

Histopathology of Neural Tube Defects

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Published Date: August 07, 2016

ABSTRACT

This chapter describes the main pathological features of neural tube defects. In particular, classification in closed and open defects is reported, as well as developmental, macroscopic, and histological characteristics are enlisted for each defect. The aim of this chapter is to provide basic but comprehensive information on features that help clinicians distinguish between the different types of neural tube defects.

INTRODUCTION

During embryonic development in vertebrates, the notochord releases soluble factors inducing the formation of the central nervous system, through the formation of the neural plate, which will form the neural tube. This process, known as neurulation, which will be completed by 28 days post fertilization in humans, starts approximately at the end of gastrulation from day 17-18 post

fertilization. At this stage, the embryo is referred to as a neurula [1,2]. The process of forming the neural tube is complex, tightly controlled and requires a number of morphogenetic events, including cell-shaping, neural folds elevation and fusion. During gastrulation, the neural plate (Figure. 1A) forms responding to signal released by the underlying notochord. It then thickens and elevates forming a typical groove (Figure. 1B) that will start closing by juxtaposing of the two neural folds (Figure. 1C). The closure will then proceed in a bidirectional wave, rostrally to form the brain and caudally to complete the spinal cord closure (Figure. 1D).

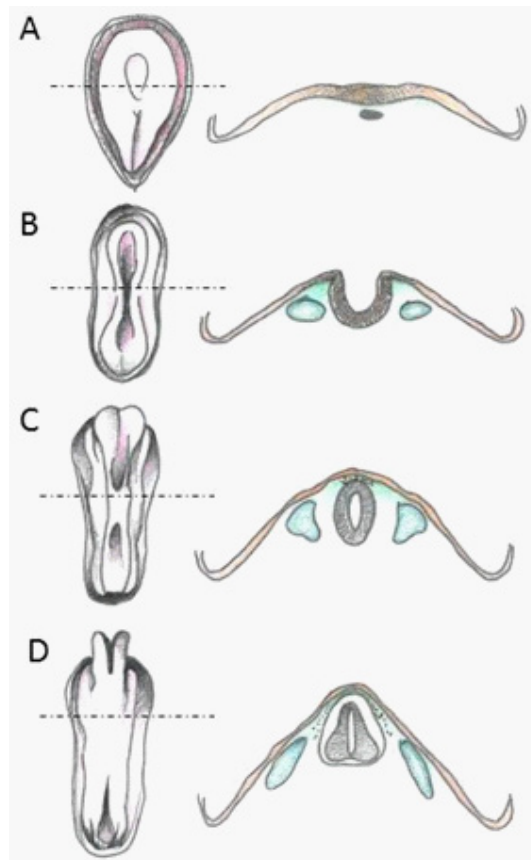


Figure 1: Schematic representation of human embryo neurulation.

Development of the neural tube in the early embryonic stages is shown from neural plate specification (A), to neural groove (B) and folds (C), that eventually close and fuse at midline to form the neural tube (D). Transverse sections are shown on the right at the level indicated by the punctuated line.

Closure of the neural tube is a multi-site process in mammals, whereby different closure points can be observed in the developing embryo. In the developing mouse embryos three closure sites can be visualized, whereas increasing evidence suggest that only two closure points (arrows in Figure. 2) characterize human embryos [3,4]. The closure mode forms in humans an anterior

neuropore (ANP) in the brain and a posterior neuropore (PNP) in the spine that reduce in size during neurulation to disappear at the end of the process. Typical normal histology of the spine including fetal vertebrae and spinal cord are shown in Figures 3 and 4.

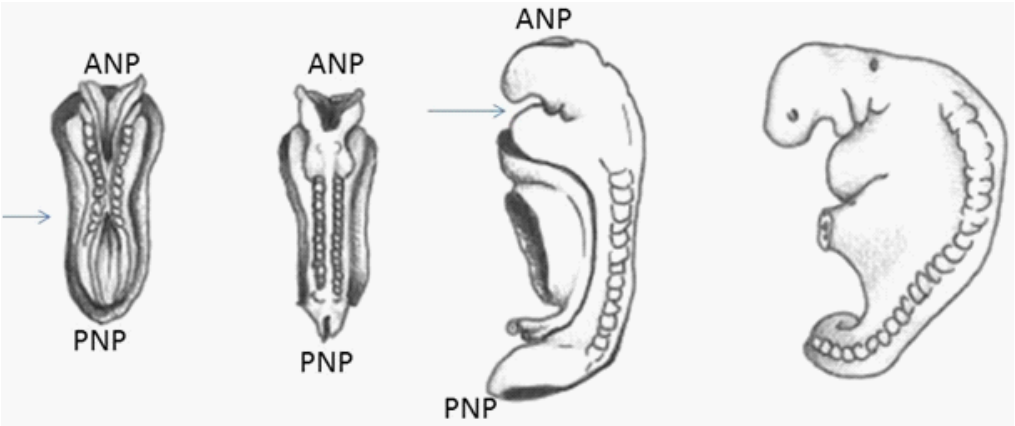


Figure 2: Schematic representation of multisite neural tube closure.

From day 17 to 28 post fertilization the neural tube closes along the embryo body with different closure site (arrows). Because of this multisite closure process and bidirectional mode an anterior (ANP) and a posterior (PNP) neuropore are formed.

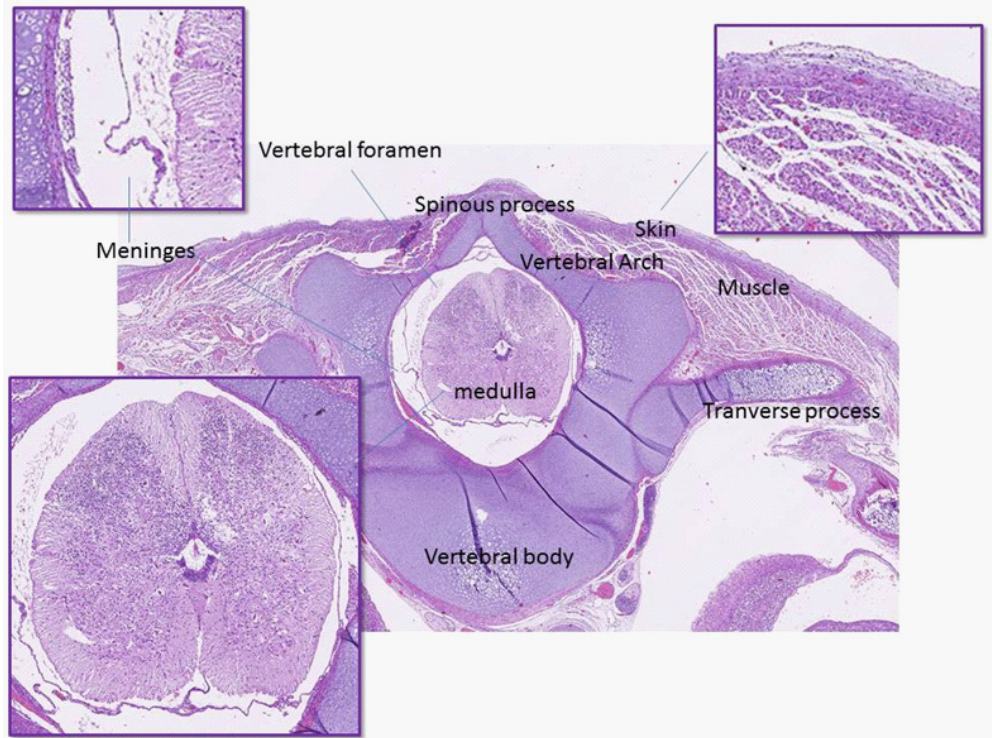


Figure 3: Transverse section of normal fetal vertebra with typical spinal cord section.

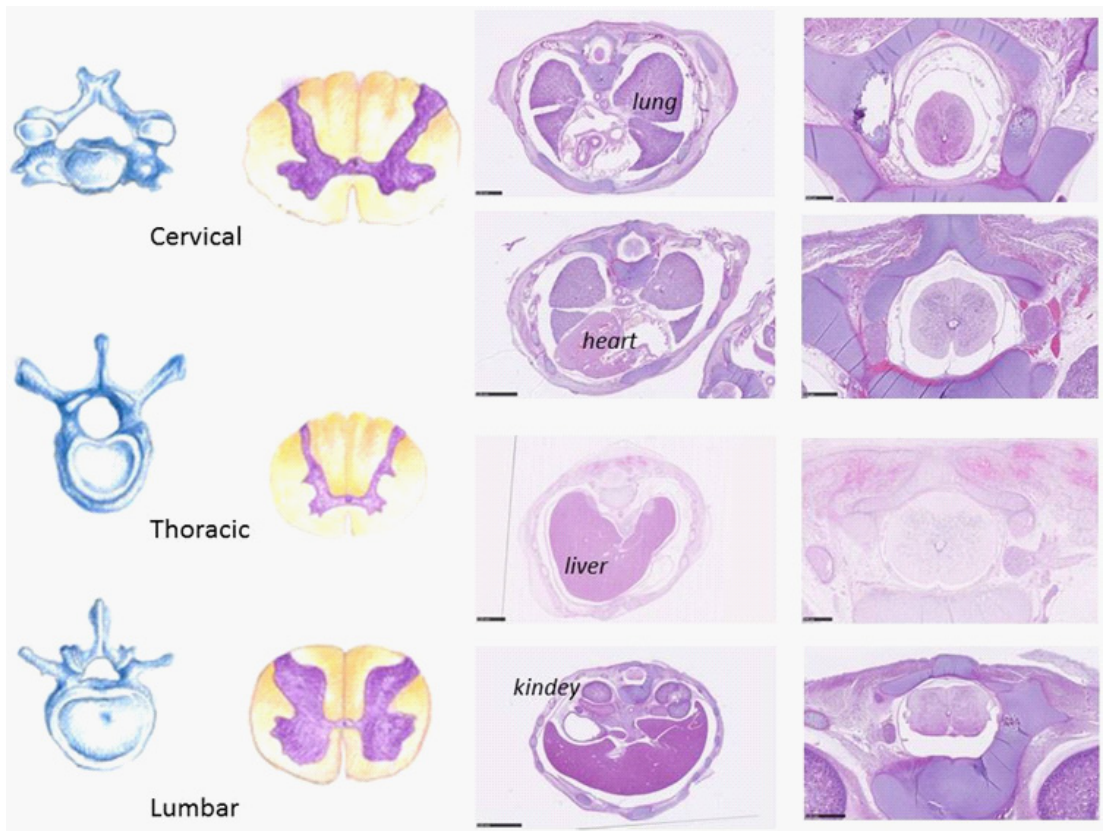


Figure 4: Different body levels of fetal vertebrae sections.

Different appearances of normal transverse sections of vertebrae and spinal cords through the column, according to the body level from cranial to caudal.

Failure of closure of the developing neural tube results in open neural tube defects (such as spina bifida and exencephaly) that can affect different levels of the neural tube length, normally reflecting the site where closure should have been completed. Indeed, craniorachischisis (i.e. open brain from the cervical region) reflects failure of the first closure site (Figure. 5), anencephaly (i.e. open brain) results from defects of closure of the rostral closure site and spina bifida occurs when the PNP does not complete closure (Figure. 5).

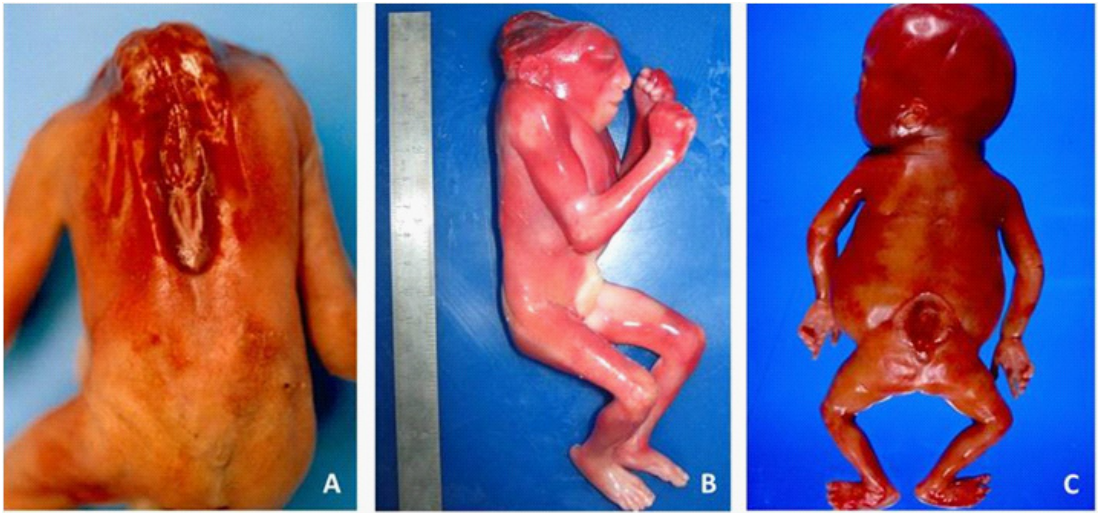


Figure 5: Open neural tube defects.

In (A) a fetus affected by craniorachischisis is shown. Note the defect of occipital bone and vertebral arch extending into upper dorsal area, resulting in anencephaly and spina bifida. In anencephaly (B) the overall brain structure is disturbed and results in an absence of forebrain and cerebrum. The nervous tissue is not covered with bones because of the absence of the cranial vault development. The brain is a mass of degenerated tissue and the skin is in continuity with the nervous tissue. A case of open spina bifida is shown in (C). It is possible to clearly visualize the bone defect in the lumbar area, related to the failure of posterior vertebral arch to fuse. Meninges and spinal cord displace through the opening.

Neural Tube Defects

A number of congenital malformations are grouped as neural tube defects (NTDs), mainly divided into *open defects* such as craniorachischisis, exencephaly-anencephaly and myelomeningocele and *closed defects* such as cephalocele, meningocele and spina bifida occulta (Table 1) [5,6]. The overall estimated prevalence of neural tube defects is around 1 in 1000 births, with regional and ethnic variations [7]. Incidence of the different types of NTDs is not always reported and spontaneous abortions or pregnancy terminations also need to be considered.

Table 1: Neural tube defects.

Classification	Open defects			Closed defects		
Name of defect	Craniorachischisis	Anencephaly	Myelomeningocele	Cephalocele	Meningocele	Spina bifida occulta
Amniotic biochemistry*	High level of amniotic fluid α-fetoprotein and amniotic fluid acetylcholinesterase	High level of amniotic fluid α-fetoprotein and amniotic fluid acetylcholinesterase	High level of amniotic fluid α-fetoprotein and amniotic fluid acetylcholinesterase	Normal level of amniotic fluid α-fetoprotein and amniotic fluid acetylcholinesterase	Normal level of amniotic fluid α-fetoprotein and amniotic fluid acetylcholinesterase	Normal level of amniotic fluid α-fetoprotein and amniotic fluid acetylcholinesterase
Intrauterine features	Easy ultrasound diagnosis: ultrasound aspects of anencephaly plus vertebral dysraphism	Easy ultrasound diagnosis made seen the absence of the upper portion of the cranial vault. Often polyhydramnios [8].	By ultrasound may be seen section of the spine demonstrating vertebral dysraphism with an associated cystic mass sometimes containing internal septations; sometimes vertebral dysraphism without posterior cyst (myelocele). Generally associated with cranial signs: abnormal shape of the skull in transverse section (lemon sign due to scalloping of the frontal bones) and abnormal shape of the cerebellum in the transverse section (banana sign) [9]. Progressive deterioration of leg movements during gestation	Ultrasound diagnosis made seen the paracranial hypo-anechogenic mass. Skull defect is often extremely small and therefore is difficult to recognize it with antenatal ultrasound. Often intracranial anomalies such as ventriculomegaly are seen [10].	By ultrasound may be seen spinal dysraphism with an associated cystic mass. The characteristics of the cystic mass generally are comparable with that of the myelomeningocele but generally there are not septa in the herniated mass and the cranial anatomy is unremarkable [9].	The prenatal diagnosis in such cases is a challenge. No secondary cranial findings [11].
Clinical aspects	Lethal	Lethal	Motor and sensorial deficit below the level of the spinal lesion. Often bladder and rectal incontinence. Hydrocephaly [12]. Intellectual disability is relatively infrequent (20-25% of cases) and related to hydrocephalus [13]. Deformity of the lower extremities.	Clinical characteristics depends on the site and extent of the lesion. Often cognitive, neurodevelopmental delay, sometimes hydrocephalus [9].	Generally normal neurologic examination without deformity of the lower extremities or sphincter dysfunction [6].	Asymptomatic in 10% of cases [12]. Possible: motor and sensorial deficit below the level of the spinal lesion. Bowel and/or bladder dysfunction. Scoliosis, foot or leg length discrepancies, deformity of the lower extremities [14].

"The primary use of maternal serum alpha-fetoprotein for neural tube defects screening should be discontinued because the low rate of detection rate. Moreover, elevated levels of maternal serum alpha-fetoprotein can be associated with other conditions, such as abdominal wall defects, fetal nephrosis, and pregnancies at an increased risk for placenta-related adverse events [15].

Open defects

Craniorachischisis. Craniorachischisis is a defect of neural tube closure that involves both the cranial and spinal portion of the neural tube [12]. In these cases both brain and spinal cord are exposed to the intra-amniotic environment resulting in destruction of the nervous tissues due to the toxicity of the amniotic fluid. The neural tube is open from the midbrain to the spine (Figure. 5A). However these cases may show a normal appearance of the forebrain.

Anencephaly. The Center for Disease Control (CDC) estimates that each year, about 3 pregnancies in every 10,000 births in the United States will be affected by anencephaly. When the developmental defect of closure involves just the cranial portion of the neural tube, exencephaly occurs as brain defect. The degeneration of the cerebral-neural tissues due to the destructive exposition of the brain to the intra-amniotic environment converts the exencephaly to anencephaly [12]. In anencephaly (Figure. 5B) the macroscopic aspect of the brain remnant appears as dark brown undifferentiated mass called area cerebrovasculosa [16]. The residual amount of brain tissues varies, generally the pituitary is present but it is hypoplastic and without the intermediate and posterior lobes, the overall brain structure is degenerated and results in an absence of forebrain (both diencephalon and telencephalon structures including thalamus and cerebrum), midbrain and hindbrain (including pons and cerebellum). The calvarium is generally absent, the base of the skull is thick and flattened and the sphenoidal bone is abnormally shaped [12] as a “bat with folded wings” [17]. The face is generally normal although the eyes are protruded because of the shallow of the orbits [12] and the forehead is absent or very short. Histologically, the residual cerebral tissue appears as an irregular mass containing vascular tissue, glia and some neuroblasts or neurons surrounded by meninges [12] even though some authors did not find neurons in the area cerebrovasculosa [18]. The overview shows a de-structured mass consisting of different caliber of venous vessels (Figure. 6A) scattered with connective tissue and islets of nervous tissue including interspersed astroglial cells, nerve cells, and cavities surrounded by the epithelium [16]. Exposed cerebral area is covered by non-keratinizing squamous epithelium that laterally is in continuity with epidermis [12] (Figure. 6B). Interestingly, even though there is a severe rostral neural tube abnormality, the spinal cord in the fetuses affected by anencephaly appears normal [16]. The differential diagnosis between anencephaly and other causes of absence of the cranial vault may include cases of head destruction related to amniotic bands [8] or cases of acephaly in monochorionic twins with an acardiac-acephalus fetus (Figure. 7).

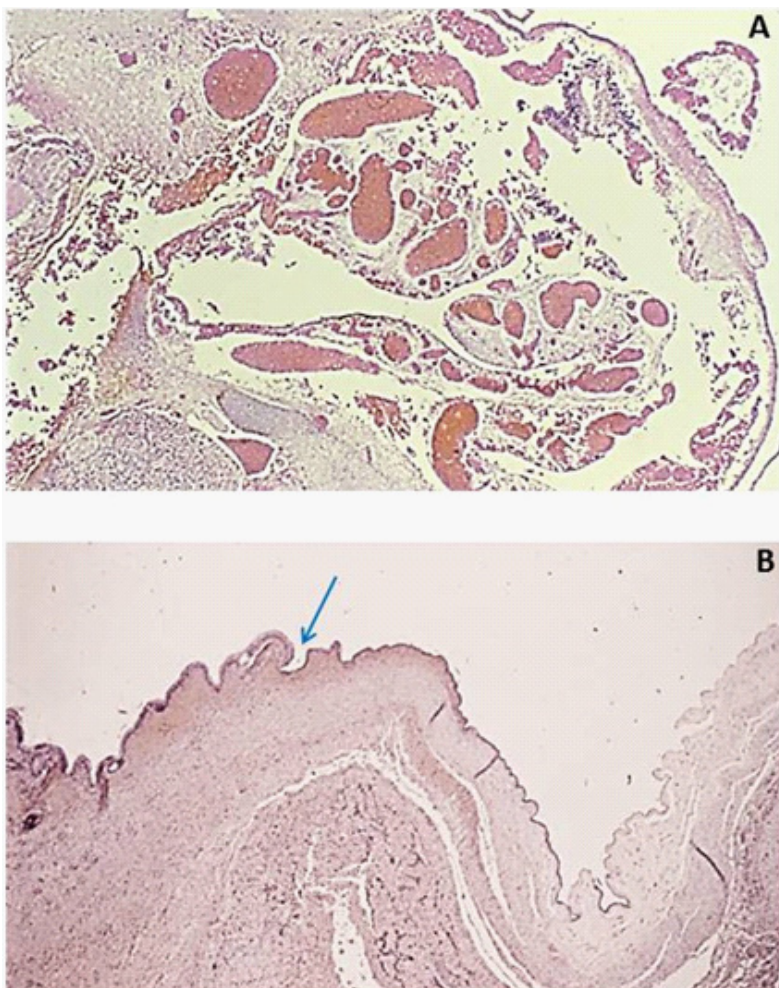


Figure 6: Histology of anencephaly.

In (A) the typical enlarged venous vessels immersed in nervous tissues are shown. Important to note the various dimension of the vessels diameters. In (B) it is possible to visualize the boundary between the squamous epithelium of the epidermis of the skin (located at the base of the skull) and the hypertrophic and hypervascularized meninges that cover the area cerebrovasculosa (arrow points at limit between the two tissues in the site of their fusion).



Figure 7: Differential diagnosis between anencephaly and other causes of absence of the cranial vault.

In (A-A1) a case of acephaly in monochorionic twins with an acardiac-acephalus fetus, in (B-B1) anencephaly, in (C-C1) head destruction due to amniotic bands.

Myelomeningocele. The most common form of spina bifida aperta in humans is defined as myelomeningocele, with an estimated incidence (by CDC) of 1.8 per 10,000 live births in the United States. In myelomeningocele, the developmental defect of closure involves the spinal portion of the neural tube, more frequently the lumbar portion. In this defect a meningeal sac herniates through a bone defect of the vertebral arch. The meningeal cystic sac contains cerebro-spinal fluid, nerve roots and spinal cord. Both spinal cord and meninges are damaged and displaced through the bones opening. Histologically, the medulla is generally hypervascularized (Figure. 8) and appears abnormal: in some cases the spinal cord could be closed with dilated central canal (Figure. 9), while in other cases the spinal cord appears open as a flat mass (Figure. 10) [12]. A clear open defect that involves the spinal cord, but without a protruding meningeal sac is defined as myelocele (Figure. 11 and 12).

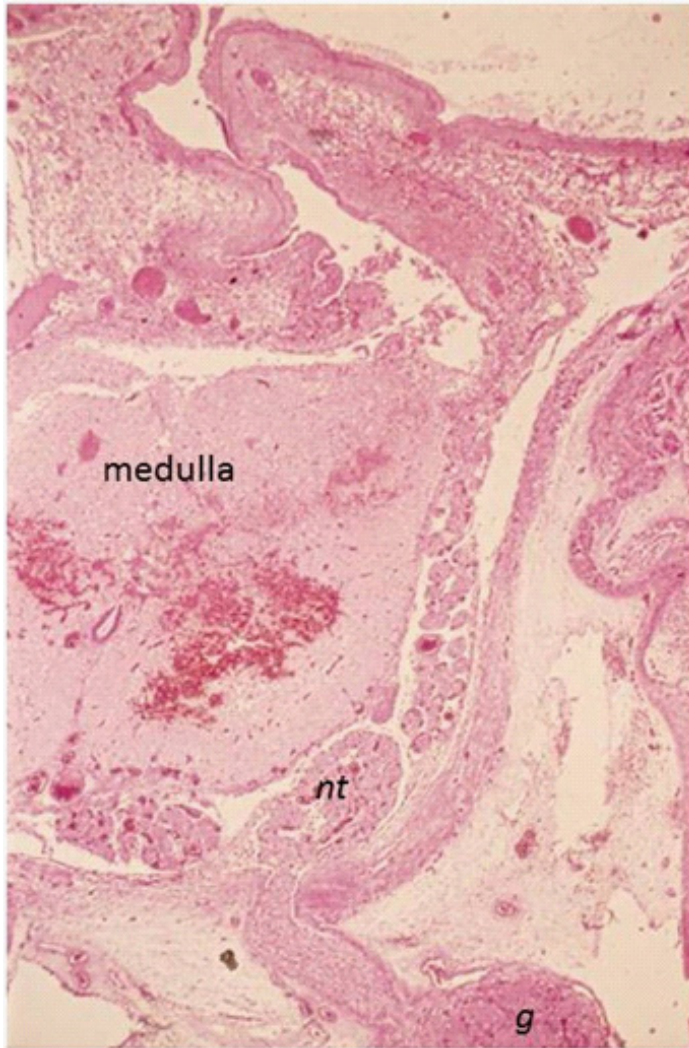


Figure 8: Histology of fetal hemorrhagic medulla.

In this section, it is possible to distinguish a medulla with clear hemorrhagic signs (red spots). It is also possible to note various nerve trunks (nt), one directed toward the first ganglion (g). The meningeal sac is fused to the epidermis and it contains medulla and nerves. Clearly detectable is the vast cleft in the epidermis. The cleft is surrounded by meninges. Hemorrhagic signs are visible also in the dermis.

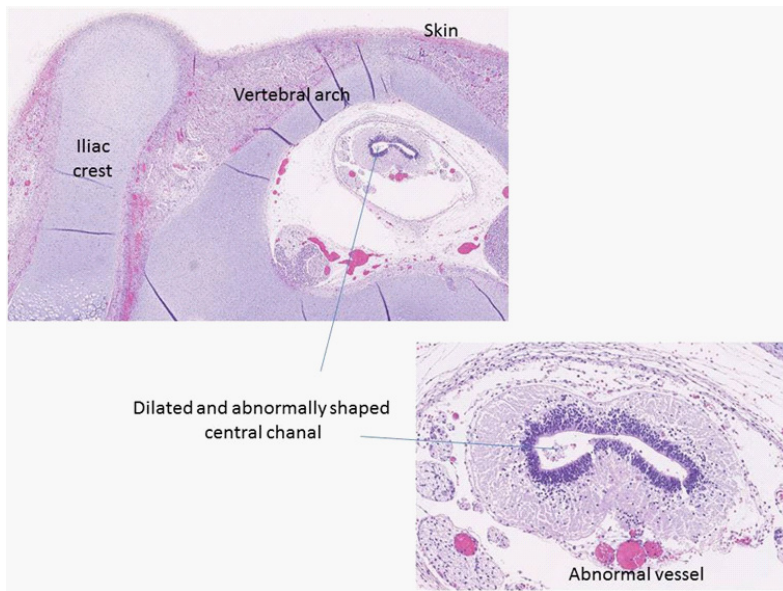
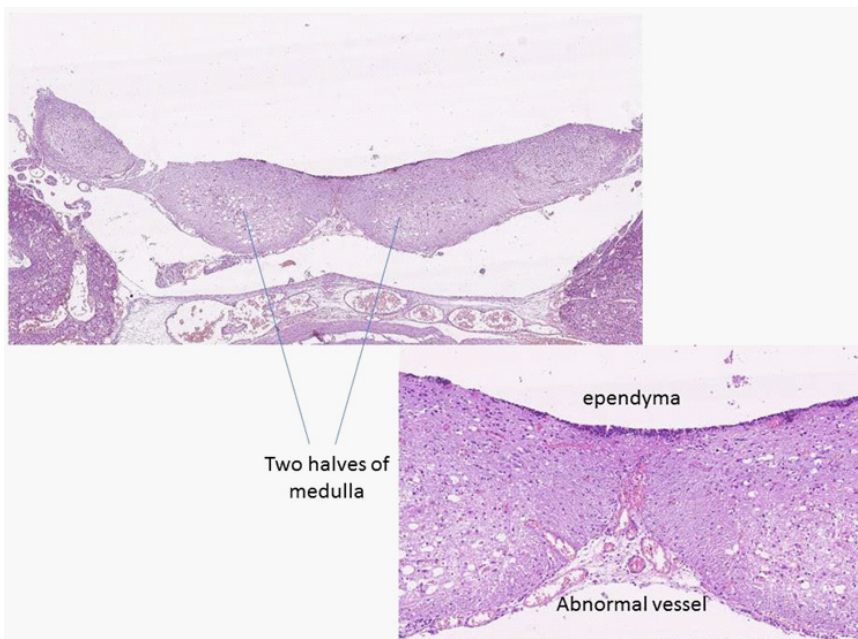


Figure 9: Histology of expanded central canal.

This section of the iliac region shows defect of medulla organization (expanded central canal) in an area caudal to the open NTD. In this section there are no defects in the vertebra and in the skin over the vertebral arches. Indeed, this is a clear example of defect in the medulla although the skeletal portion is not affected.



Spinal cord is not closed but is divided in two halves appearing as an “open book”. The remnant of the ependymal layer is visible at the surface, covering the center of the two halves.



Figure 11: Macroscopic aspect of open neural tube defects.

Fetal MRI image of open spina bifida at 21 weeks of gestation (arrow). T2-weighted sagittal section shows the lumbar-sacral vertebral defects. Arnold-Chiari II malformation is evident at the craniocervical junction. Autopsy confirmed MRI diagnosis: the fetus presented a clear open defect in the lumbar-sacral region (L1-S1). It is possible to visualize the spinal cord in the depth of the cleft. The defect is open and no meningeal sac is protruding.

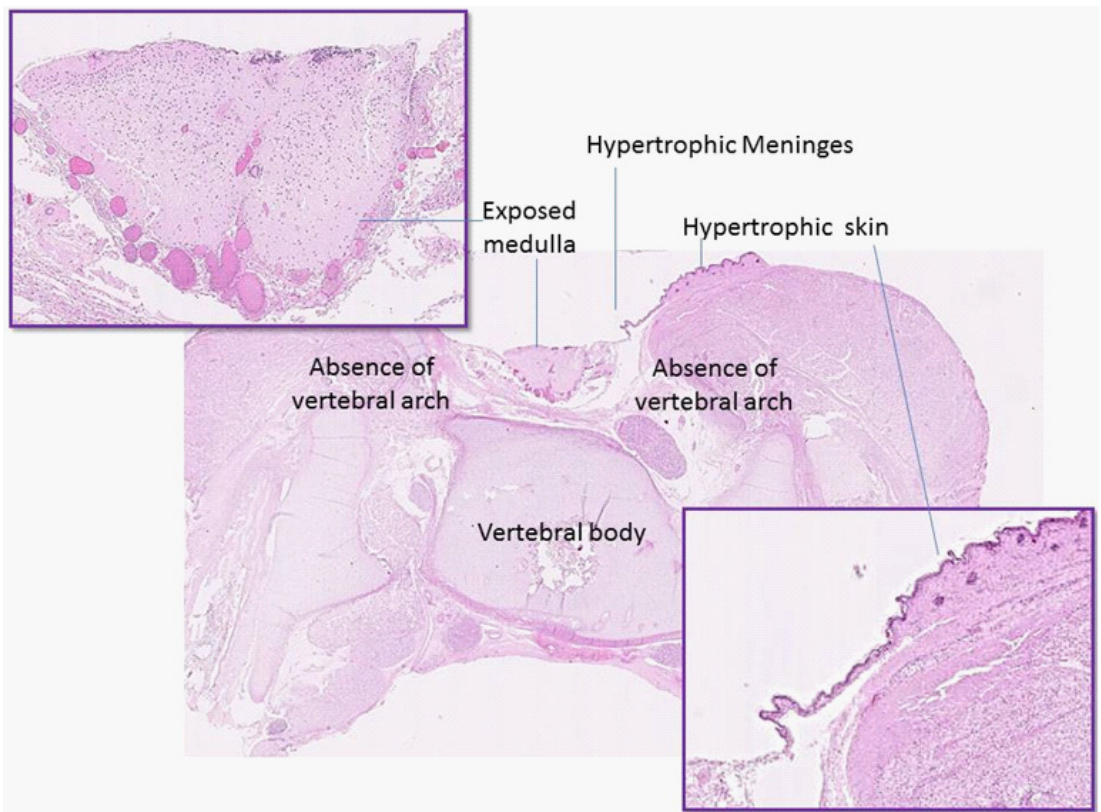


Figure 12: Histology of myelocoele.

Exposed spinal cords are clearly visible through the bone defect of the vertebral arches. Note the abnormal shape of the hypervascularized medulla (upper inset on the left) and the fusion between the hypertrophic meninges and the skin (bottom inset on the right).

Myelomeningocele and myelocoele as open spina bifida is usually associated with Arnold-Chiari malformation (Figure. 13) because of the traction of the brain stem from below due to tethering of the open spinal cord through the vertebral defect. Indeed the brain stem is elongated, the cerebellar vermis is displaced into the foramen magnum and therefore the normal flow of cerebro-spinal fluid through the ventricles is compromised causing hydrocephalus.

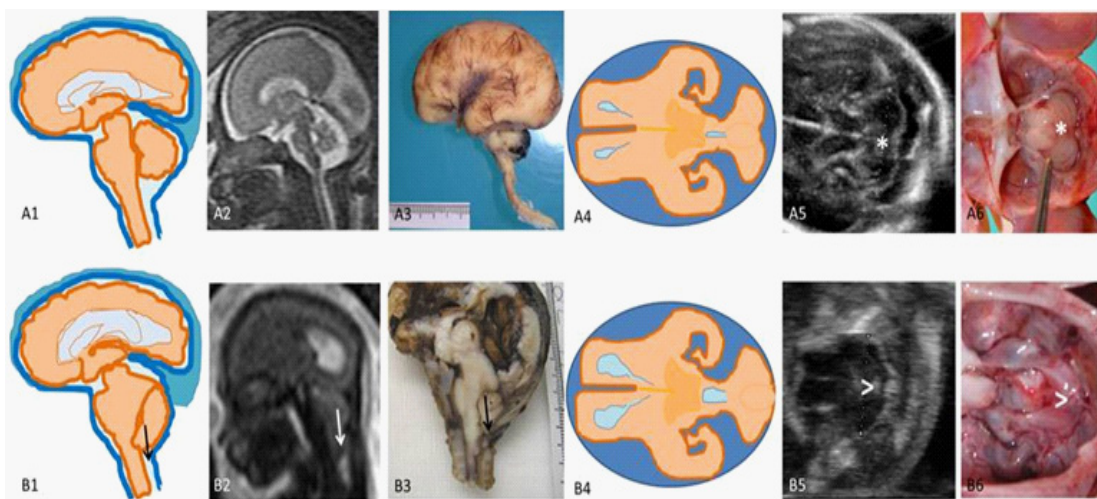


Figure 13: Arnold-Chiari malformation.

A schematic representation (A1, A4), prenatal images (A2, A5) and autoptic features (A3, A6) of normal brains in sagittal (A1-3) and transverse (A4-6) view.

In the lower panel, comparable sections in case of Arnold-Chiari malformation are shown. Schemes (B1, B4), prenatal images (B2, B5) and autoptic features (B3, B6) of brains in sagittal (B1-3) and transverse (B4-6) view in cases affected by spina bifida. Note the herniation of cerebellar vermis through the foramen magnum as characteristic appearances of Arnold-Chiari malformation (arrow). The differences between the normal appearance of transverse section of cerebellum at the ultrasound scan (typical butterfly shape, asterisk) and the appearance of the herniated cerebellum in Arnold-Chiari malformation (characteristic banana shape, arrowhead) are clearly recognizable.

Closed Defects

Cephalocele. Cephalocele is a herniation such as a sac-like protrusion of brain and/or the meninges through an opening in the skull. CDC estimates that each year about 1 out of every 10,000 babies are born with cephalocele in the United States.

According to the type of tissue involved in the herniation, cephaloceles are classified (Figure. 14) in meningocele (herniation of meninges), encephalomeningocele (herniation of meninges and brain), and encephalomeningocystocele (herniation of meninges, brain and ventricle) [10]. Cephalocele can occur in any part of the cranial vault (Figure. 15); cystic mass passes through a cleft of the calvarium squamae (grey line in figure 15) and approximately 90% of cases involve the midline. The vast majority of cephaloceles are covered by skin (pink line in figure 15).

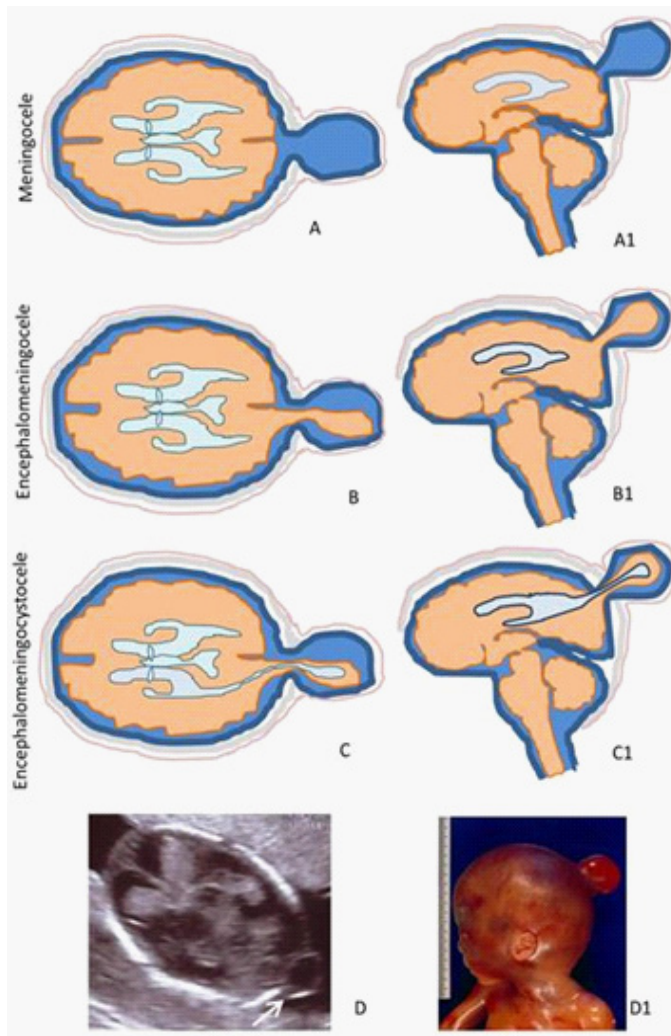


Figure 14: Closed neural tube defects.

The figure shows different cephaloceles. Meningocele (A and A1, transverse and sagittal view respectively), encephalomeningocele (B and B1, transverse and sagittal view respectively), and encephalomeningocystocele (C and C1, transverse and sagittal view respectively). In (D) an early ultrasound screening scan is shown, presenting a para-cranial anechogenic sac at 13 weeks of gestation. The diagnosis was confirmed after delivery, by macroscopic examination (D1).

The posterior shape of choroid plexus is abnormal and it is not completely clear if some brain tissues herniated through the opening of the skull. Arrow: paracranial cystic mass indicating the protruded meningeal sac.

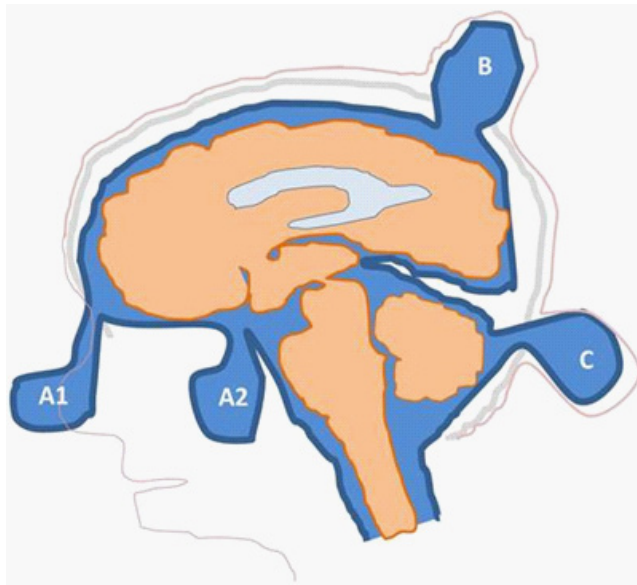


Figure 15: Cephalocele localization.

A schematic representation of possible cephalocele sites. A: anterior; B: parietal and C: occipital.

Two sub-types of anterior cephalocele are shown: the frontal (A1) and basal (A2) varieties. According to the site of the herniation, cephaloceles are classified as: *anterior*, with lesion located between the bregma and the anterior of the ethmoid bone; *parietal*, with lesion located between the bregma and the lambdoid suture and *occipital*, with lesion located between the lambdoid suture and foramen magnum. This location is the most common, including about the 75% of cephaloceles.

The anterior encephalocele is also sub-classified into frontal, sincipital and basal varieties, according to the localization. The frontal cephaloceles are external lesions that cause craniofacial abnormalities and are localized near the so-called glabella, the root of the nose. They are subdivided into naso-frontal, naso-ethmoid and naso-orbital types. Basal cephaloceles are internal lesions which occur within the nose, the pharynx, or the orbit. They are normally classified into three types: speno-orbital, speno-maxillary and speno-pharyngeal [10].

Meningocele. Although macroscopically similar to myelomeningocele, meningocele is a closed spina bifida, comparable to cephalocele, whereby the defect consists in herniation of meninges through the vertebral column (Figure. 16). Macroscopic differential diagnosis between myelomeningocele and meningocele is described in Table 2. In meningocele, the dura and the arachnoid herniate through the vertebral arch defect whereas the spinal cord remains in the normal position into the spinal canal (Figure. 17). The herniated mass is covered by skin that is characterized by atrophic epidermis without skin appendages [12].

Table 2: Differential diagnosis between meningocele and myelomeningocele.

	Meningocele	myelomeningocele
Ultrasound aspects		
Posterior anechogenic sac-like protrusion from the spine	Yes	Yes
Frequent presence of septa in the sac	No	Yes
Abnormality of vertebral bones (absence of the arches)	Yes	Yes
Frequent abnormality of the shape of skull (lemon sign)	No	Yes
Frequent abnormality of the shape of cerebellum (banana sign)	No	Yes
Frequent association with Arnold Chiari malformation type II	No	Yes
Frequent association with hydrocephalus	No	Yes
Frequent association with clubfoot	No	Yes
Macroscopic aspects of the lesion		
Absence of vertebral arches	Yes	Yes
Meningeal herniation through the bones defect	Yes	Yes
Presence of neural tissues in the meningeal sac (medulla and/or nerves)	No	Yes

Although macroscopically similar, meningocele and myelomeningocele represents two opposite types of spina bifida, a closed and open defect respectively with different prognosis. Because of the similar macroscopic aspect of the herniated sac, prenatal ultrasound differentiation of the cystic lesion may be difficult.

In myelomeningocele, septas in the fluid-filled posterior bulging of meninges forming the cystic sac are frequently seen due to the nerve roots arising from the medulla and allocating the herniation.

The lemon sign represents the scalloping of the frontal bones: loss of the convex outward shape of the frontal bones with mild flattening. It is present in virtually all fetuses with myelomeningocele between 16 and 24 weeks of gestation.

The banana sign is due to downward traction on the cerebellum related to tethering of the spine through the spinal opening.

Clubfoot is a foot deformity, an abnormal foot position in which the foot is internally rotated. It is due to the damage of the nerve roots and virtually always involves both the feet.

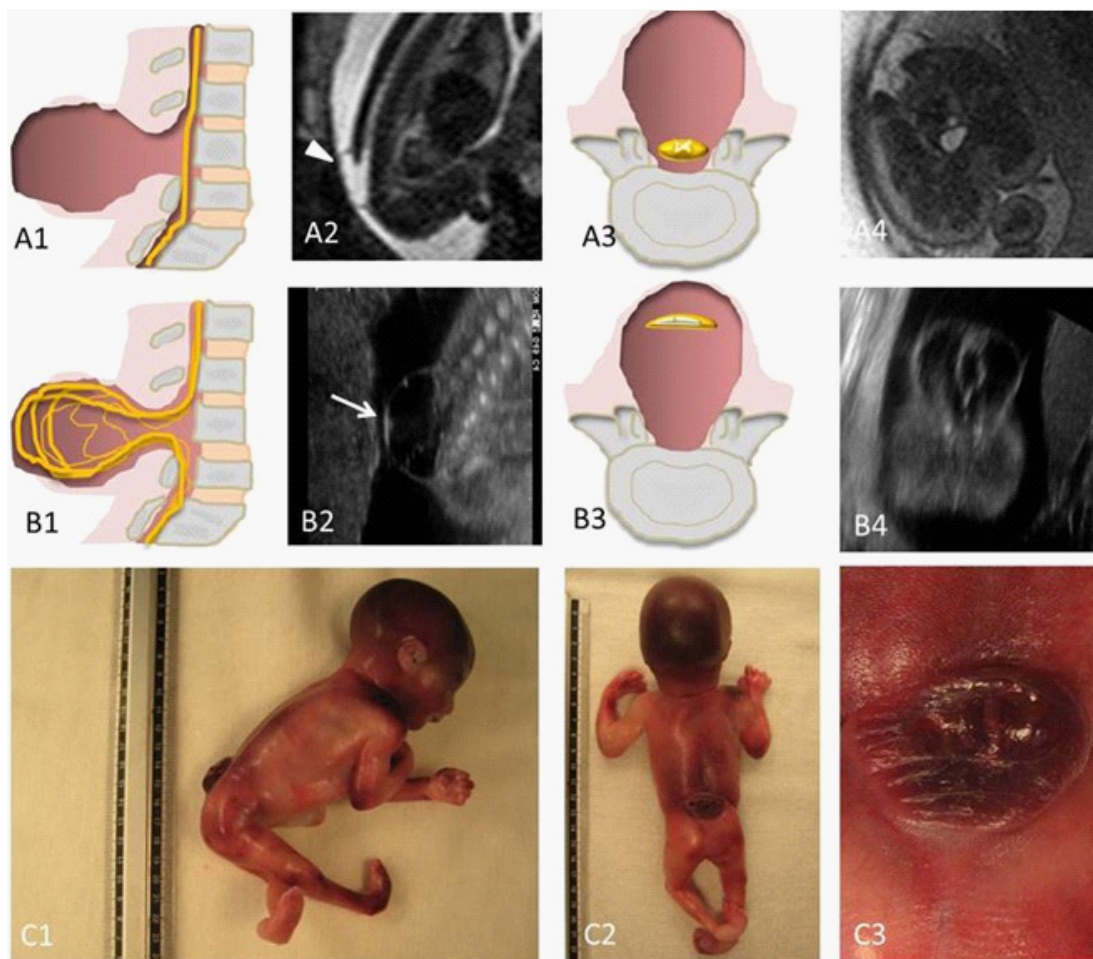


Figure 16: Myelomeningocele and meningocele.

Schematic representations (A1, A3) and prenatal magnetic resonance images (A2, A4) of meningocele in sagittal (A1, A2) and transverse (A3, A4) view: note the protrusion of the meninges through a bone defect of the vertebral arches. In this neural tube defect spinal cord is not involved in the protrusion therefore is usually normal whereas meninges are displaced through the bone opening and damaged. In (B1, B3) schematic representations and images from prenatal ultrasound (B2, B4) of myelomeningocele in sagittal (B1, B2) and transverse (B3, B4) view: the meningeal cystic sac contains cerebro-spinal fluid, nerve roots and spinal cord. In the lower panel, postnatal aspect of myelomeningocele in lateral (C1) and dorsal (C2, C3) views are presented: note the translucent appearance of the neural tissues through the protruded meningeal sac. Note also in the fetus the abnormal position of the feet, related to the compression of the medulla and damage of the spinal nerves. Arrowhead in A2: cystic mass of herniated meninges. Arrow in B2: septated cystic mass of herniated neural tube.



Figure 17: Histology of meningocele.

In figure a typical meningocele is shown. The protrusion is in continuity with epidermal tissue. The herniating mass is formed by extremely thick meninges containing vascularized stromal tissue. It is also possible to distinguish a vast blood clot in the central area of the mass.

Note the absence of skin appendages in the herniated mass

Spina bifida occulta. Spina bifida occulta is a spectrum of a spinal cord abnormalities related to abnormal development of the embryonic tail bud involving the low lumbar and sacral regions and resulting in closed defects with incomplete vertebral arches (Figure. 18) often associated with other skeletal defects such as sacral agenesis. This group of defects represents the less severe form of malformations: more often, this problem may be detected only later in life, because nerves and spinal cord are not affected and therefore it does not usually cause disabilities. In other cases spinal cord anomalies may occur and include hydromyelia (overdistension of the central canal), diplomyelia (longitudinal duplication of the spinal cord), diastematomyelia (longitudinal split of the spinal cord) and tethering of the lower end of the cord [12]. The closed spinal dysraphism is often associated with intradural lipoma (Figure. 18) and this aspect may affect the prenatal diagnosis: during the intra-uterine life, meningoceles and lipomas have a very similar appearance and may be very difficult or impossible to distinguish. In fact, by ultrasound, lipomas typically appear as echogenic masses [9] as well as meningocele.

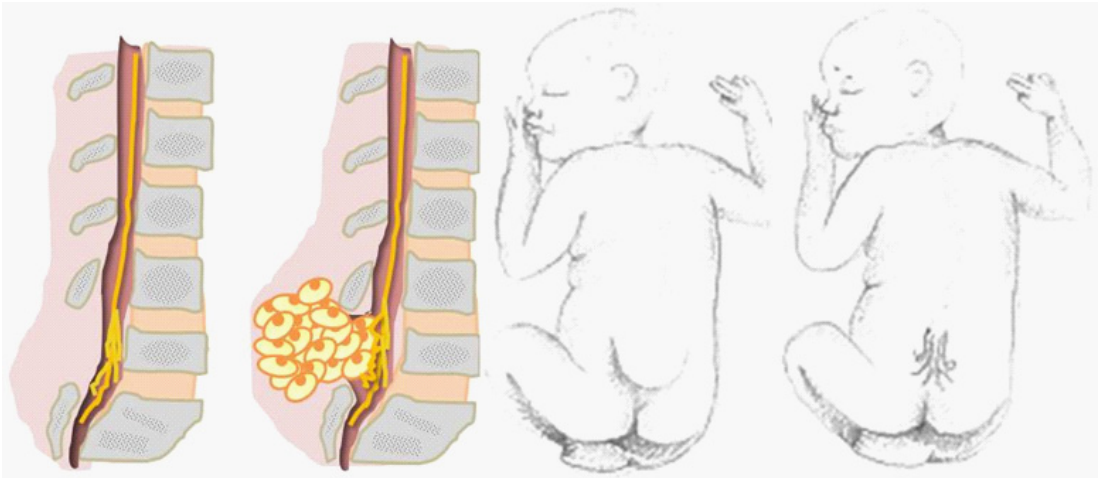


Figure 18: Spina Bifida Occulta.

The internal lesion is covered by intact skin. The skin overlying the defect may be totally normal or hyperpigmented or covered by hairs (hypertrichosis). The back often appears asymmetric and may present an external mass, usually in the sacral or lumbar region. Spinal cord may be unaffected or could present different abnormalities such as tethering, duplication, split and thickening of the filum terminale.

In conclusion, neural tube defects are a vast and heterogeneous group of congenital malformations representing the second most common birth defects in human. The embryonic origin and histology deeply vary among different types of defects as well as the associated prognoses, hence a good differential diagnosis is fundamental for good counseling and clinical management.

ACKNOWLEDGEMENTS

We would like to express our deep gratitude to Ms Dawn Savery for commenting the manuscript and to Ms Susanna Brusa for graphic assistance with skillful drawings. This work has been supported by Fondazione Cariplo, grant n. 2015-0783.

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