to visualise astrocytes, immunohistochemistry with a polyclonal antibody against glial fibrillary acidic protein (GFAP, Dakopatts 1:500) was used. The Purkinje cell, cerebellar cortex and other neurones were identified with antibody to calcium binding protein calbindin — D28k (Sigma, 1:200). Additionally, Ulex Europeans lectin was used for detection of the network of brain blood vessels (Vector, 1:500). The brain of the second twin was cut coronally into representative slides. Sections were stained with H&E, cresyl violet, Klüver-Barrera methods.

**NEUROPATHOLOGIC DESCRIPTION**

**Case 1. Acardiac foetus**

The examination of the rudimentary brain tissue demonstrated considerable structural disorganisation. Two main morphological forms of various appearances were distinguishable. The tissue consisted of a highly cellular central part and a highly vascular peripheral part (Fig. 2).

In the centre, we observed a scarcely vascularised mass of tissue containing mature (Fig. 3) and immature neurones, glial cells and randomly distributed fibres. The mass of tissue appeared poorly differentiated, although there were some arrangements reminiscent of cerebral structures. Clusters of neurones provided a slight suggestion of nuclear structure. The neuronal arrangement and shape resembled the cochlear nucleus. However, it was hard to decide whether the tissue belonged to the midbrain or represented material destined for diencephalic structures. The cerebellar cortex was the only well recognisable structure. In the mixture of grey and white matter without a regular topographic arrangement, we observed fluctuating bands of cerebellar tissue (Fig. 4). Strong immunoreactivity for calbindin was found in the cerebellar Purkinje neurones. Intensive immunopositivity of the Purkinje cell bodies as well as processes were observed. The separated fragment of well-developed cerebellum was developmentally adequate for the gestational age (28/30 weeks of gestation). In the middle portion of the rudimentary brain, a few linearly arranged small cysts lined with ependymal epithelium were differentiated. In the vicinity, a few germinial neuroblasts were seen. There were only a few matrix cells in all the examined nervous tissue. In the other fragment of tissue, slit cavity with ependymal outline and well-developed choroid plexus were found (Fig. 5).

Weak immunoreactivity to GFAP within the central part of the nervous tissue was stated. We found a few GFAP-positive cells scattered within the undifferentiated mass of tissue. The cells were only slightly immunopositive, astrocytic perikarya stained better than processes. The vessels were few and localised without any specific arrangement.
The scarcely vascularised disorganised tissue was surrounded by highly vascularised one. It included many thin-walled sinusoid vessels. In some places, they were so concentrated that they resembled cavernous haemangioma. The vessels were large and abnormal. They were surrounded by undifferentiated tissue composed mainly of mesenchymal elements. Immunocytochemically, this tissue was positive immunoreactive with antibodies to glial fibrillary acidic protein. Distinctly stained GFAP-positive cells were observed around the vessels. A few calbindin-positive cells were dispersed in the strands of tissue between the vessels. The cells were of triangular or fusiform shape. The spinal cord appeared comparatively well organised with a slightly dilated central canal. It displayed a mixture of grey and white matter, showing relatively symmetric clustering of neurones, but anatomical topographic arrangement was indiscernible.

Case 2. Second twin

Upon neuropathological microscopic examination, it was affirmed that the brain development is compatible with the developmental age. Recent perinatal lesions were revealed: congestion and haemorrhages. In the brain hemispheres generalised hyperaemia was stated. Small focus of haemorrhage was seen in the periventricular matrix. In the nervous tissue, disseminated neuronal changes diagnosed as hypoxic encephalopathy were found. Dispersed acute neuronal damage was present in the cerebral cortex, mainly in the temporal lobe. Also in the temporal lobe gyrus discrete cortical malformations were seen. Small foci of marked disar-

Figure 3A, B, C. Mature pyramidal neurones with well visible nucleus and cytoplasmic granules of tigroid. Cresyl violet, × 60.

Figure 4. Bands of cerebellar tissue; matured Purkinje cells and granular layer neurones. Calbindin and cresyl violet, × 100.

Figure 5. Well-developed choroid plexus placed in the ventricle lined with ependymal cells. Cresyl violet, × 60.
rangement of cortical neurones with irregular laminar structure and ectopic neurones in the first cortical layer were observed. Loss or ischaemic and pycnotic damages of neurones were localised mainly in the magnocellular part of the reticular formation in the medulla oblongata. Rarefaction of the subcortical white matter with hypertrophied glial cells and not many macrophages was observed. In the periventricular white matter, the area of tissue rarefaction was also seen. Small malformation of the vessel sub forma glomerulous, thin-walled vascular conglomerate was found inside the cortical frontal sulcus.

**DISCUSSION**

Monozygotic twins are particularly at risk of in utero damage of the brain. The cause of these destructive lesions has never been clear and probably varies among cases. The twin reversed arterial perfusion (TRAP) sequence found in monozygotic twins is a consequence of primary or secondary cardiac development disruption and direct arterioarterial and venovenous placental anastomoses [14]. Similar findings include the presence of a single umbilical artery (66%) [9, 11] and chromosomal abnormalities in the acardiac twin (33%) [4, 6]. The recipient twin shows different degrees of developmental arrest of internal organs, including the brain. Anomalies of the central nervous system can be considered to be primary, i.e., due in some way to the abnormal twinning, or secondary, i.e., due to the haemodynamic imbalance and hypoperfusion. A most likely cause of the brain lesions is failure of perfusion due to perturbations in the shared circulation, which results in ischaemic-hypoxic damage. It is important to consider the development of cerebral structures under conditions of increasing ischaemia. The development of the CNS can be divided into a number of phases, each of which is characterised by particular developmental disorders. In embryonic development the brain anlage with its neuromeric organisation forms from the anterior part of the neural plate, which develops into the neural tube between 18 and 28 days of gestation [30]. The anterior part of the neural plate receives impact to form the forebrain (prosomeres p1–p6) from the prechordal mesoderm [31]. When one considers the great number and kinds of genetic interactions that must occur to properly pattern the developing neural tube and forebrain, it is not surprising that aetiology and morphological appearance of cerebral malformations are extremely heterogeneous.

The morphological picture of the rudimentary brain tissue resembled the picture of anencephaly or area cerebrovasculosa. Area cerebrovasculosa denotes abnormal spongy, vascular tissue admixed with glial tissue ranging from a thin membrane to a large pseudoencephalic mass simulating cerebral tissue, which is composed of connective tissue, haemorrhagic vascular channels, glial nodules, and disorganised choroid plexuses. It always occurs with the absence of the cranial vault bones. Some authors [18, 22, 25] suggest that a mesodermal defect is primary in the development of this malformation. The mesenchymal defect results in failure of the whole neural fold to elevate, thus causing the loss of a buttressing effect to the neuroectoderm, which leads to failure of neural tube closure. Norman’s position is that both neuroectoderm and mesoderm are needed for normal development and that there is manifestly abnormal development of both neuroectoderm forming the neural tube and of the mesoderm contributing to the coverings of the neural tube in anencephaly and opened neural tube defects [23]. How these abnormalities are determined and interrelated is unknown. It is probably that both neuroectoderm and mesoderm are affected. The timing of the mesenchymal defect is 18–20 days in the development of the human foetus.

In the reported case, mature pyramidal neurones and mature calbindin positive neurones were found in the nervous tissue. Histological and immunohistochemical studies of experimental anencephaly suggest neuronal over-maturation [24]. The authors suggest the pathogenic development of the „area cerebrovasculosa” in the neural placode as a phenomenon resulting from hyper-vascularisation in response to neuronal overgrowth, as seen in human cases of exencephaly or anencephaly.

However, the morphological picture of the presented case resembles area cerebrovasculosa, the poorly developed but intact skull differentiates this case from anencephaly. The formation of the skull indicates that the neurulation was complete. A failure of growth of the anterior end of the neural tube leads to subsequent morphogenesis of apropencephaly or holoprosencephaly. In the neuropathological picture, there was no lateral ventricle, a structure that its formation is associated with holoprosencephaly [23]. However, a few linearly arranged small cysts lined with ependymal epithelium were differentiated in the solid nervous tissue mass that may state rudimentary lateral ventricle. The remnants of forebrain constituted a solid mass, differing from a vesicular wall described in pseudo-apropencephaly [28]. In the presented solid mass of disorganised nervous tissue, there were some arrangements reminiscent of cerebral structures. Clusters of neurones provided a slight suggestion of nuclear structures. Silt cavity lined with ependymal cells and well-developed choroid plex-
us seemed to represent the 3rd ventricle. The bands of cerebellar tissue consisting of Purkinje cells and granular neurones were well differentiated and matured adequately for the gestational age. The mature neurones were intensive calbindin positive. This picture is compatible with aprosencephaly [13, 28]. The appearance of nervous mass tissue is similar to Harris’s description of aprosencephalic case. In his presentation, neuropathological examination showed virtual absence of the cerebral hemispheres with an incomplete diencephalon. Microscopic examination revealed disorganised neuropil with a proliferative vasculopathy [12]. According to Kakita [13] it seems likely that aprosencephaly covers a spectrum of cases with severe but variable failures of prosencephalon formation.

The morphological features imply that the failure occurs after neurulation and before the prosencephalic began to grow. This malformation is due to improper specification and formation of the forebrain during early development.

Lack of oxygen has been shown to be a very effective teratogen, causing disturbance of normal development, if it interferes with early stages of embryonic development. Teratogens may alter gene expression, hinder differentiation of tissue, or damage the induced progenitor cells, which cannot be regenerated in sufficient amounts, resulting in matrix deficiency defects. The same may be caused by vascular factors, inducing lack of oxygen supply or nutritional deficiency.

Hypoxia, due to impaired cerebral flow, has hazardous effects on brain structure and function. Among the regulatory proteins, hypoxia-inducible factor-1 (HIF-1) appears to have a key role. Lee’s data clearly show that hypoxia marker immunoreactivity was highly detected in developing neural tubes, heart, and intersomitic mesenchyme at an early stage of organogenesis, suggesting that hypoxia may exist in the early stages of embryo development [16]. It was also found that hypoxia inducible factor-1 alpha and vascular endothelial growth factor (VEGF) were spatiotemporally co-localised with possible hypoxic regions in embryos. The VEGF/VEGFR system induced by hypoxia leads to the growth of new vessels after cerebral ischaemia [19]. The abundance of vessels in highly vascularised peripheral part of nervous tissue may be the effect of hypoxia-induced angiogenesis. During development, VEGF is expressed in multiple embryonic and foetal tissues with the highest levels found in the lung, kidney and heart. VEGF is also expressed in placental tissues and foetal membranes, and this expression increases with advancing gestation. In the foetal heart and placenta, VEGF expression is inducible by hypoxia. The presence of VEGF and its receptors in placental tissues throughout gestation strongly suggests that VEGF plays an important role in the development and maintenance of placental vascular function during pregnancy [8].

Appropriate growth and development of the placenta is essential for foetal growth and well-being. The perturbations in VEGF expression may result in abnormal formation of the vascular system. There is evidence that VEGF plays an essential role in the development and differentiation of the cardiovascular system [10]. This molecule plays an important role in the regulation of physiological and pathological vessel formation and its defect may result in abnormal vascularisation. The development of vascular supply is essential for organ development and differentiation during embryogenesis.

Alterations of brain development result from noxious intrauterine signals as oxygen deprivation due to ischaemia. The anomalous formation of the nervous system is the deleterious consequence of the ischaemia. The haemodynamic abnormal placental circulation inducing lack of oxygen supply and nutritional deficiency and altered foetal haemodynamics due to abnormal foetal cardiovascular system implies the morphological pattern of brain anomaly in the acardiac foetus. The noxious effect of the abnormal placental circulation on the central nervous system was also seen in the cotwin. Cerebral hypoperfusion led to chronic hypoxic-ischaemic brain lesions stated in the second twin. This information is agreement with that in the literature [14]. The haemodynamic disturbances due to abnormal placental circulation of TRAP syndrome resulting in ischaemia and hypoxia determined the malformation of the nervous system structures in acardius and chronic hypoxic-ischaemic lesions in the second twin.

REFERENCES