Aus dem

Department für Frauengesundheit Tübingen
Universitäts-Frauenklinik

Gesichtsprofil bei Feten mit Spina Bifida

Inaugural-Dissertation
zur Erlangung des Doktorgrades
der Medizin

der Medizinischen Fakultät
der Eberhard Karls Universität
zu Tübingen

vorgelegt von

Prodan, Natalia Carmen

2023

Dekan: Professor Dr. B. Pichler

1. Berichterstatter: Professor Dr. K.O. Kagan
2. Berichterstatter: Professorin Dr. L. Springer

Tag der Disputation: 16.11.2023

Contents

List of Tables

List of Figures

List of Abbreviations

NTD neural tube defects

MTHFR 5,10 - methylenetetrahydrofolate reductase

PCP planar cell polarity

VANGL Van Gogh - like protein

MMC myelomenigocele

AFP alpha 1-fetoprotein

FMF Angle frontomaxillary facial angle

PFSR prefrontal space ratio

PFS prefrontal space

CCM distance between madibulomaxillary axis and corpus callosum

MNF mandibulo-naso-frontal axis

MoM multiple of median

IQR interquartile range

BMI body mass index

CI confidence interval

ROC receiver operating characteristic

AIC Aikake information criterion

BIC Bayesian information criteria

DR detection rate

FPR false positive rate

AUC area under the ROC curve

1 Introduction

The neural tube defects (NTDs) are among the most serious antenatally detectable fetal abnormalities and the second most frequent after cardiac abnormalities (Kaplan et al. 2005). They comprise of anencephaly, cephalocele, iniencephaly, open and closed spinal dysraphisms, diastematomyelia and lipomeningocele (Botto et al. 1999). Ancencephaly and spina bifida are the most frequent of them and account for approximately 95% of NTDs. They have a reported worldwide incidence of 1 to 10 in 1000 births and in 2018 had a prevalence rate of 1.075 in 1000 live births in Europe (Dolk et al. 2010) (Au et al. 2010) (EUROCAT 2021).

The prevalence of NTDs was markedly higher in the past, with distinct geographic variations. According to EUROCAT the prevalence in Europe in the 1980s was 3.074 in 1000 live births for NTDs in general and 1.728 in 1000 live births for spina bifida. This decreased in the 1990s due to folic acid supplementation and food fortification introduced after observational and randomised controlled studies showed a benefit in preventing the disease (Honein et al. 2001). The timely ultrasound diagnostic of such defects has reduced the prevalence in live births even further, as some of the parents with babies affected by spina bifida in utero will choose termination of pregnancy (Botto et al. 1999). The current prevalence in Europe as of 2018 is reported at 1.075 in 1000 live births for all NTDs and 0.49 in 1000 live births for spina bifida (EUROCAT 2021).

While some of these abnormalities, like anencephaly, are lethal, the ones that allow survival of the fetus and the child into adulthood, such as spina bifida, imply a lifelong associated morbidity and thus pose great challenges for the affected persons, for their families and eventually for the society as whole, which makes their timely diagnosis of crucial importance.

1.1 Anatomy of the Spine

The vertebral column or spine is an essential and complex structure composed of a variety of tissues. It functions as a support for the whole body and as a conduit for neural elements, ensuring their connection to the brain. The osseous spine is composed of 33 vertebrae: 7 cervical vertebrae, 12 thoracic vertebrae, 5 lumbar and sacral, respectively, and 4 coccygeal. The first

24 vertebrae are separated and articulated with one another through the intervertebral discs and the sacral and coccygeal ones are fused, forming the sacrum and the coccyx, respectively. The vertebra typically comprises a body and an arch, which together enclose the foramen, through which runs the spinal cord (Cho 2015). The lower regions of sacrum and coccyx lack a central foramen. Attached to the vertebral arch there are transverse and spinous processes.

The vertebral column is additionally composed of ligaments extending along the whole spine anteriorly and posteriorly and between the vertebrae, joining their arches, as well as the spinous and transverse processes.

The whole osseous and ligament structures enclose the spinal cord. In the adult it extends from the brain stem until the lumbar region of the vertebral column where it tapers into the conus medullaris. It then continues with the cauda equina, composed of the roots of the spinal nerves, and filum terminale, which is an extension of the pia mater. This arrangement occurs because of the disproportionate extension of the meninges of the spine and of the vertebral column compared to that of the neural tube (Bican et al. 2013).

1.2 Embryology of the Spine

The embryo goes through several developmental stages, each of which last for about 2-3 days and together unfold over 9 weeks. During its development it undergoes a series of cellular processes and collective cellular movements which lead to the narrowing along one axis and extension along another axis, the so called convergent extension (Tada & Heisenberg 2012). Central to the development of the spine are the processes that unfold starting with the third week of embryogenesis (O'Rahilly & Müller 2010).

1.2.1 The First Two Weeks of Embryogenesis

The first week of embryogenesis begins with the ovulation and continues after the ovum is fertilised with the formation of the zygote, which undergoes a series of cleavages, generating smaller cells called blastomeres. As this compact group of cells in the form of the morula, at the stage of sixteen cells, enters the uterine cavity in the third to fourth day after fertilisation, a small cavity filled with fluid appears within and the blastocyst emerges. It contains a smaller inner cell mass called embryoblast, which will later form

the embryo, and an outer cell mass, which envelops the inner mass and the blastocyst cavity, and will form the trophoblast. At the same time, the blastocyst will hatch from the zona pellucida, a remnant of the oocyte, and will begin implanting in the endometrium during the 6th day after fertilisation (Adé-Damilano et al. 2008, Module 6).

During the second week of embryogenesis the trophoblast differentiates into the cytotrophoblast, an inner mass of actively proliferating cells and the syncytiotrophoblast, which invades maternal vessel and by the end of the second week of embryogenesis will establish the primitive uteroplacental circulation. The embryoblast meanwhile also differentiates into a bilaminar disc composed of the epiblast, which will give rise to the embryo and the cells that will line the amniotic cavity, and the hypoblast, which, together with the extracoelomic membrane, will line the primitive yolk sac (Sadler et al. 2019, S.50-58).

1.2.2 The Third Week of Embryogenesis - The Gastrulation

The development of the vertebral column proper begins in the third week of embryogenesis, with the crucial process of epithelio-mesenchymal transition, also known as gastrulation. It entails the formation of the three germ layers of ectoderm, mesoderm and endoderm in the epiblast. Gastrulation begins with the development of the primitive streak (comprised of a groove, a pit and a node) on the surface of the epiblast at its caudal end. The epiblast cells will start to invaginate through the primitive streak and move towards the hypoblast. These invaginating cells will finally displace the hypoblastic cells in the bilaminar disc and will form the endoderm, while the remnant epiblast will form the ectoderm. A subsequent wave of cells which comprises the majority of invaginating cells will migrate between the ecto- and the endoderm to form the mesoderm. At the cephalic and caudal end of the embryo the ectoderm and endoderm come in close contact forming the oro-pharingeal and cloacal membranes, respectively. The cranially migrating cells will form the prechordal plate, a thickened region of the ectoderm at the cephalic end of the embryoblast (Kaplan et al. 2005).

Around the 19th day of embryogenesis the cells that invaginate at the level of the primitive node and move cranially will develop in the form of a dense cylinder, called the prechordal process. It will elongate cranially over the next few days until it reaches the prechordal plate (Kaplan et al. 2005). For a brief period of time on the 18th - 23rd day the chordal process will coalesce with the

underlying ectoderm, opening the so called neurenteric canal, which connects the amniotic cavity with the yolk sac and balances the pressure of liquids in both cavities. Then on the 20th - 25th day the edges of the prechordal process will come together and fuse again, forming the notochord (Nolting et al. 1998), which lies amidst the mesoderm and plays a role in the induction of the ectoderm that stretches over it in its differentiation into the neural plate, as well as in the genesis of the vertebral body. In the postembryonic life the notochord will be replaced by other tissues and will eventually regress and become incorporated into the nucleus pulposus of the intervertebral disk.

At the same time at the level of the intraembryonic mesoderm cells multiply on both sides of the median line and by the 17th day of embryogenesis condense in the form of the paraaxial mesoderm. At the extreme edge of the intraembryonic mesoderm the layer of cells is still thin and forms the lateral plate and between these two mesodermal regions lies the intermediate mesoderm (Reghunath et al. 2019).

At the beginning of the 3rd week the paraaxial mesoderm will organise into segments of concentrically arranged cells, which are known as somites in the caudal region. As almost all other processes involved in the formation of the spine, this process proceeds in a craniocaudally fashion. The first somites emerge around the 20th embryogenesis day and afterwards new ones will rhythmically appear in a craniocaudal sequence with a frequency of approximately three pairs per day so that at the end of the fifth week 42 to 44 pairs are present: 4 occipital, 8 cervical, 12 thoracic, 5 lumbar, 5 sacral, and 8 to 10 coccygeal (Kaplan et al. 2005). Some of them later degenerate, leaving the remaining somites to form the base of the occipital bone and the axial skeleton, including the vertebrae, ribs, muscles, ligaments and skin. The cells that constitute the somites will first undergo a process of epithelisation.

1.2.3 Fourth Week of Embryogenesis

At the beginning of the 4th week the cells in the ventral and medial wall of each somite will reverse to being mesenchymal cells and change their arrangement to surround the neural tube and notochord. These cells thus form the sclerotome from which the vertebrae, ribs and cartilages will evolve. The rest of the somite will form the dermomyotome, which will give rise to the dermis of the skin of the back, the musculature of the back, the intercostal muscles and some of the limb muscles (Scaal 2016). Irrespective of where the cells of each myotome and dermatome will later migrate and develop,

they will retain their innervation from the segment of origin. The sclerotome will arrange around the neural tube and the notochord and will fuse with the opposite somite. It will also subdivide into a cephalic and a caudal compartment. Subsequently a resegmentation process occurs and thus the caudal part of each sclerotome merges with the cephalic part of the underlying sclerotome, forming the definitive vertebrae. This is an especially important aspect of the development of the spine, as the muscles which derive from each myotome will thus attach to two adjacent somites which become fused, allowing for a range of motion across the spine. Mesenchymal cells between cephalic and caudal halves of the original sclerotome segment fill the space between two vertebral bodies and contribute to formation of the intervertebral disc. As mentioned earlier, the notochord disappears in the sclerotome segments but persists in the region of the intervertebral discs as the nucleus pulposus (Lawson & Harfe 2017).

1.2.4 The Development of the Nervous System. Primary and Secondary Neurulation

The development of the nervous system starts on the 19th day of embryogenesis with the formation of the neural plate and then unfolds parallel to and under the influence of the notochord and the underlying mesoderm and prechordal plate until the 6th week of embryogenesis. Through several biochemical signalling processes and mechanical forces the neural plate grows in a craniocaudal direction and narrows along the opposite axis, through the process of convergent extension (Ossipova et al. 2015). It is wider at the cranial end which will give rise to the central nervous system and narrower at the caudal end which will form the spine (O'Rahilly & Müller 2006). The adjacent structures secrete inducing factors which will determine the differentiation of the neural plate into neuroectoderm, and this represents the first step of the neurulation. The lateral edges of the neural plate start to rise, forming neural folds which enclose a depressed mid-area called the neural groove.

The primary neurulation unfolds during the 4th week of embryogenesis (O'Rahilly & Müller 1999). The neural folds will coalesce in the mid-line starting from the cervical region at the level of the 5th somite, approximately in the middle of the embryo, and this process continues cranially and caudally until the neural tube is formed. Until approximately the 25th day of embryogenesis the neural tube is still open, communicating through the cranial and caudal neuropores with the amniotic cavity. However, once the cranial neuropore

closes on the 25th day and the caudal one on the 28th day, the neural tube is established in the form of a closed tubular structure with a narrower caudal end, the spinal cord, and a broader cranial region with several dilatations, the brain vesicles (O'Rahilly & Müller 2010).

Afterwards the cell mass at the caudal end of the spinal cord undergoes an epithelial-mesenchymal transition and condensation of cells to form the lower end of the spinal cord, called the conus medullaris, in what is known as the secondary neurulation (Eibach & Pang 2020) (Deora et al. 2019).

An additional important process is the formation of the neural crest from the cells on the lateral edges of the neural plate, which detach themselves and undergo an epithelial-mesenchymal transition. They will give rise to the peripheral nervous system, including the ganglia and the peripheral nerves, as well as contribute to the formation of the meninges (O'Rahilly & Müller 2007).

The embryological development of the spine with its multiple steps and intricacies explains the pathophysiology of neural tube defects.

1.3 Aetiology of Spina bifida

The causes of NTDs and spina bifida specifically remain elusive. Epidemiological studies indicate that genetic and environmental factors play a crucial role in NTDs as they interact to produce variable phenotypes of the disease (Botto et al. 1999).

1.3.1 Environmental Factors

Several environmental factors are known to cause NTDs, among them maternal diet, especially folate deficiency, drug exposure to possible teratogens and glucose metabolism, especially maternal diabetes and obesity (Hernández-Díaz & Mitchell 2000) (Copp & Greene 2013). It must be noted however that environmental factors alone explain less than 50% of the NTDs and less than 30% of cases of spina bifida (Agopian et al. 2013).

Folate and its metabolism have long been known to be responsible for NTDs (Smithells et al. 1976). Folate functions as acceptor and donor of one-carbon units, which are necessary in the synthesis of adenine, guanine and thymine, and also in the methylation processes and thus is crucial in times of rapid cellular growth (Blom et al. 2006). Inhibitors of its uptake such as

carbamazepine and trimethoprim, as well as a diminished dietary availability, increase the risk for NTDs (Hernández-Díaz & Mitchell 2000).

Autoantibodies against folate receptor and against folate binding protein will also inhibit the uptake of folate in the embryonic tissue and were shown to be associated with NTDs (Cabrera et al. 2008) (Denny et al. 2013).

Teratogenic factors causing NTDs are well documented in animal models, however only some of them have been conclusively identified as causative agents in humans. Some agents disturb key signalling pathways in the process of neurulation. Valproic acid is one of the better known among them and its potent inhibition of histone deacetylase leads to imbalance of histone acetylation and deacetylation processes, which causes the failure of closure of the neural tube. The intake of valproic acid in the first trimester of pregnancy leads to a 10-fold increase in the risk of NTDs (Detrait et al. 2005).

An altered glucose metabolism has been associated with increased risk for spina bifida. An insufficient glycemic control in the mother in the early stages of embryonic evolution likely exposes the developing tissues to an overload of glucose at a stage when, lacking a pancreas, the embryo is unable to moderate such excess (Carmichael et al. 2010). Additionally it has been shown that maternal diabetic status suppresses and deregulates a series of NTD - associated genes (Salbaum & Kappen 2010). There are strong indicators that pregestational diabetes increased the risk of NTDs four-fold compared to non-diabetic mothers (Mowla et al. 2020). Obese mothers have a 2.2 to 3.5 increased risk of having a pregnancy affected by spina bifida as revealed by several studies and meta-analyses, and the association remains relatively strong even when corrected for potential confounding factors such as ethnicity, socioeconomic status, age, diabetes (Watkins et al. 2003) (Carmichael et al. 2010) (Stothard et al. 2009).

1.3.2 Genetic Factors

The variance in the prevalence of NTDs is due in a great proportion to genetic factors, with several observations strongly suggesting a genetic background. For instance there is a high risk of recurrence in siblings of affected cases, up to 25 to 50% more than in the general population (Melvin et al. 2000). Furthermore a woman with two or more pregnancies affected by spina bifida will have a significantly increased risk, up to 10%, of recurrence in subsequent pregnancies. Same-sex twins, a proportion of which are monochorionic, have a higher prevalence of spina bifida than twins of different sex (Copp & Greene

2013).

NTDs are known to be associated with aneuploidies such as trisomy 13 and triploidy. They are also present as a feature of several genetic syndromes, such as Meckel-Gruber syndrome, Waardenburg syndrome, Siegel-Bartlet syndrome, Fryns microphthalmia syndrome and other rare diseases (Chen 2008).

Several genes were studied as potential candidates in the aetiology of NTDs in cohorts of patients (Bassuk & Kibar 2009). One focus were genes involved in folate metabolism, especially the 5,10 methylenetetrahydrofolate reductase (MTHFR). It has been shown that the homozygous presence of the MTHFR mutation which replaces a cytosine with thymine at the 677th position, also known as MTHFR C677T, increases the risk of the carrier woman of having a child affected by spina bifida by 60% (Blom et al. 2006). Moreover, children who exhibit MTHFR C677T will have a 1.9 odds ratio of developing spina bifida. Other genes active in folate metabolism were investigated but studies failed to show their mutations might harbour a serious risk for NTDs (van der Linden et al. 2006).

A second group of genes potentially associated with spina bifida are those belonging to the planar cell polarity (PCP) pathways that regulate convergent extension and the rearrangement of the embryonic tissue along the body axes and are involved in the gastrulation and neurulation processes in human embryos (Wallingford & Harland 2002). Homozygous and compound heterozygous mutations in genes that control the PCP pathways have been identified in animal models of NTDs. VANGL-1 and 2 genes have been extensively studied in animal models where an association between their mutation and NTDs has been documented (Galea et al. 2018) (Doudney et al. 2005). These observations have been also investigated and confirmed in human patients (Kibar et al. 2011) (Bartsch et al. 2012). The Prickle-1 gene has also been suggested as a candidate gene responsible for NTDs in a subset of patients (Bosoi et al. 2011).

However, the phenotype heterogeneity in humans with mutations in these genes suggest that mutations of single genes are unlikely to explain a major proportion of cases with NTDs. Rather it is likely that several associated weak factors, as well as gene-gene interactions or environmental effects on a genetic predisposition have a larger contribution on the aetiology of NTDs (Copp & Greene 2010).

1.4 Pathophysiology of Spina bifida and Associated Malformations

The cascade of events leading to the appearance of neural tube defects, including spina bifida, starts with the incomplete closure of the neural tube below the 5th somite during the primary neurulation in the fourth week of embryogenesis. If for instance the posterior or caudal neuropore fails to close, a form of spina bifida will occur, whereas anencephaly results from a closure failure of the anterior or cranial neuropore.

Because the neural tube has a inductive effect on the evolution of the sclerotomes, a developmental defect of the neural tube will reverberate on the vertebral formation and will lead to a defect in the vertebral arch. This results in variable degrees of opening of the spinal canal or spinal dysraphism, which can be broadly divided into open and closed spinal defects. Common to all types of spina bifida is an absent closure of the vertebral arch.

Spina bifida occulta is characterised by a lack of development of the vertebral arch alone and is a result of a defective secondary neurulation (Basaloglu et al. 2017) (Copp & Greene 2013). It is seldom diagnosed prenatally due to a very subdued ultrasound appearance and has a good long-term outcome for the affected child.

Spina bifida aperta on the other hand is defined by a defect in the closure of osseous structures along with abnormalities of the meninges and of the spinal cord itself. There are several types of spina bifida aperta. The myelomeningocele (MMC) is the most frequent, accounting for approximately 90% of them (Au et al. 2010). In a myelomeningocele the spinal cord, covered by meninges and sometimes by a very thin skin layer, protrudes outside of the vertebral arch. Another type of spina bifida aperta is the myeloschisis, also known as rachischisis. It is the most severe form of spina bifida aperta and is caused by a failure in the closure of the neural fold and lacks a dermal or meningeal covering, thus completely exposing the nervous tissue.

Individuals affected by spina bifida aperta will exhibit loss or reduction of motor and sensory neurological functions, such as weakness or paralysis of lower limbs, absence of sensations that increase the risk of injuries, urinary and faecal incontinence, talipes, joint contractures or dislocations, scoliosis and kyphosis (Copp et al. 2015). The spinal cord malformations due to the failure of the embryonic processes of primary neurulation account for a part of these deficits. However the exposure of neural tissue to the outside environment consisting of amniotic fluid has a potentially greater pathophysiolog-

ical effect, in the so-called two-hit hypothesis (Meuli & Moehrlen 2014). The amniotic fluid is neurotoxic and leads to degeneration of neural tissue, with subsequent loss of neurological function at and below the level of the lesion (Copp & Greene 2010). Earlier observations suggested that the lesions caused by the contact with amniotic fluid intensify beginning with the late second trimester and during the third trimester (Stiefel et al. 2007). However new research shows that the loss of neural motors is observable on post-mortem specimens long before that and as early as 16 weeks of gestation (Ben Miled et al. 2020).

Furthermore, the continuous leakage of cerebro-spinal fluid in the amniotic cavity, which characterises the open spinal defects, will cause hypotension in the subarachnoid spaces, thus leading to a downward displacement of the midbrain and subsequent collapse of the posterior fossa with obliteration of cisterna magna and herniation of the cerebellum into the foramen magnum (Ghi et al. 2006). Consecutively the upstream circulation of the cerebro-spinal fluid will be affected, leading to obstructive hydrocephalus (Van den Hof et al. 1990). Almost all cases of open spina bifida will present these associated cerebral abnormalities. This pathophysiological aspect of the spinal defect is known as Arnold-Chiari or Chiari II malformation and both defects constitute parts of the same sequence of malformation (Miller & Huisman 2019).

1.5 Diagnostic of Spina bifida

1.5.1 Biochemical Testing

In the 1970s following observations that the amniotic fluid of fetuses with NTDs showed high levels of a specific fetal protein, the alpha 1-fetoprotein (AFP), several studies have proved the validity of using elevated levels of amniotic fluid AFP obtained after an amniocentesis at 16 - 18 weeks of gestation as a marker for spina bifida and anencephaly (Wald et al. 1977) (Brock 1976).

As in the second trimester, the AFP was shown to be a useful first trimester marker for spina bifida (Bredaki et al. 2012). An increase serum value could guide the sonographer to a thorough assessment of the face and spine or to a later reevaluation in suspicious situations.

However, as ultrasound markers are readily assessed and AFP is not currently integrated in the first trimester screening, the ultrasound offers a more straightforward way to screen for spina bifida during the pregnancy.

1.5.2 Ultrasound Diagnostic: Direct and Indirect Signs

A new era of prenatal testing and diagnosis began with the advent of ultrasound for medical purposes in the 1950s and the introduction of real-time imaging in 1970s (Carlson & Vora 2017). Ultrasound guided investigations and testing allow for a timely diagnosis of fetal and obstetrical problems and can guide the appropriate counselling and therapy.

The accuracy of ultrasound compared to biochemical testing in the screening or spina bifida in the second trimester of pregnancy was established in a landmark research published in 1980 (Nicolaides et al. 1980).

The second trimester ultrasound was used to directly identifying the lesion, the myelomeningocele, seen in fig. 1, and the sensitivity and specificity in experienced hands were remarkably high.

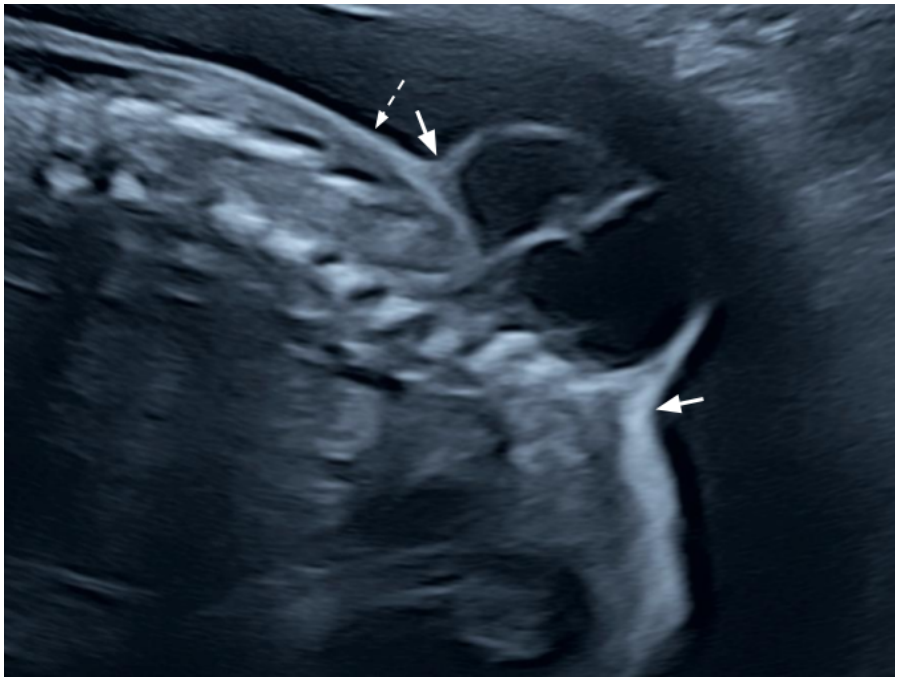

Figure 1: Mid-sagittal View of the Fetal Lower Back with Myelomenigocele (MMC) between the two continuous arrows, the dashed arrow points at the normal skin of the back

When compared to a normal spine, seen in fig. 2, the MMC sac is easily recognisable.

Additional observations indicated that there are indirect sonographic markers for spina bifida at the level of the fetal head. These are easily recognisable and could be used by less experienced sonographers. The reduction of the bi-

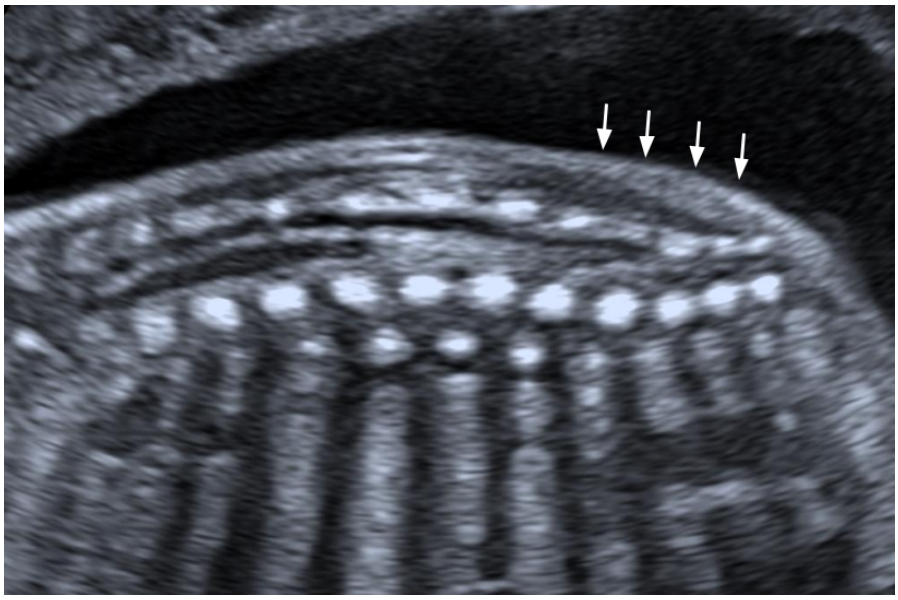

Figure 2: Mid-sagittal View of the Fetal Spine (the arrows point at the intact skin of the back)

parietal diameter and head circumference, the scalloping of the frontal bones, which was called the "lemon sign", as well as the widening of occipital horns of the lateral ventricles are markers visible in the transverse plane of the fetal head and are illustrated in fig. 3. The normal fetal head is depicted in fig.4 and the differences are salient.

Another marker at the level of the fetal head in the region of the posterior fossa is the collapse of the cisterna magna and anterior curvature of cerebellar hemispheres, also known as "banana sign", seen in fig. 5, which, when compared to the normal appearance of these structures, illustrated in fig. 6, shows an obvious difference.

These markers were firstly thoroughly defined as a result of patho - physiological changes induced in the brain by the presence of spina bifida downward. They were then investigated retrospectively, as well as prospectively in high risk patients against normal controls and were proven to have a high diagnostic accuracy in numerous studies, as seen in table 1, adapted after (Bahlmann et al. 2015). Thus sonographic markers soon replaced the biochemical ones in the diagnosis of spina bifida.

In the 1990s due to technical improvements in the quality of ultrasound and the call for an earlier diagnosis of fetal structural and chromosomal abnormalities, efforts were made to shift the diagnosis of spina bifida to the first trimester of pregnancy (Chaoui & Nicolaides 2010).

The ultrasound markers visible in the transverse plane of the head first

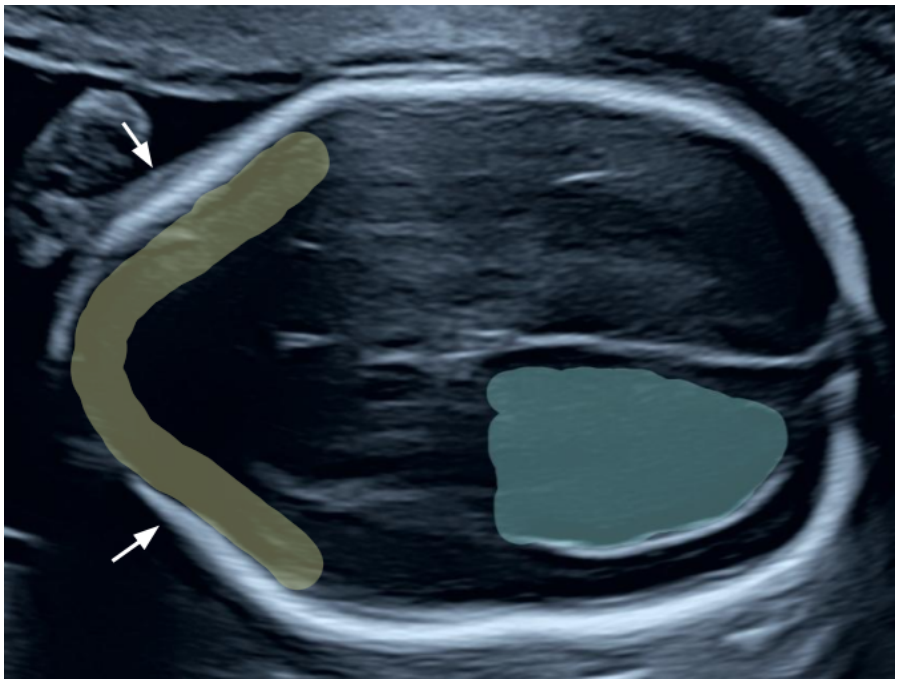

Figure 3: Transverse View of the Fetal Head with Typical Indirect Signs of Frontal Scalloping or "lemon sign" (in yellow between the two arrows) and Ventriculomegaly (in blue)

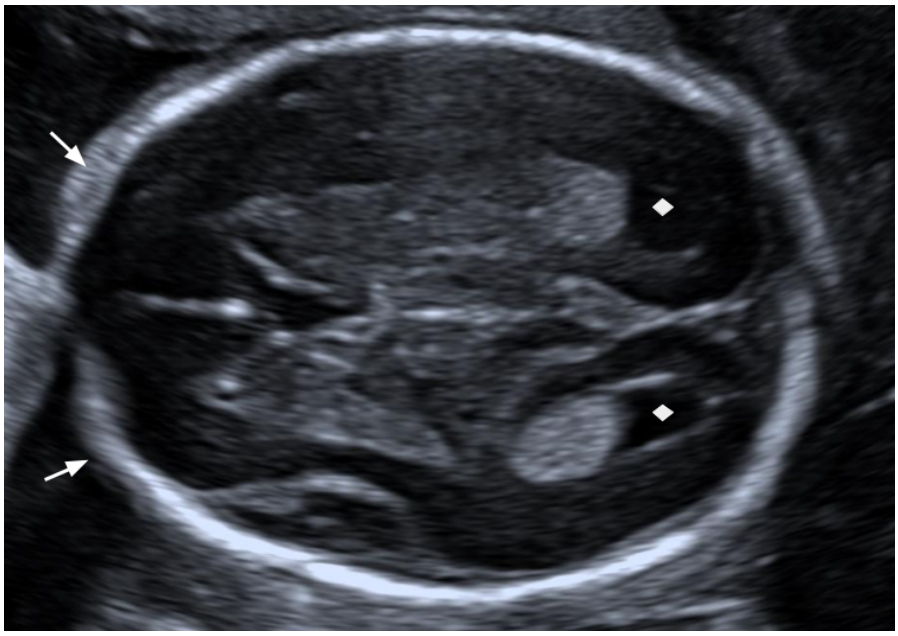

Figure 4: Transverse View of the Normal Fetal Head (the arrows point at the normal frontal bones, the diamond shapes are placed in the occipital horns of the lateral ventricles)

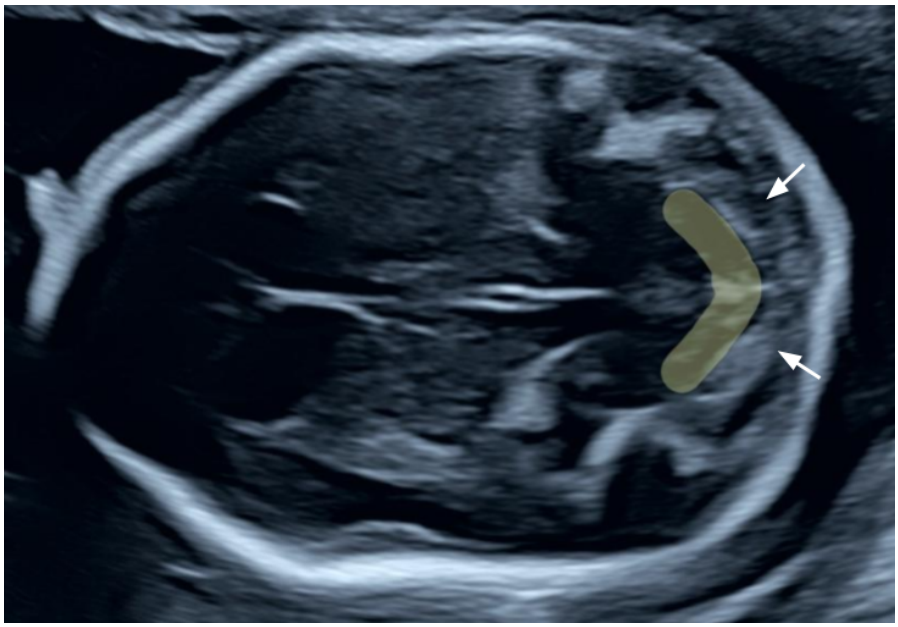

Figure 5: View of the Posterior Fossa of the Fetal Head with Collapsed Cerebellum and Cisterna magna ("banana sign" marked in yellow, arrows point at the collapsed cisterna magna)

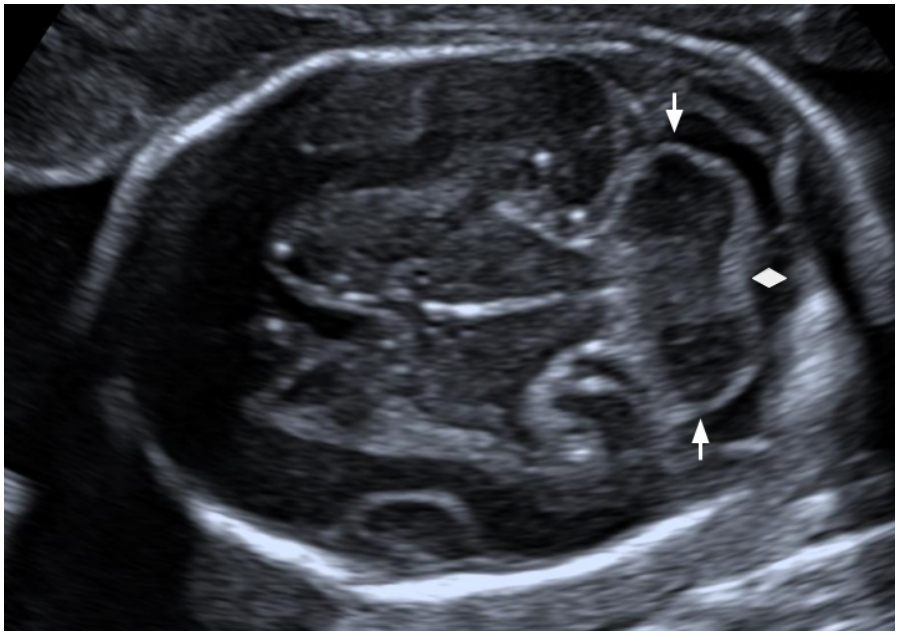

Figure 6: View of the Normal Posterior Fossa of the Fetal Head (the normal cerebellum is shown between the arrow, the diamond shape is placed in the cisterna magna)

Table 1: Detection Rates of Second Trimester Sonographic Markers

Study	Week	n	BPD < 5 centile (%)	HC < 5 centile (%)	Banana Sign (%)	Lemon Sign (%)	VM (%)	CM (%)
(Nicolaides et al. 1980)	16-23	70	61	26	95	100	85	95
(Campbell et al. 1987)	16-23	26	62	35	62	100	54	
(Nyberg et al. 1988)	< 24	27				89	82	
(Thiagarajah et al. 1990)	16-24	16	69			100	63	100
(Van den Hof et al. 1990)	<24	107			72	98		96
(Ghi et al. 2006)	16-34	53					64.2	
(D'Addario et al. 2008)	18-28	49				53	81	96
(Karl et al. 2012)	16-24	23	69					
(Khalil et al. 2014)	20-22	39	56				64.1	
(Bahlmann et al. 2015)	18-22	588	52	69.7	97.1	88.6	46.1	96.7

VM = Ventriculomegaly, CM = Cisterna magna

reported in the second trimester for the detection of spina bifida, such as lemon sign, were investigated in the first trimester as well, however with limited results (Sebire et al. 1997), as the changes noted at the level of the brain in the second trimester are gradual and not yet evident in the first trimester.

A new approach suggested investigating the mid-sagittal facial plane already used in the first trimester screening for fetal aneuploidies to identify a novel marker for spina bifida (Chaoui & Nicolaides 2010). Apart from the nuchal translucency and nasal bone, this plane will allow the visualisation of the brain-stem, the fourth ventricle and the future cisterna cerebellomedullaris or cisterna magna. The fourth ventricle is represented by the intracranial translucency, a sonolucent region which runs parallel to the nuchal translucency and is delineated by two echogenic lines (Chaoui et al. 2009), as seen in fig. 7.

The upper echogenic line is the dorsal part of the brain stem. The choroid plexus of the fourth ventricle forms the lower echogenic line. The second thinner sonolucent area below the fourth ventricle and above the occipital bone is the cisterna magna.

In the fetuses with spina bifida the fourth ventricle is compressed as a result of the caudal displacement of the mid-brain and consequently the intracranial translucency should not be visible, at least in some cases (Chaoui et al. 2011), as seen in fig 8.

This feature amounts to a precursor of the known Arnold-Chiari malformation. The ratio of the brainstem to the distance from the brainstem to the

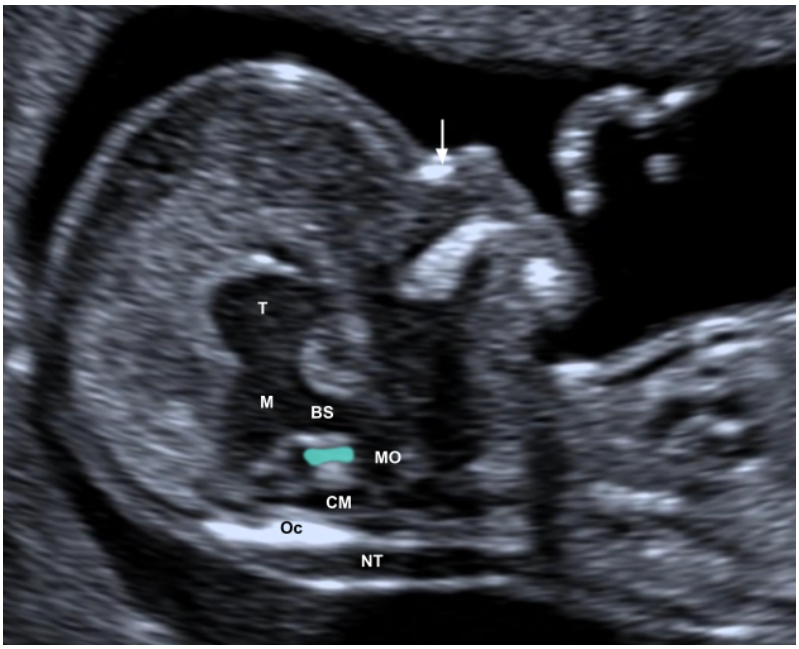

Figure 7: Mid-sagittal View of the Normal Fetal Profile (fourth ventricle or "intracranial translucency" in green, T=thalamus, M=midbrain, BS= brain stem, MO=medulla oblongata, Oc=occipital bone, CM=cisterna magna, NT=nuchal translucency, arrow-nasal bone)

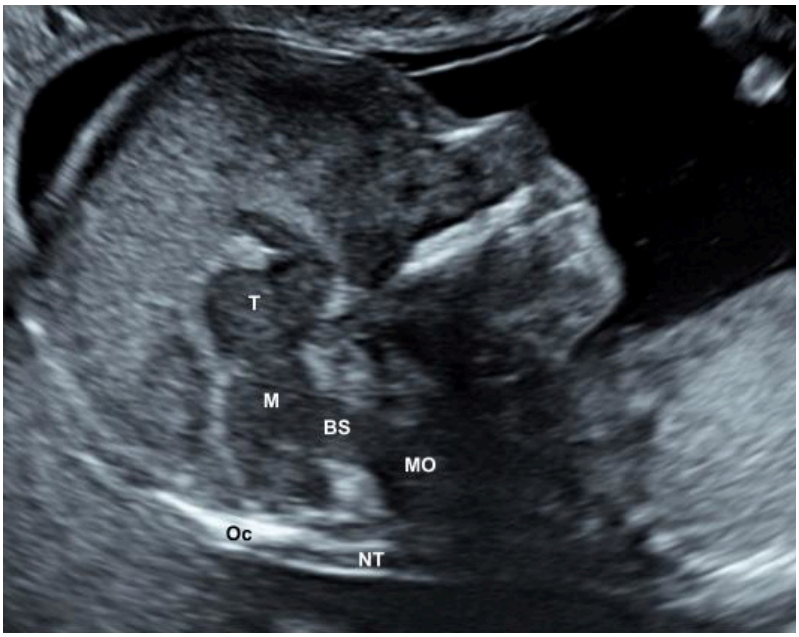

Figure 8: Mid-sagittal View of the Fetal Profile in a Fetus with Spina Bifida: collapsed intracranial translucency, disappearance of Cisterna magna (T=thalamus, M=midbrain, BS=brain stem, MO=medulla oblongata, Oc=ocipital bone, NT=nuchal translucency)

Table 2: Comparison of mean delta values and standard deviations (SD) of brain stem (BS) diameter, brain stem to occipital bone (BSOB) diameter and the ratio of BS to BSOB in normal and affected fetuses in the first trimester

Measurement	Control n=1000	Spina bifida n=30	p-value
Delta BS diameter mean (SD)	0.000 (0.303)	1.297 (0.420)	<0.0001
Delta BSOB diameter mean (SD)	0.000 (0.468)	-1.772	<0.0001
Delta BS to BSOB ratio mean (SD)	0.000 (0.081)	0.769 (0.265)	<0.0001

occipital bone is a measurable parameter which helps in the early diagnosis of spina bifida: if the ratio is above 1 there is a strong suspicion of spinal defect, compared to normal fetuses, as seen in table 2, adapted after (Lachmann et al. 2011).

The posterior fossa is amenable in the first trimester to an axial plane assessment as well, however the expert opinion suggests taking a step-wise approach: the mid-sagittal plane should be assessed first, as it is easier and more convenient, and the axial plane should be investigated as a second step in suspicious cases (Chaoui & Nicolaides 2011).

The collapse of the posterior fossa in open spina bifida can thus be recognised in the ultrasound examination as the banana sign in the second trimester and disappearance of the intracranial translucency in the first trimester.

1.5.3 Ultrasound Diagnostic: Fetal Profile in Fetuses with Spina bifida

The mid-sagittal view of the fetal face is one of the most important planes for the evaluation of fetal development and assessment of risks of chromosomal abnormalities and fetal defects in the first trimester, as recommended by many international and national guidelines for fetal ultrasound (ISUOG Practice Guidelines 2013) (von Kaisenberg et al. 2016). The fetal profile was extensively investigated for potential markers for fetal abnormalities, such as trisomies and fetal malformations.

One research project (Sonek et al. 2007a) investigated the frontomaxillary facial (FMF) angle in the prediction of trisomy 21 in the first trimester. This parameter is obtained in the same mid-sagittal plane in which the nuchal translucency, nasal bone and intracranial translucency are visible and defined as the angle between the upper surface of the maxilla and the line which goes through the upper corner of the anterior part of the maxilla and the most anterior point of the external surface of the frontal bone, as in fig. 9. In fetuses with trisomy 21 this angle is significantly larger than in normal

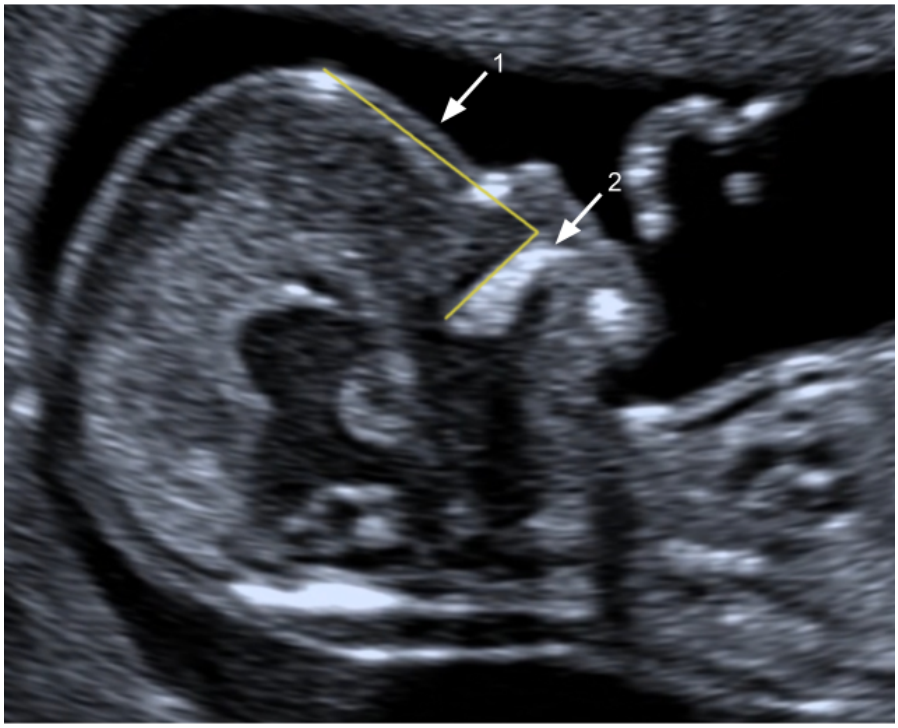

Figure 9: Mid-sagittal View of the Normal Fetal Profile (fronto-maxillary-facial angle shown between the two lines. 1.frontal bone, 2.anterior process of maxilla)

fetuses.

The FMF angle was additionally studied in cases with trisomy 18 in the first trimester (Borenstein et al. 2008), and similarly, the angle was greater in affected fetuses than in normal fetuses.

Significantly, one study (Lachmann et al. 2010) found the FMF angle to be relevantly decreased in fetuses with spina bifida compared to controls and posited that it might be useful in the first trimester screening for this abnormality. The hypothesis was that the incipient Arnold Chiari malformation and the caudal shift of the midbrain lead to an underdevelopment of the frontal bones, which will consequently determine a modified profile and FMF angle, as seen in fig.10.

Interestingly, one study (Plasencia et al. 2007) found that this parameter is highly reproducible in the first trimester ultrasound.

In the second trimester the assessment of the fetal facial profile is also a requirement for the systematic evaluation of the fetal anatomy and it is included in most international and national fetal ultrasound guidelines (Merz et al. 2012) (Salomon et al. 2011).

Similar to the first trimester ultrasound, one research project evaluated the

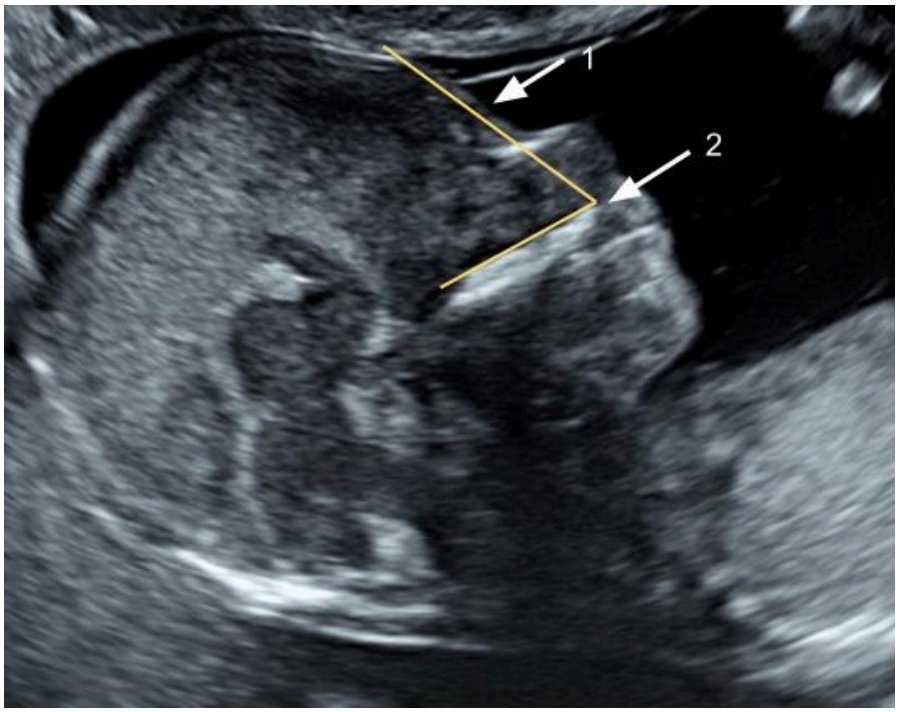

Figure 10: Mid-sagittal View of the Fetal Profile in the First Trimester in a Fetus Affected by Spina bifida (fronto-maxillary-facial angle shown between the two lines. 1.frontal bone, 2.anterior process of maxilla)

utility of the FMF angle in screening for trisomy 21 in the second trimester (Sonek et al. 2007b). The FMF angle was similarly defined as the angle between the upper part of the maxilla and the leading edge of the bony frontal skull. Additionally, the group described a second angle between the upper maxilla and the skin of the forehead. Both are depicted in fig. 11 and both of them were found to be significantly increased in fetuses with trisomy 21, so much so that the researchers concluded that the FMF angle could be the single most sensitive second trimester marker for trisomy 21.

Another study (Sonek et al. 2012) looked at an additional parameter of the fetal profile, the prefrontal space ratio (PFSR), in screening for trisomy 21 in the second trimester. This was defined as the ratio between the distance from the leading edge of skull to prenasal skin (D1) and the distance from prenasal skin to the point where the mandibulomaxillary axis is intercepted (D2), as seen in fig. 12. The researchers found that the ratio was also significantly reduced in fetuses with trisomy 21 compared to normal controls, and concluded that this measurement could also be used for screening for trisomy 21 in the second trimester, with a high sensitivity and specificity.

In conclusion, multiple studies have shown that parameters of fetal facial

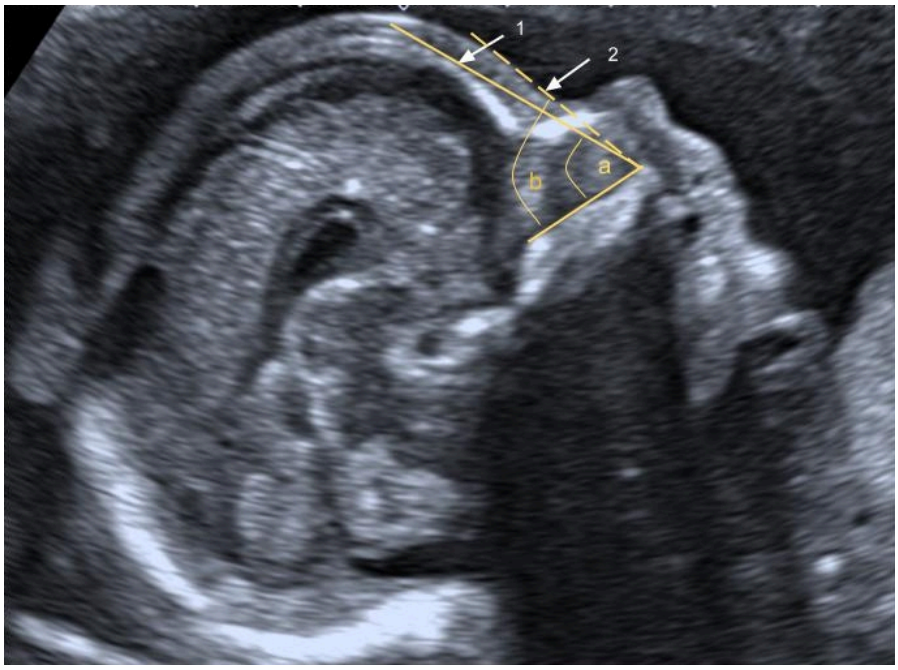

Figure 11: Mid-sagittal View of the Normal Fetal Profile in the Second Trimester with FMF Angle (1.frontal bone, 2.skin covering the forehead, a.fronto-maxillary-facial angle to the frontal bone, b.fronto-maxillary-facial angle to the frontal skin)

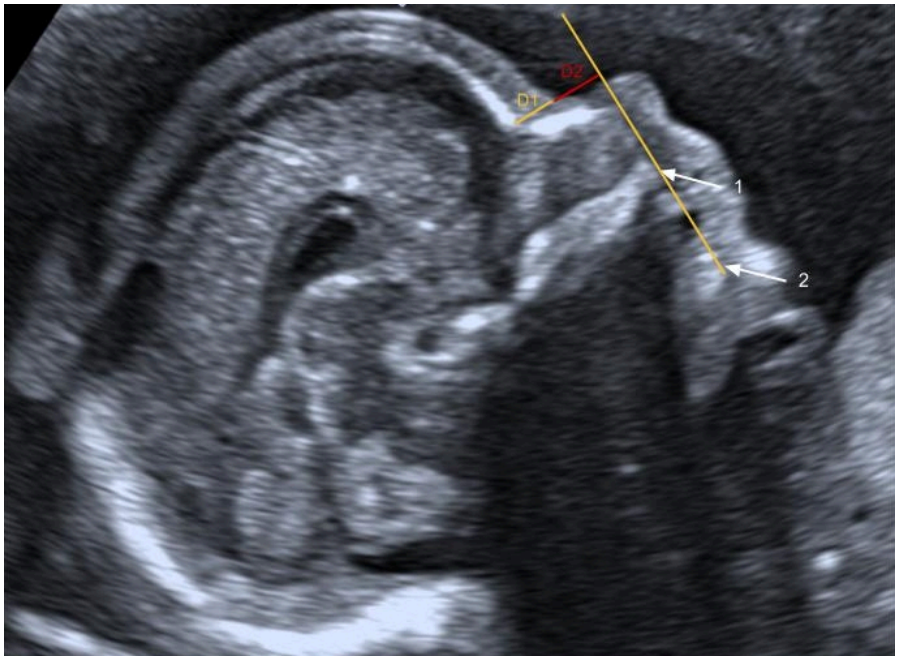

Figure 12: Mid-sagittal View of the Normal Fetal Profile in the Second Trimester with PFSR (1.leading edge of the maxilla, 2.edge of the mandible, yellow line-mandibulomaxillary axis)

profile can be used to screen for chromosomal abnormalities, like Down's syndrome. This is most likely due to the fact that aneuploidies are associated with certain morphological characteristics of the face, such as, in case of trisomy 21, a thickened prenasal skin and midface hypoplasia.

Spina bifida is also associated with changes of the fetal face, especially due to the caudal displacement of the midbrain and to the Arnold-Chiari malformation. As discussed above, these features were already shown to be relevant in the first trimester screening for spina bifida. Our question was if the same changes in the fetal profile would be marked enough to be helpful in the screening for spina bifida in the second trimester ultrasound.

1.6 Aim of Research

The prevention modalities, as well as diagnostic capabilities and therapeutic success for fetuses and children affected by spina bifida, although greatly improved in the last decade, are still unfortunately limited.

Sonography is a relatively inexpensive prenatal diagnostic tool and can be extremely useful in the hands of accomplished diagnosticians, but also in those of less experienced technicians, provided there is sufficient and continuous training and clear guidelines for anatomical assessment. Ultrasound diagnostic is the cornerstone in the current management of spina bifida patients before birth. As previously shown, it has seen great progress in later years, and this has led to earlier and more precise diagnosis, allowing for better support and counselling of parents, earlier interventions and improved outcomes for patients.

The present research is aimed at defining second and third trimester ultrasound markers for spina bifida based on the anatomical plane of the fetal profile, which is commonly assessed during the routine second trimester scan and is illustrated in fig. 13.

We hypothesise that the fetal profile is modified in the fetuses with spina bifida in the second trimester (fig. 14) due to the same pathological changes already described above and that certain parameters of this anatomical plane, angles as well as distances, could discriminate between fetuses with spina bifida and unaffected fetuses.

This might improve diagnostic output, especially in the presence of difficult fetal position, less experienced sonographers or challenging technical conditions (Prodan et al. 2020). Two of these parameters were investigated

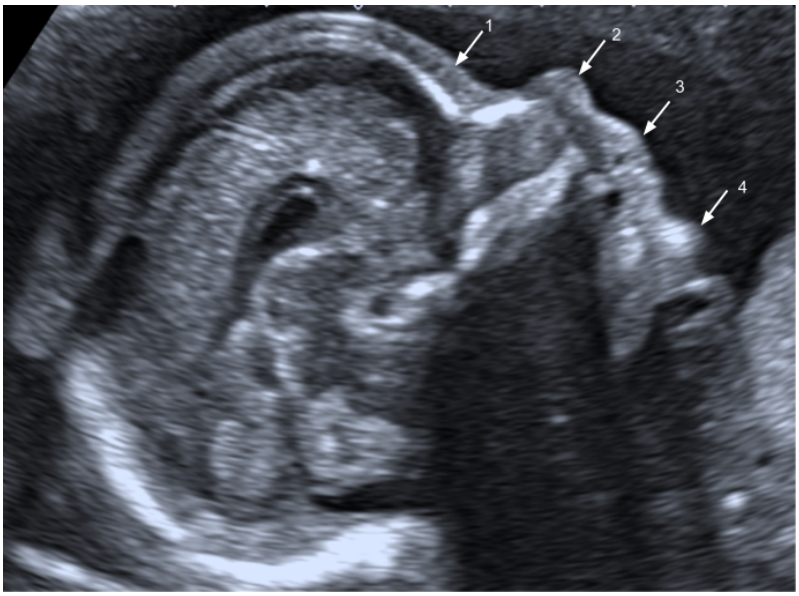

Figure 13: Mid-sagittal View of the Normal Fetal Profile in the Second Trimester of Pregnancy (1.frontal bone, 2.tip of the nose, 3.upper lip, 4.chin)

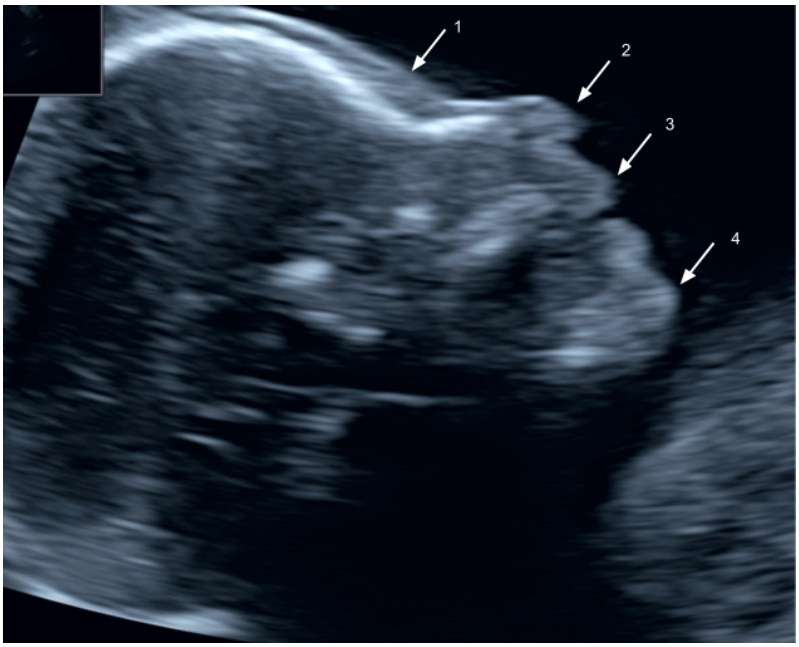

Figure 14: Mid-sagittal View of the Fetal Profile in the Second Trimester of Pregnancy in a Fetus with Spina Bifida (1.frontal bone, 2.tip of the nose, 3.upper lip, 4.chin)

before (FMF angle and PFSR) and three were defined de novo.

2 Material and Methods

This project was a retrospective study of 71 spina bifida cases seen between 14+4 weeks and 37+3 weeks of gestation in the Prenatal Diagnosis Department of the University of Tübingen, Germany between 2007 and 2017 and 279 controls matched for gestational age. The study was approved by the ethical committee of the University of Tübingen (357/2019BO2).

All images were acquired during clinical ultrasound examinations performed by the prenatal medicine specialists of our department using Voluson 730, E6 and E8 ultrasound machines, ™ of General Electric, as well as Affiniti 50 and 70 ultrasound machines, ™ of Philips.

For data acquisition, we searched our database for examinations in the second and third trimester of pregnancy in patients where a diagnosis of open spina bifida had been made in the current pregnancy and in which the presence of abnormal karyotyping and malformative syndromes was excluded. Our team used the Viewpoint 5 ultrasound data management system, ™ of General Electric.

In each case additional parameters, such as maternal history, gestational age and fetal head biometry were recorded for further analysis and in addition, for the affected cases, we also recorded the level of the spinal defect.

For the assessment, the images had to meet certain quality criteria: a true midsagittal section, visible corpus callosum where possible, clear anterior edges of the maxilla, of the mandible and of the leading edge of the frontal skull, as well as clearly delineated skin covering the forehead, as seen in fig. 15. A sufficient magnification, so that the profile filled the majority of the image, was also an important criterion. In cases where more than one examination was performed, we only assessed the earliest image where the quality criteria were met.

We defined five parameters to be assessed on stored two-dimensional images of the midline view of the fetal face. Four of them were numerical parameters: the prefrontal space ratio (PFSR), the frontomaxillary facial angle (FMF angle), the prefrontal space (PFS) and the distance between the mandibulomaxillary axis and the corpus callosum (CCM). One variable, the mandibulonaso-frontal axis (MNF) was binary.

The parameter measurements in the control group were performed by the author. In the affected group they were performed independently by the author and another experienced operator, in order to be able to assess the

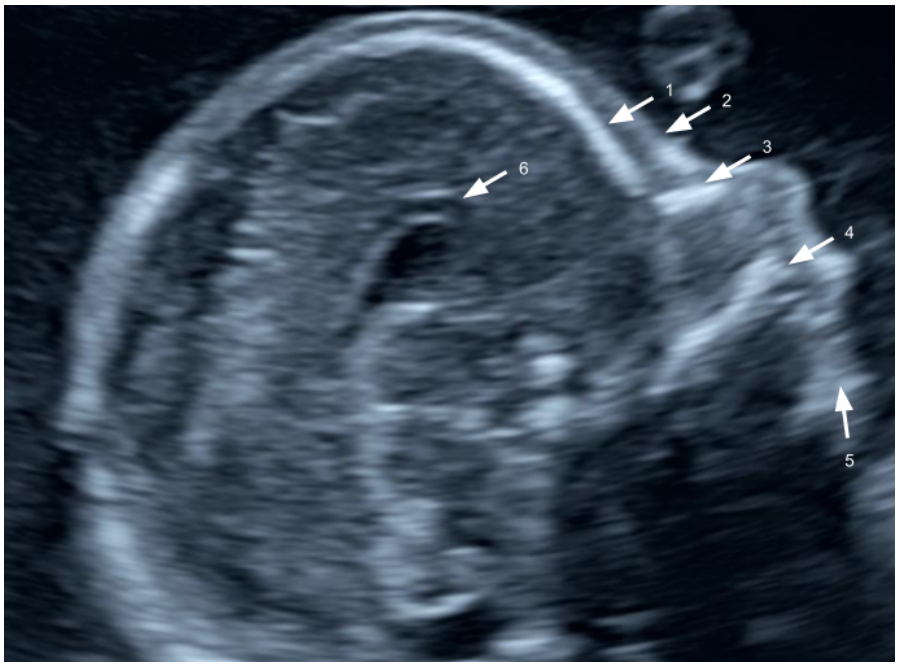

Figure 15: Anatomy of Fetal Profile with Sonographic Landmarks (1.edge of the frontal skull, 2.edge of skin over the forehead 3.nasal bone, 4.anterior edge of the maxilla 5.anterior edge of the mandible, 6.genu of corpus callosum)

inter-operator variability. The image viewer programme used for this task was OsiriX Lite DICOM Viewer ™ , the demo version of the OsiriX medical images viewer, developed by Pixmeo SARL, a Swiss company.

All data were collected using Excel spreadsheets (© Microsoft).

The statistical analyses were performed using version 4.1.2 of the statistical language R (*R: A Language and Environment for Statistical Computing* 2021), © of the R Foundation for Statistical Computing, as well as SPSS statistical software, ™ of IBM.

The inter-observer variability of the numerical variables was assessed with paired t-tests and represented by Bland-Altman plots. For the binary variable we assessed the inter-observer variability by the McNemar test. The measurements of the numerical parameters were compared between the affected and control groups by a with a Wilcoxon–Mann–Whitney two-sample rank-sum test. For the categorical variable a two-sample test for comparison of proportions was used.

To assess the performance of screening for spina bifida based on each parameter, we computed the individual ROC curves. For that, we assigned 60% of cases and controls randomly to the test group.

Subsequently we developed prediction models based on combinations of

parameters. To allow for model comparison we only analysed cases that had measurements for all parameters. To identify the model with the most most predictive power while being the most parsimonious we proceeded to build a series of nested models using sets of most relevant predictors, as well as a model including all parameters. The models were compared using likelihood ratio tests, Akaike information criterion and Bayesian information criterion.

2.1 Prefrontal Space Ratio

The first parameter we analysed is the prefrontal space ratio (PFSR), obtained by dividing the distance between the leading edge of skull to prenasal skin (D1) to the distance from prenasal skin to the point where the mandibulomaxillary axis is intercepted (D2), as seen in fig. 16 (Sonek et al. 2012). A previous research published the normal range for the PFSR, deriving it from the assessment of 276 normal fetuses (Yazdi et al. 2013). The group found that the mean value for PFSR in euploid fetuses was 0.97 ± 0.29 and that the value was below the 5th centile in 14 (5.0%) of them.

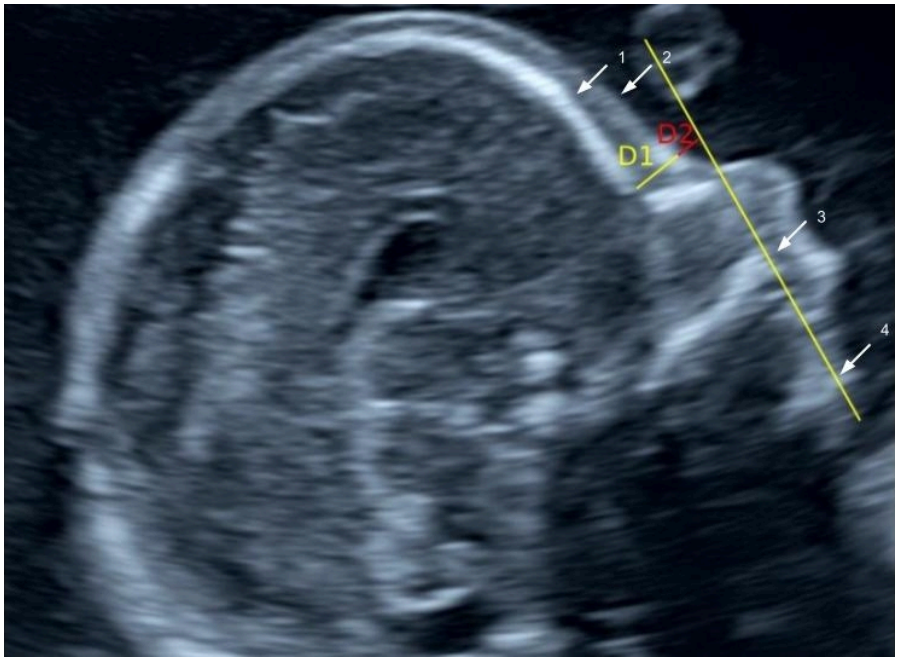

Figure 16: Prefrontal Space Ratio in a Normal Fetus (1.edge of the frontal skull, 2.the skin covering the forehead 3.anterior edge of the maxilla 4.anterior edge of the mandible,)

We compared the values in the normal group to those of fetuses with spina bifida (fig. 17)

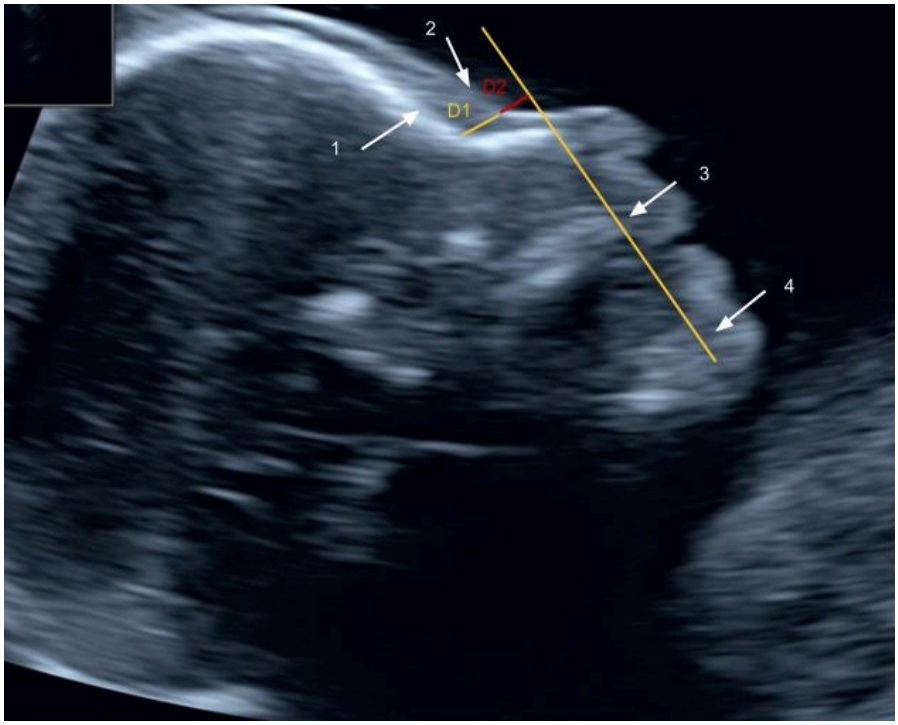

Figure 17: Prefrontal Space Ratio in a Fetus with Spina bifida (1.edge of the frontal skull, 2.skin over the forehead 3.anterior edge of the maxilla 4.anterior edge of the mandible,)

2.2 Frontomaxillary Facial Angle

We then measured the frontomaxillary facial angle (FMF Angle) defined by (Sonek et al. 2007a) (Lachmann et al. 2010) as the angle between the upper surface of the palate and the frontal bone in a midsagittal view of the fetal face, both in unaffected fetuses (fig. 18), as well as in fetuses with spina bifida (fig. 19).

For the purpose of this thesis we derived the normal range for the FMF Angle by assessing the same control group consisting of 276 patients which analysed for the PFSR measurements (Yazdi et al. 2013). Regression analysis was used to search for significant covariates and the normal range for gestational age groups was computed.

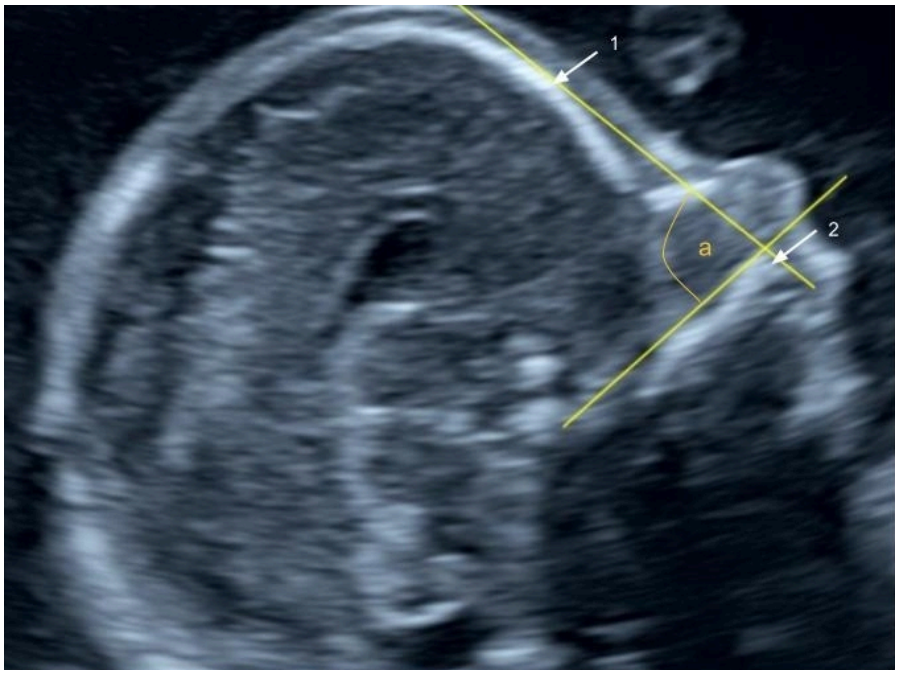

Figure 18: Frontomaxillary Facial Angle in a Normal Fetus (1.edge of the frontal skull, 2.anterior edge of the maxilla, a= FMF angle)

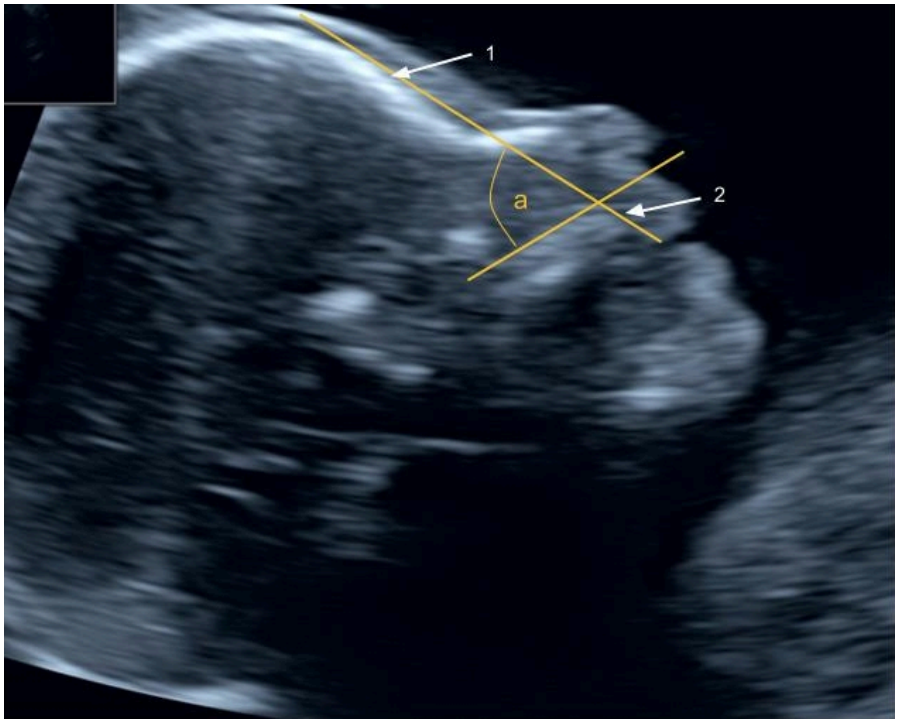

Figure 19: Frontomaxillary Facial Angle in a Fetus with Spina bifida (1.edge of the frontal skull, 2.anterior edge of the maxilla, a=FMF angle)

2.3 Prefrontal Space

Thirdly we measured the prefrontal space (PFS) defined as the distance between the mandibulomaxillary line and the most prominent edge of the frontal skull, as in fig. 20 in the normal group, and also in the affected grous (fig. 21). The normal range for PFS was also computed using measurements performed on the images from the control group. Regression analysis was used to search for significant covariates.

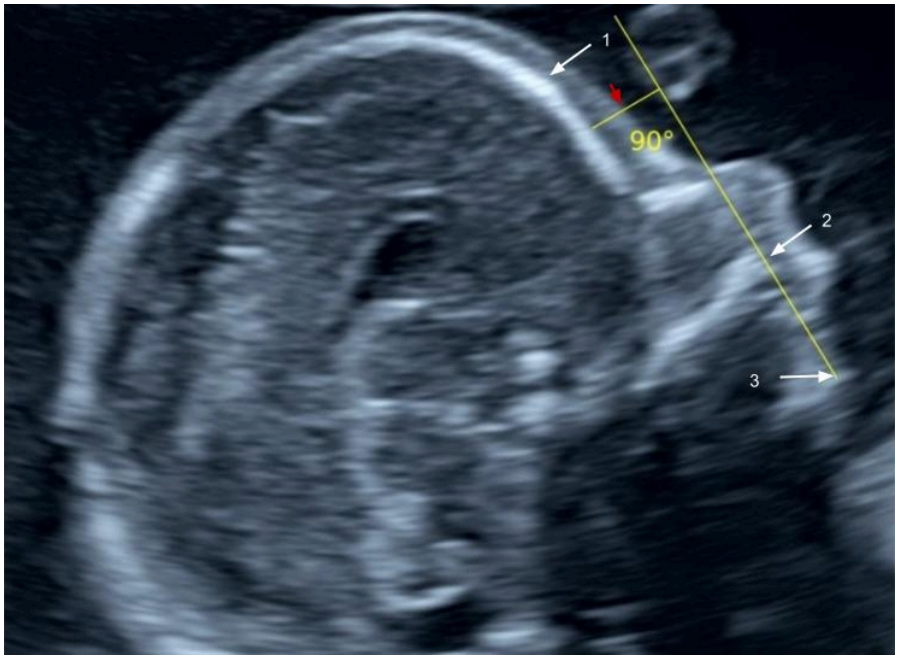

Figure 20: Prefrontal space in a Normal Fetus 1.edge of the frontal skull, 2.anterior edge of the maxilla, 3.anterior edge of the mandible, the red arrow points at the PFS

2.4 Distance Between Mandibulomaxillary Axis and Corpus Callosum

The fourth parameter we analysed was the distance between the mandibulomaxillary axis and the genu of corpus callosum (CCM), both for controls (fig. 22) and for affected fetuses (fig.23). As for previous parameters, we computed the normal range using measurements based on the images of our control group.

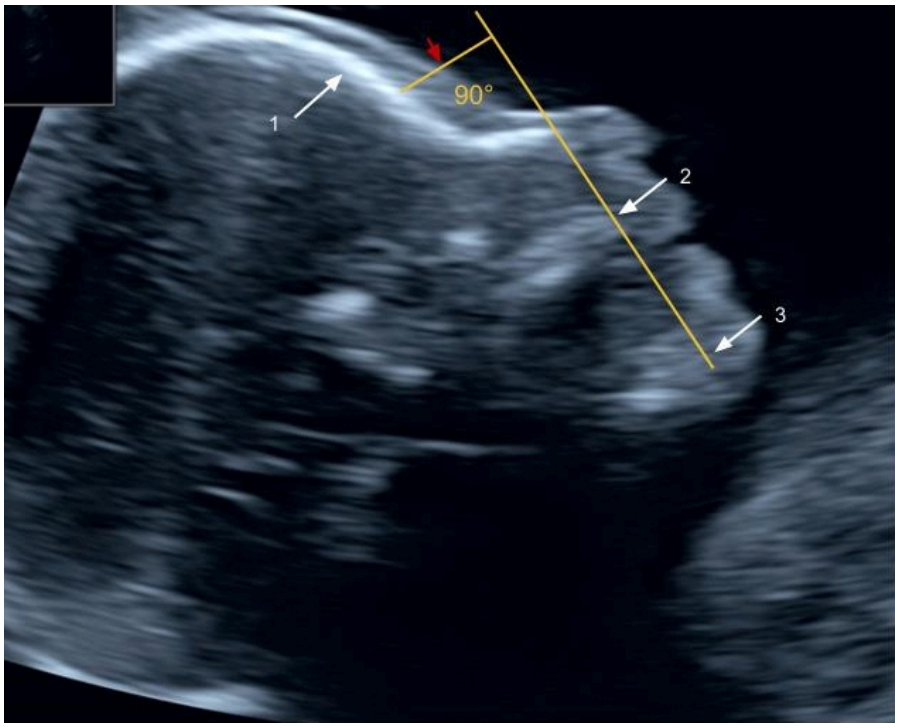

Figure 21: Prefrontal space in a Fetus with Spina bifida 1.edge of the frontal skull, 2.anterior edge of the maxilla, 3.anterior edge of the mandible, the red arrow points at the PFS

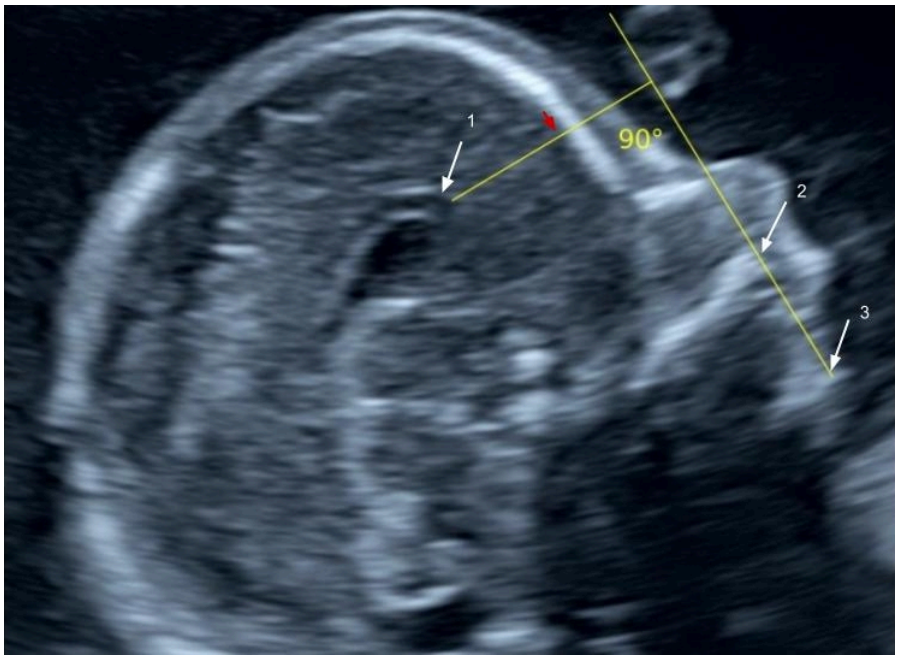

Figure 22: Distance Between Mandibulomaxillary Axis and Corpus Callosum in a Normal Fetus 1.genu of corpus callosum, 2.anterior edge of the maxilla 3.anterior edge of the mandible, the red arrow points at the PFS

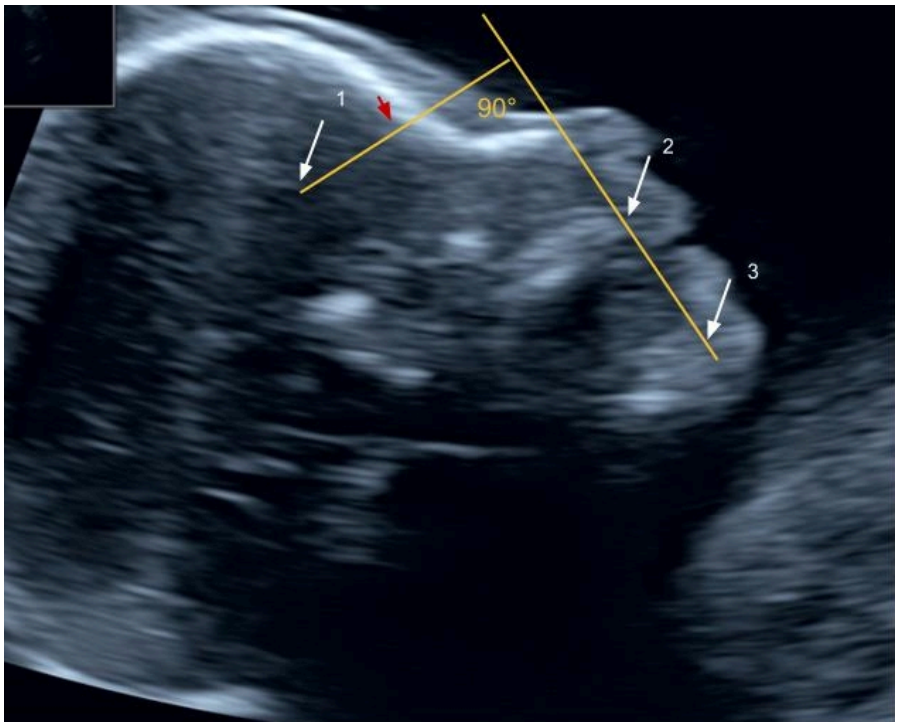

Figure 23: Distance Between Mandibulomaxillary Axis and Corpus Callosum in an Affected Fetus 1.genu of corpus callosum, 2.anterior edge of the maxilla 3.anterior edge of the mandible, the red arrow points at the PFS

2.5 Mandibulo-Naso-Frontal Axis

We also assessed the mandibulo-naso-frontal axis (MNF), defined as the straight line between the anterior edge of the mandible and the point corresponding to the fronto-nasal suture, as seen in fig. 24, and ascertained whether the axis falls before (defined as negative) or behind (defined as positive) the leading edge of the skull. We compared the results with those obtained in fetuses with spina bifida (fig.25)

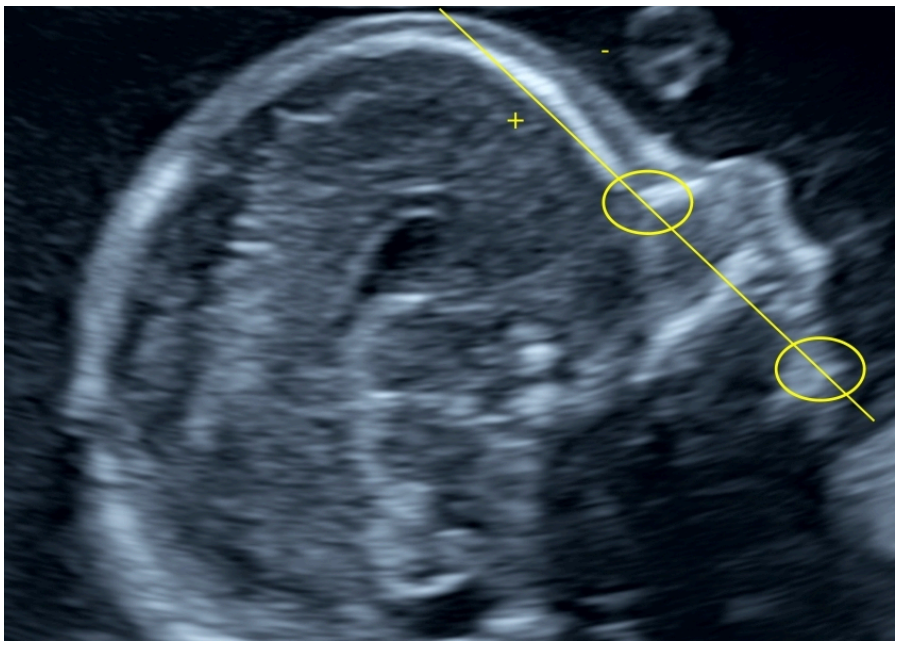

Figure 24: Mandibulo-Naso-Frontal Axis in a Normal Fetus 1.fronto-nasal suture 2.anterior edge of the mandible

The underlying cause for the alteration of fetal profile which leads to a change in the measurements of the above mentioned parameters is the caudal displacement of the midbrain and subsequent collapse of the posterior fossa. This anatomical abnormality determines a reduction in FMF angle as demonstrated in the first trimester by Lachmann et al. (Lachmann et al. 2010). We expect therefore to see changes in several parameters related to the fetal profile in the second and third trimester as well.

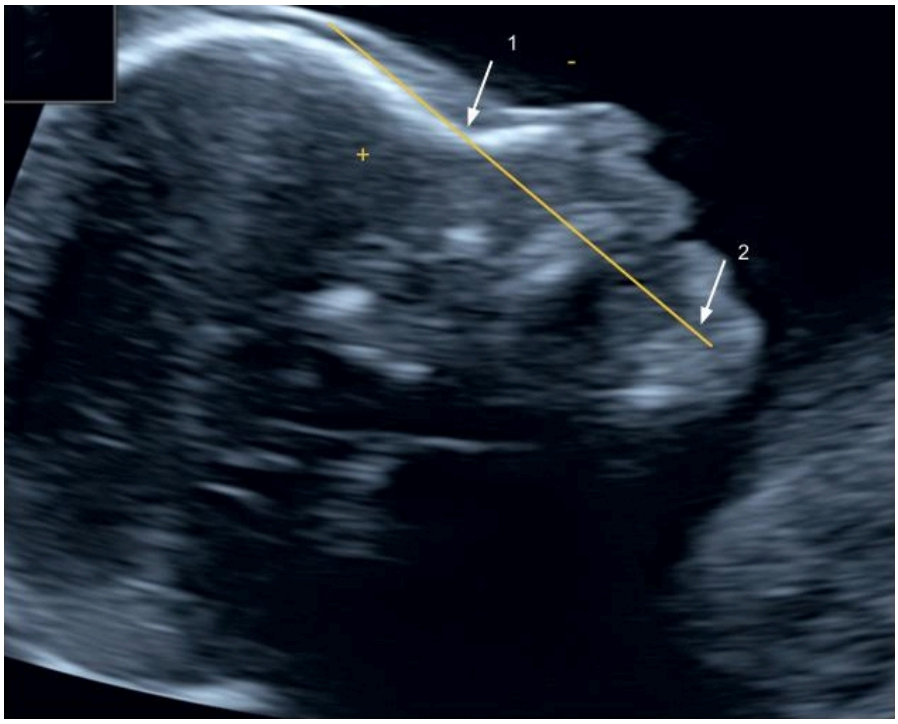

Figure 25: Mandibulo-Naso-Frontal Axis in a Fetus with Spina bifida 1.fronto-nasal suture 2.anterior edge of the mandible

3 Results

3.1 Group Characteristics

Eighty fetuses with open spinal defect who had stored images of fetal profile that met the quality criteria mentioned above were identified in our database. Nine of them were excluded due to additional chromosomal abnormalities or other facial defects. Seventy one fetuses were thus included for further analysis. Fifteen fetuses had a cervical or thoracic defect, twenty seven fetuses had a lumbar defect and twenty nine a sacral spina bifida.

The control group comprised 279 normal fetuses. To allow for a more accurate comparison and to avoid potential biases introduced by the normal development of the fetal face, we chose to match the control cases with the affected cases for gestational age in the second trimester as follows: 8 cases of spina bifida in the early second trimester were matched with 21 cases of unaffected fetuses between 14+1 and 17+6 pregnancy weeks and 187 fetuses in the late second trimester between 18+0 and 26+6 pregnancy weeks were chosen as match for the 47 affected cases with a comparable gestational age.

The maternal characteristics are detailed in table 3. The median gestational age in the normal and the affected groups was 21.5 and 21.7 weeks, respectively. Maternal age was similar in both groups, as was the BMI.

Table 3: Maternal Characteristics

	Normal n=279	Spina bifida n=71
Maternal age years median (IQR)	30.6 (27.2-34.7)	31.8 (27.8-34.9)
Gestational age in weeks median (IQR)	21.5 (20.4-25.7)	21.7 (20.1 – 25.1)
Second trimester examination n(%)	212 (75.9)	56 (80.0)
Third trimester examination n(%)	67 (24.0)	15 (20.0)
BMI kg/m^2 median (IQR)	25.1 (22.0 – 27.5)	26.1 (22.9 – 29.3)

The similitude of the two groups in terms of maternal characteristics are evident in figures 26 to 28.

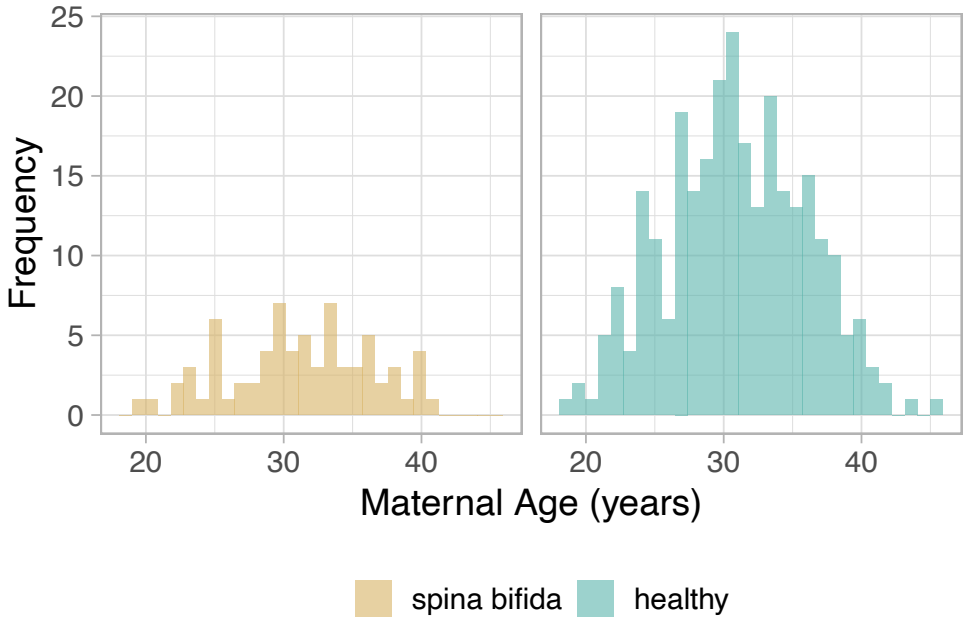

Figure 26: Distribution of Maternal Age (years) for Affected and Unaffected Fetuses

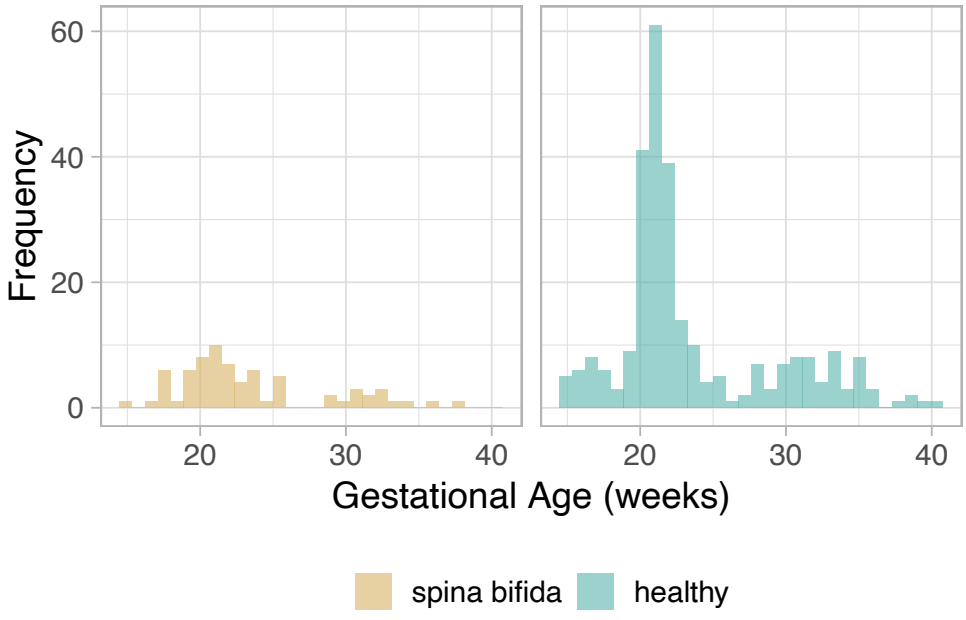

Figure 27: Distribution of Gestational Age (weeks) for Affected and Unaffected Fetuses

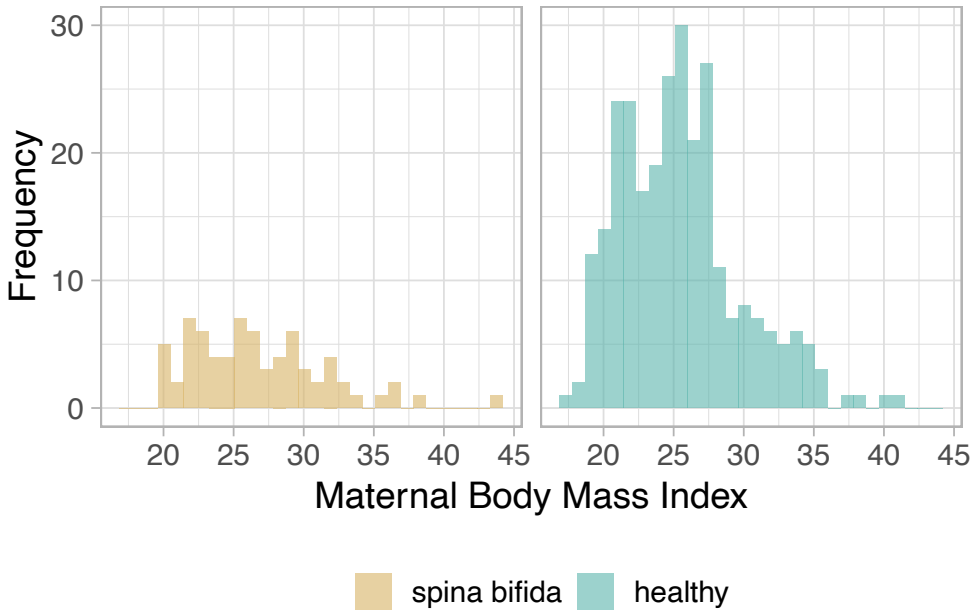

Figure 28: Distribution of Maternal Body Mass Index for Affected and Unaffected Fetuses

3.2 Parameters and Analysis

The measurements for the spina bifida cases were performed by two experienced operators as detailed in the Material and Methods Section.

The inter-observer variability was assessed with a paired t-test for the PFSR, FMF angle, CCM and PFS. No significant difference was observed for the first three parameters, however the inter-observer variability for the last parameter was unsatisfactory. This is detailed in table 4 and fig. 29 to 32.

Table 4: Inter-Observer Variability (Paired T-Tests)

	p value	confidence interval
PFSR	0.85	(-0.26, 0.31)
FMF Angle	0.33	(-0.42, 1.23)
PFS	0.01	(0.07, 0.65)
CCM	0.20	(-0.88, 0.20)

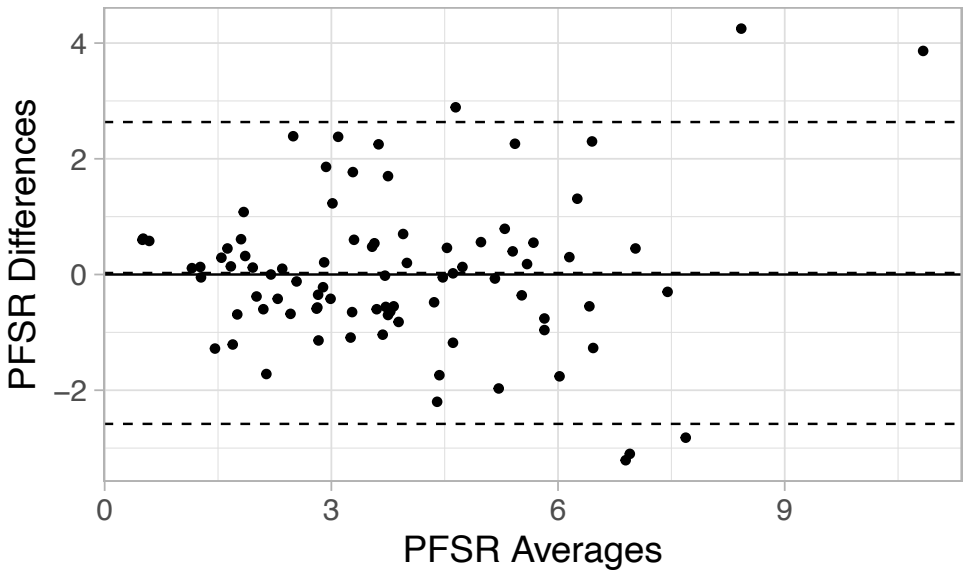

Figure 29: Inter-observer Variability of Prefrontal Space Ratio

Regarding the MNF, the inter-observer variability was evaluated by a Mc-

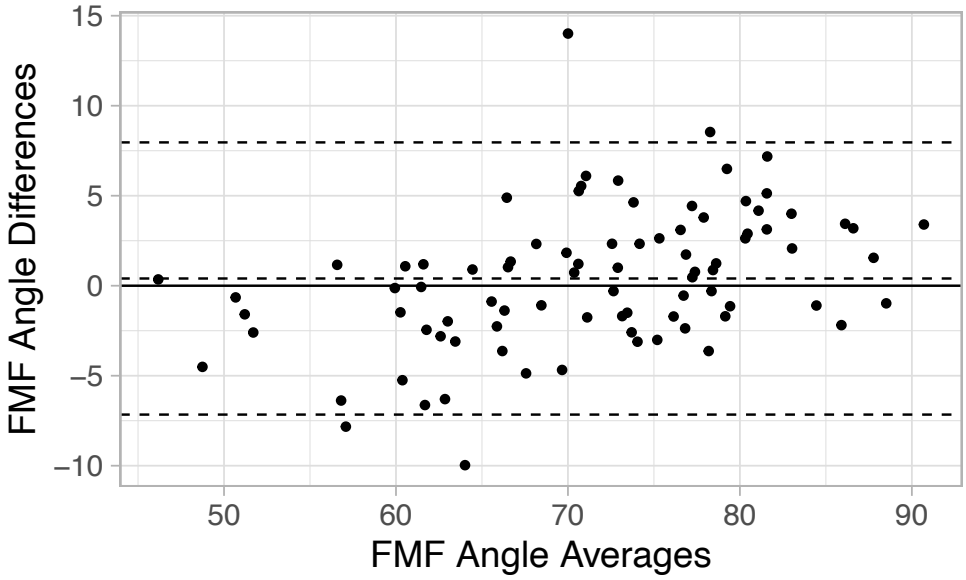

Figure 30: Inter-observer Variability of FMF Angle

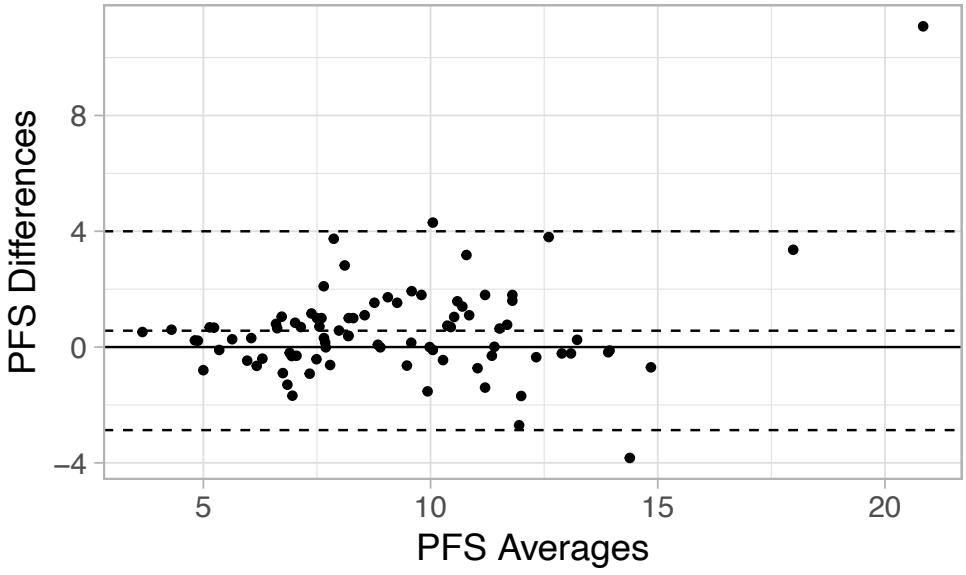

Figure 31: Inter-observer Variability of Prefrontal Space

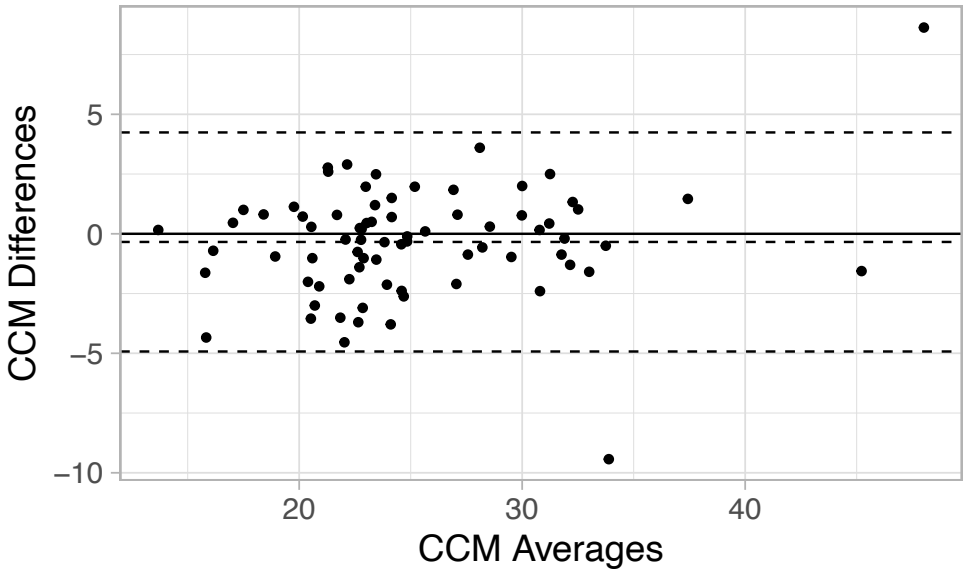

Figure 32: Inter-observer Variability of Corpus callosum - Mandibulomaxillary Axis

Nemar test and the difference was not significant (p-value = 0.1306).

For the numerical parameters PFSR, FMF angle, PFS and CCM the results of both groups were shown as median and interquartile range (IQR) and were compared with a Wilcoxon–Mann–Whitney two-sample rank-sum test. A p-value of < 0.05 was set as significance threshold. For the categorical variable MNF a two-sample test for comparison of proportions was used.

We assessed the correlation of each parameter with gestational age, as the incidence of spina bifida is expected to decrease in later pregnancy due to elective termination of pregnancy or intrauterine death, but at the same time the diagnosis might be more difficult, due to inadequate fetal lie. We also evaluated the relation with the head measurement, as it is known that the fetuses with spina bifida have smaller measurements compared to controls, but also that the measurements change with gestational age. We used the biparietal-head measurement as a substitute for the size of the fetal head.

3.3 Analysis of the Prefrontal Space Ratio

The median PFSR value in the normal group was 0.95 and the IQR 0.34. The median D1 and D2 distances and the total D distance in the normal group were 4.30, 4.30 and 8.60 mm respectively. In the affected group the median PFSR value was 0.85 and the IQR 0.51. The distributions of the two groups did not differ significantly (Mann-Whitney U = 11216, n1 = 71, n2 = 279, p-value = 0.06 two-tailed) (Prodan et al. 2020).

The PFSR was independent of gestational age, as seen in fig. 33 (correlation coefficient 0.005).

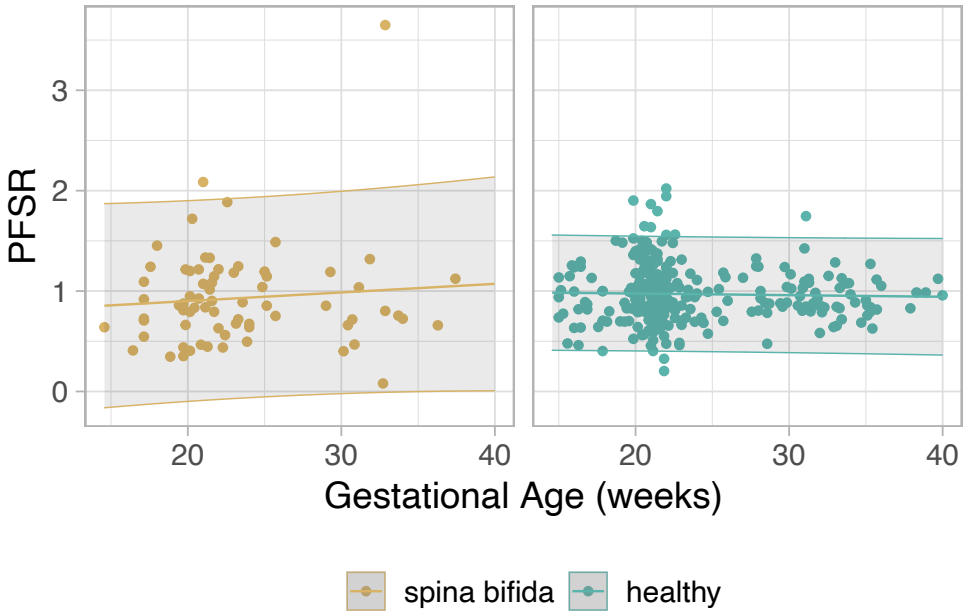

Figure 33: Association of PFSR with Gestational Age in Healthy and Affected Patients (mean, 5th, 95th centile)

However, as expected, D1, D2, and total D distance increased with advancing gestational age (PFSR p= 0.632, D1= -0.275 + 0.205 x gestational age, p<0.0001, r=0.866; D2= -0.079 + 0.188 x gestational age, p<0.0001, r=0.649; total D = -0.275 + 0.205 x gestational age, p<0.0001, r=0.866). Based on these normal ranges, the median MoM value for the D1, D2, and the total D measurements were 0.99, 0.97 and 0.99, respectively.

The relation of PFSR to the head measurements was also evaluated in both groups and, again as expected, showed no correlation (fig. 34), with a

correlation coefficient of 0.03.

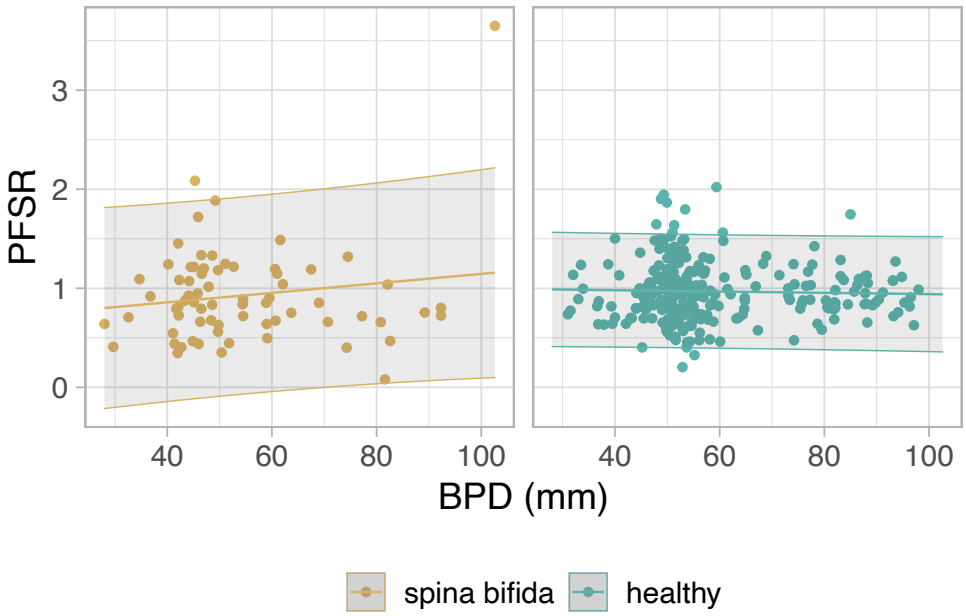

Figure 34: Association of PFSR with BPD in Healthy and Affected Patients (mean, 5th, 95th centile)

The gestational age-adjusted PFSR values were similar in the normal and the spina bifida cohorts, as seen in fig. 35. The D1, D2 and total D measurements were also similar in the two cohorts.

In order to assess the usefulness of predicting spina bifida using PFSR, we used the following logistic regression model:

$$\log\left(\frac{\pi}{1-\pi}\right) = \text{intercept} + \beta_{PFRS}\text{PFSR} \tag{1}$$

where π is the probability of the outcome being spina bifida, given the measurement of PFSR.

The logistic regression model for PFSR indicates that the parameter alone is not useful in screening for spina bifida, as seen in table 5 and fig.36

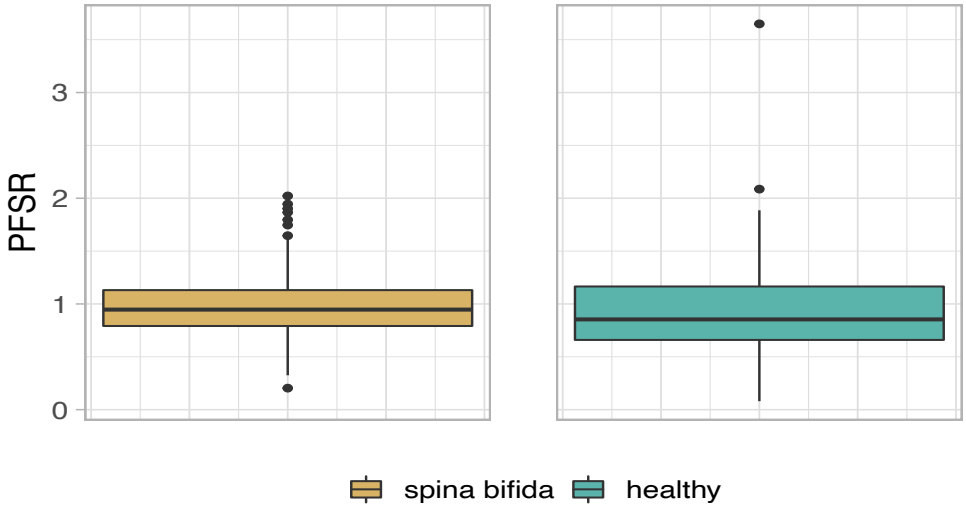

Figure 35: Comparison of PFSR Measurements in Healthy versus Affected Patients

Table 5: Logistic Regression Model Coefficients for PFSR

	estimate	std.error	statistic	p.value	2.5 %	97.5 %
(Intercept)	0.85	0.43	1.95	0.05	-0.02	1.68
β_{PFSR}	0.45	0.45	1.02	0.31	-0.37	1.37

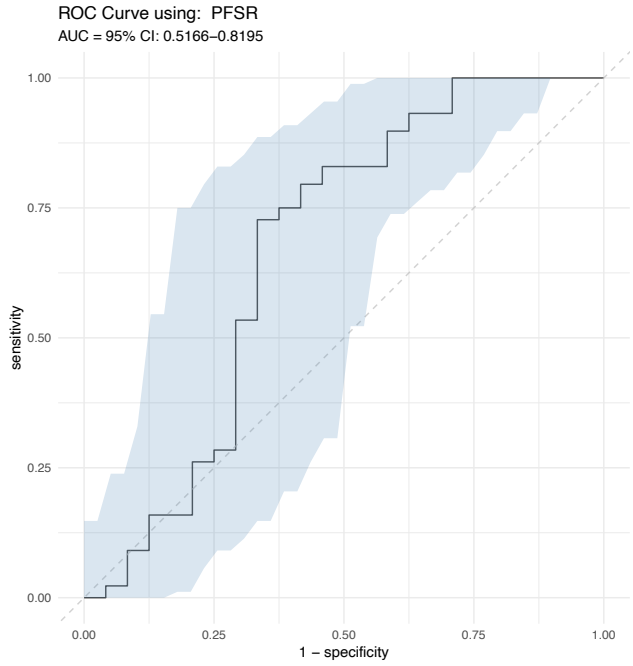

Figure 36: ROC Curve with 95% Confidence Interval for the Area under the Curve for PFSR

3.4 Analysis of the Frontomaxillary Angle

The median FMF frontomaxillary angle was 79.6° in the normal group and 72.9° in the spina bifida group.

Both in the normal and affected group the angle was independent of gestational age (p - value =0.098 for the normal group and p=0.265 for affected group), as seen in fig. 37.

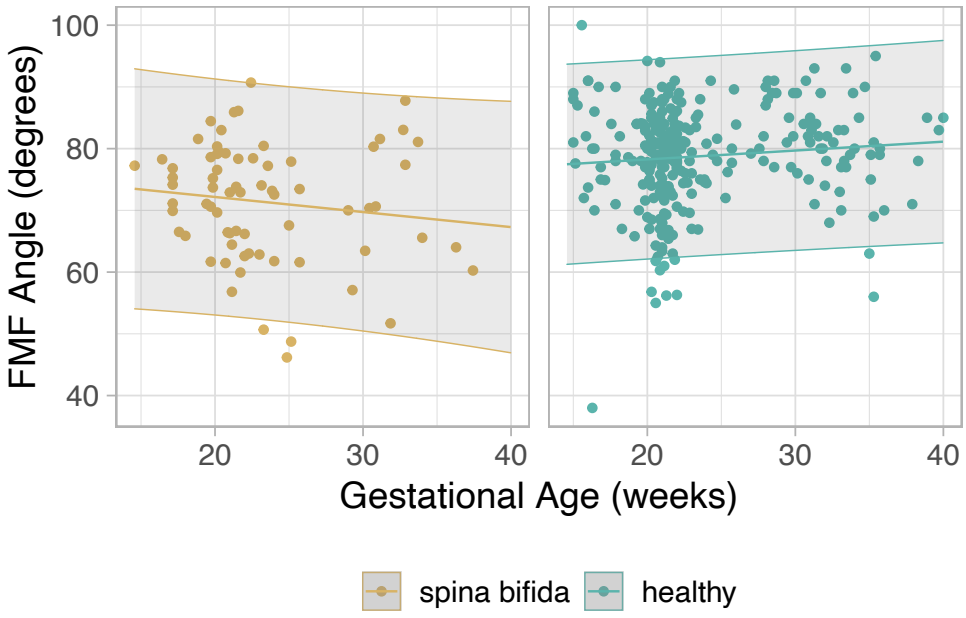

Figure 37: Association of FMF Angle with Gestational Age in Healthy and Affected Patients (mean, 5th, 95th centile)

There was no relation between the measurements and the biparietal measurement of the fetal head in either group, as seen in fig. 38.

The angle was also not affected by the size of the defect (p=0.340) and by its level (using sacral defect as reference: lumbar defect p=0.367, thoracic and cervical defect p=0.288).

In 16 (22.5%) of the affected cases, the measurement was at or below the 5th centile of the normal population.

The FMF angle was significantly smaller in the spina bifida than in the normal population (Mann-Whitney U = 14438, n1 = 71, n2 = 279, p-value<0.0001, two-tailed), as seen in fig. 39 (Prodan et al. 2020).

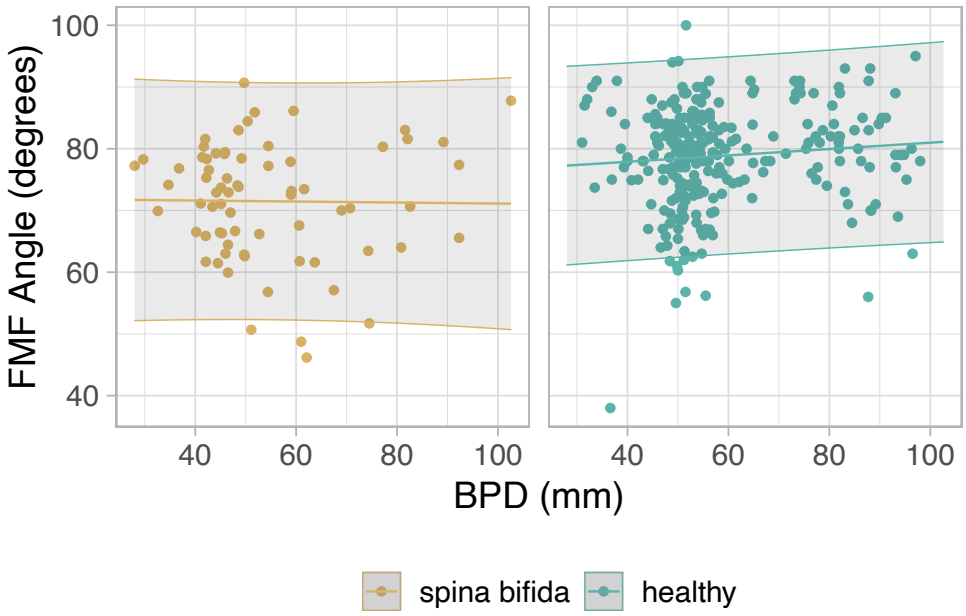

Figure 38: Association of FMF Angle with BPD in Healthy and Affected Patients (mean, 5th, 95th centile)

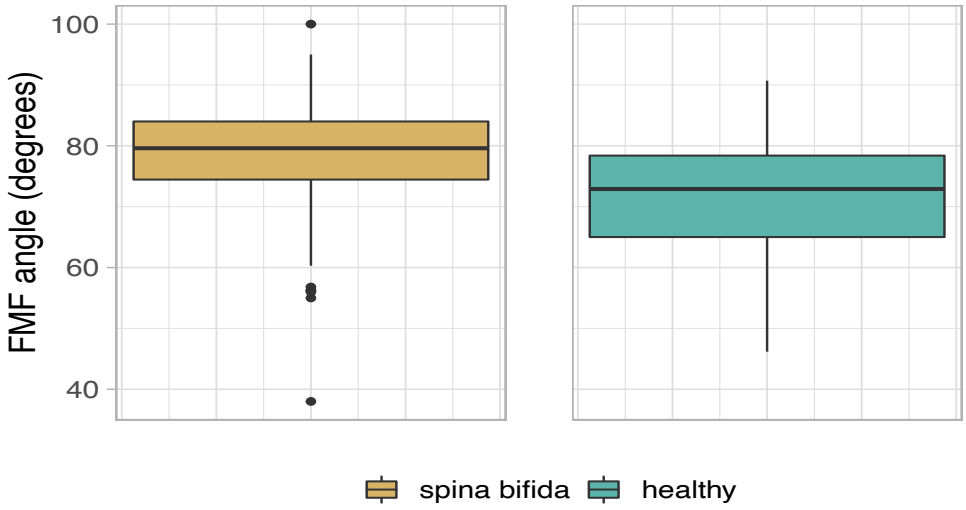

Figure 39: Comparison of FMF Angle Measurements in Healthy versus Affected Patients

The following logistic regression model was used to asses the performance of FMF angle in predicting spina bifida :

$$\log\left(\frac{\pi}{1-\pi}\right) = \text{intercept} + \beta_{FMFangle}\text{FMFangle} \tag{2}$$

where π is the probability of the outcome being spina bifida, given the measurement of FMF angle.

The FMF angle appears to be useful in screening for spina bifida based on the logistic regression model, as seen in table 6 and fig.51.

Table 6: Logistic Regression Model Coefficients for FMF Angle

	estimate	std.error	statistic	p.value	2.5 %	97.5 %
(Intercept)	-5.12	1.35	-3.80	0.00	-7.85	-2.55
$\beta_{FMFAngle}$	0.08	0.02	4.69	0.00	0.05	0.12

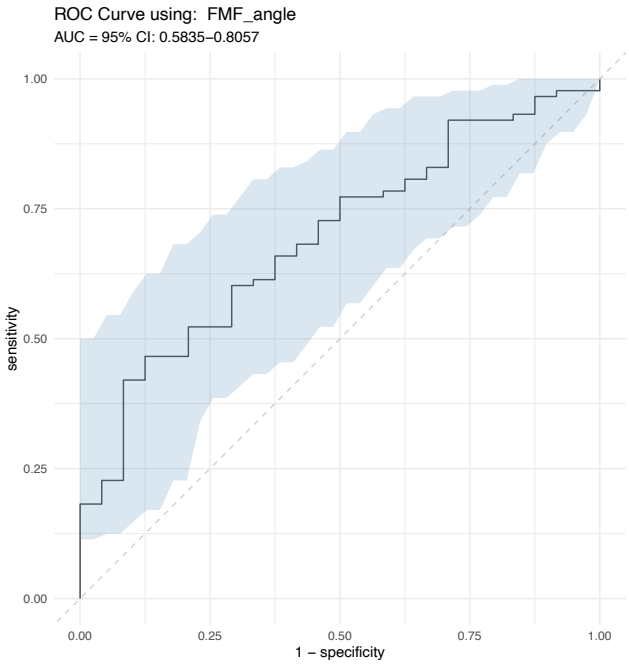

Figure 40: ROC Curve with 95% Confidence Interval for the Area under the Curve for FMF Angle

3.5 Analysis of the Prefrontal Space

In the normal group the median PFS was 9.9 mm and IQR 3.16. In the affected group the median PFS value was 8.9 mm and the IQR was 3.682.

As expected, the PFS values were strongly correlated with the gestational age, both for the spina bifida ($r = 0.744$, $p < 0.001$) and the control group ($r = 0.672$, p - value < 0.001), as seen in fig. 41.

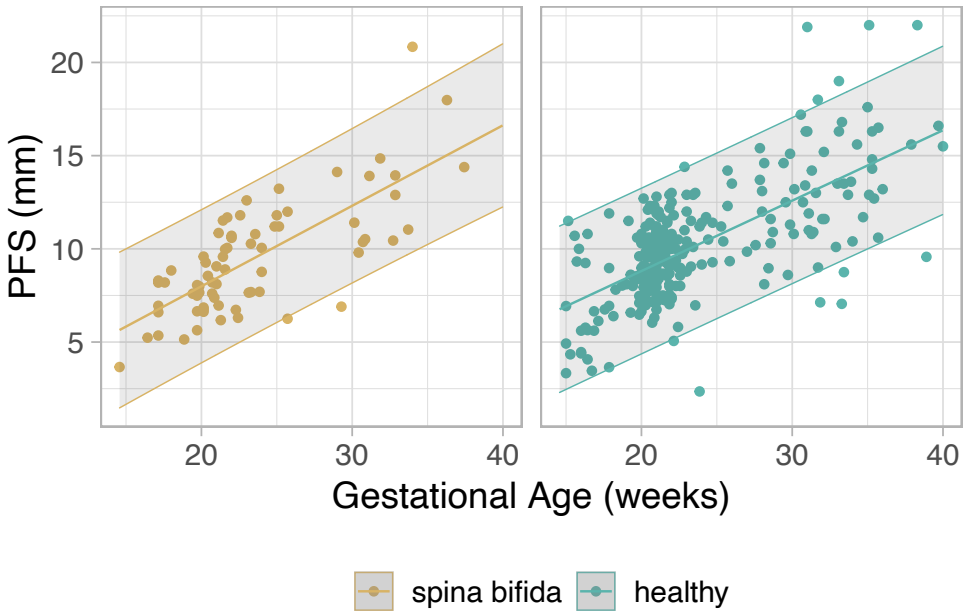

Figure 41: Association of PFS with Gestational Age in Healthy and Affected Patients (mean, 5th, 95th centile)

There was also a strong correlation of PFS measurement with the biparietal measurement, both for the control group ($r=0.73$, $p<0.001$) and for the affected group ($r=0.707$, $p <0.001$), as illustrated in fig. 42.

The distribution of the two groups for PFS differed marginally (Mann-Whitney $U = 11311$, $n1 = 71$, $n2 = 279$, p-value = 0.04 two-tailed). A comparison between the two distributions is seen in fig. 35 and it suggests a slight difference between the mean values. However, as it was demonstrated earlier, the PFS has a relatively high inter-observer variability. As such, although the difference was shown to have a statistical significance, this is possibly a spurious effect.

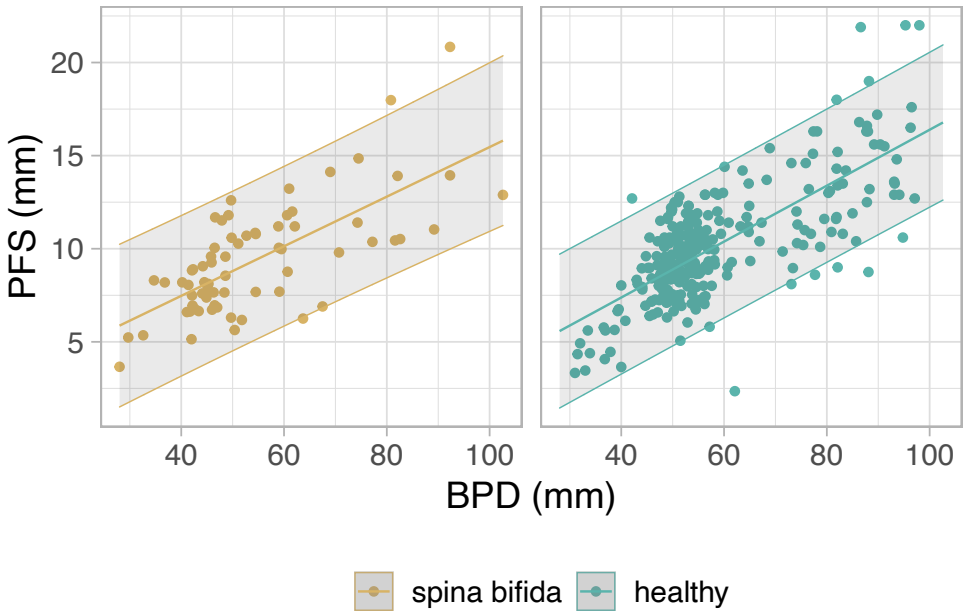

Figure 42: Association of PFS with BPD in Healthy and Affected Patients (mean, 5th, 95th centile)

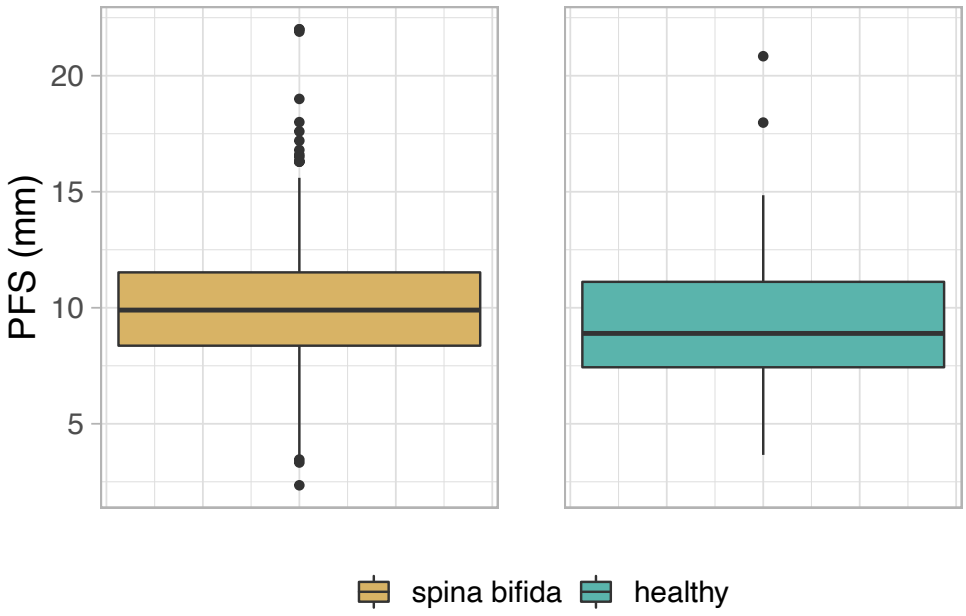

Figure 43: Relation of PFS Measurements in Healthy versus Affected Patients

The following logistic regression model was used to estimate the value of PFS in predicting spina bifida:

$$\log\left(\frac{\pi}{1-\pi}\right) = \text{intercept} + \beta_{PFR}\text{PFS} \tag{3}$$

where π is the probability of the outcome being spina bifida, given the measurement of PFS.

The logistic regression model for PFS indicates that this parameter alone is also not useful in screening for spina bifida, as seen in table 7 and fig.44

Table 7: Logistic Regression Model Coefficients for PFS

	estimate	std.error	statistic	p.value	2.5 %	97.5 %
(Intercept)	0.38	0.58	0.65	0.51	-0.76	1.52
β_{PFS}	0.10	0.06	1.57	0.12	-0.02	0.22

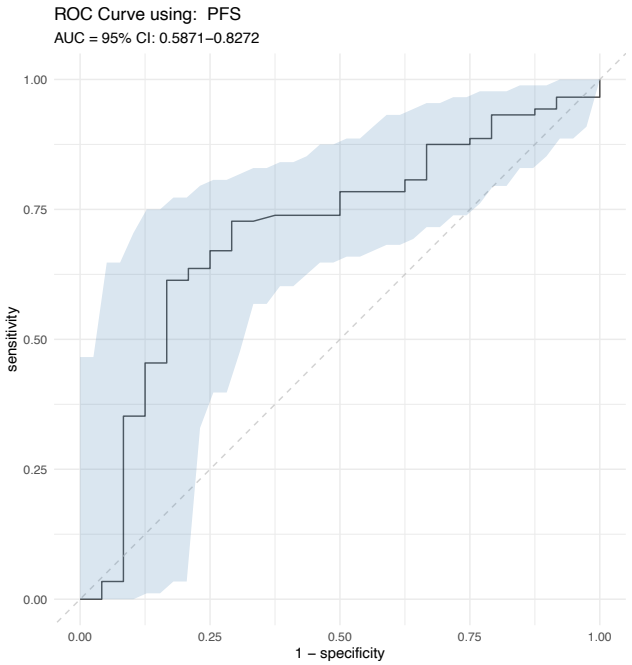

Figure 44: ROC Curve with 95% Confidence Interval for the Area under the Curve for PFS

3.6 Analysis of the Distance Between Mandibulomaxillary Axis and Corpus Callosum

The median CCM measurement was 25.85 mm and the IQR 5.275 for the control group. For the group with spina bifida the mean value was 23.42 mm and the IQR 7.541.

The CCM values were strongly correlated with the gestational age, both for the control group ($r = 0.717$, $p < 0.001$) and the control group ($r = 0.764$, p - value < 0.001), as seen in fig. 45.

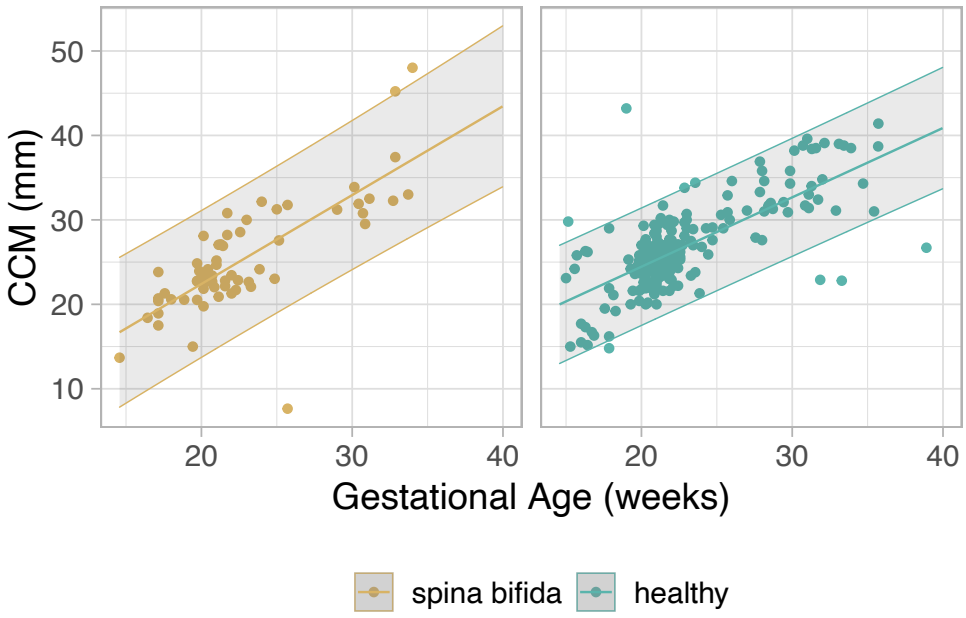

Figure 45: Association of CCM with Gestational Age in Healthy and Affected Patients (mean, 5th, 95th centile)

Moreover, as for the PFS, there was a strong correlation of CCM to the head measurements (in the control group $r=0.809$, $p < 0.001$ and in the affected group $r=0.769$, $p < 0.001$), as seen in fig. 46.

We observed a statistically significant difference between the distribution of the two groups (Mann-Whitney $U = 8310.5$, $n1 = 71$, $n2 = 279$, p-value = 0.0125 two-tailed). This is illustrated in fig. 47.

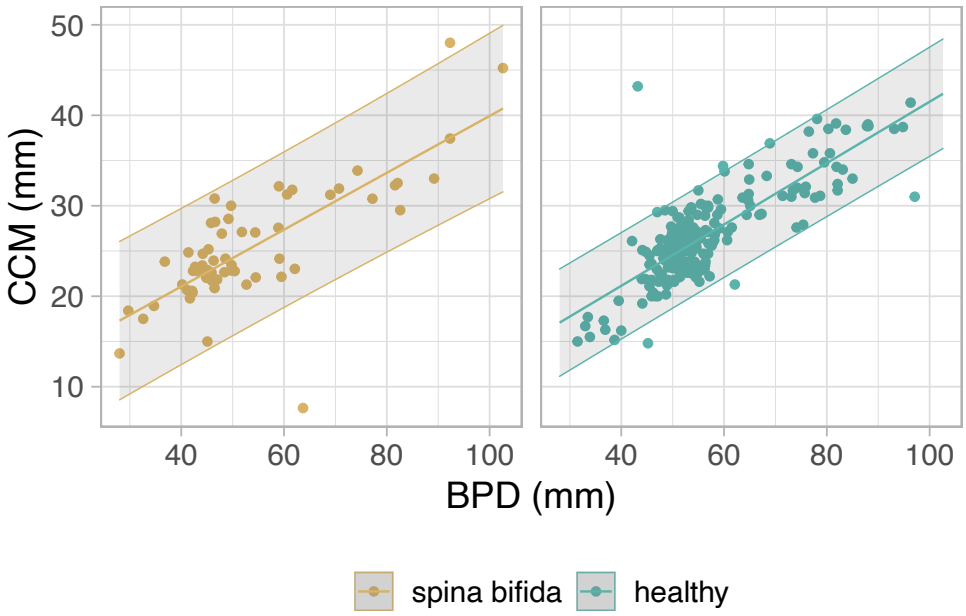

Figure 46: Association of CCM with Biparietal Diameter in Healthy and Affected Patients (mean, 5th, 95th centile)

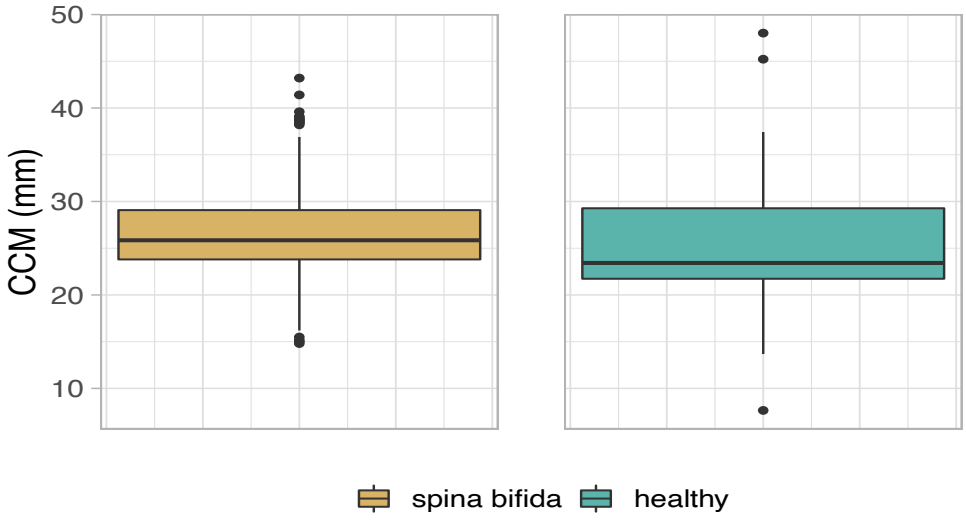

Figure 47: Relation of CCM Measurements in Healthy versus Affected Patients

To evaluate the significance of CCM in assessing the risk of spina bifida we also used the logistic regression model stated below:

$$\log\left(\frac{\pi}{1-\pi}\right) = \text{intercept} + \beta_{CCM}\text{CCM} \tag{4}$$

where π is the probability of the outcome being spina bifida, given the measurement of CCM.

This logistic regression model shows that CCM alone is also not useful in screening for spina bifida, as seen in table 8 and fig.48

Table 8: Logistic Regression Model Coefficients for CCM

	estimate	std.error	statistic	p.value	2.5 %	97.5 %
(Intercept)	-0.00	0.74	-0.00	1.00	-1.47	1.43
β_{CCM}	0.05	0.03	1.74	0.08	-0.01	0.11

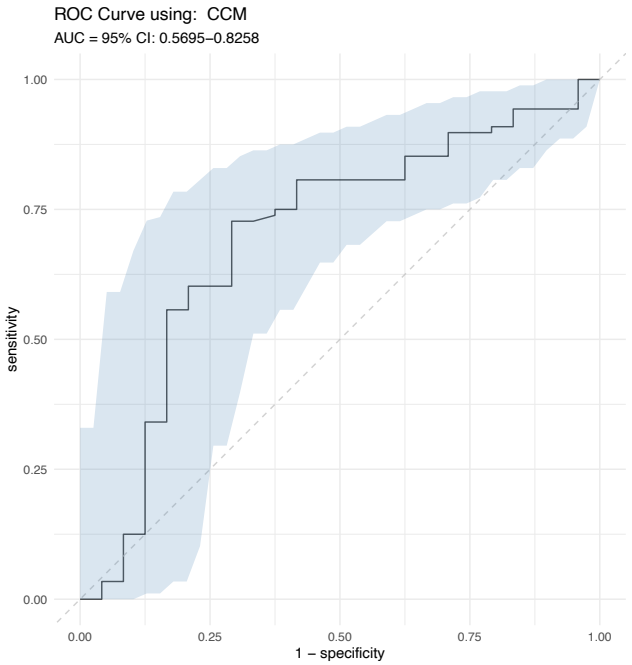

Figure 48: ROC Curve with 95% Confidence Interval for the Area under the Curve for CCM

3.7 Analysis of the Mandibulo-Naso-Frontal Axis

A two-sample test for equality of proportions with continuity correction indicates that the proportion of positive axis (defined as falling behind the leading edge of the skull) was approximately 20% in both groups. There was no significant difference (p-value > 0.05) either, as it easily noticeable in fig. 49.

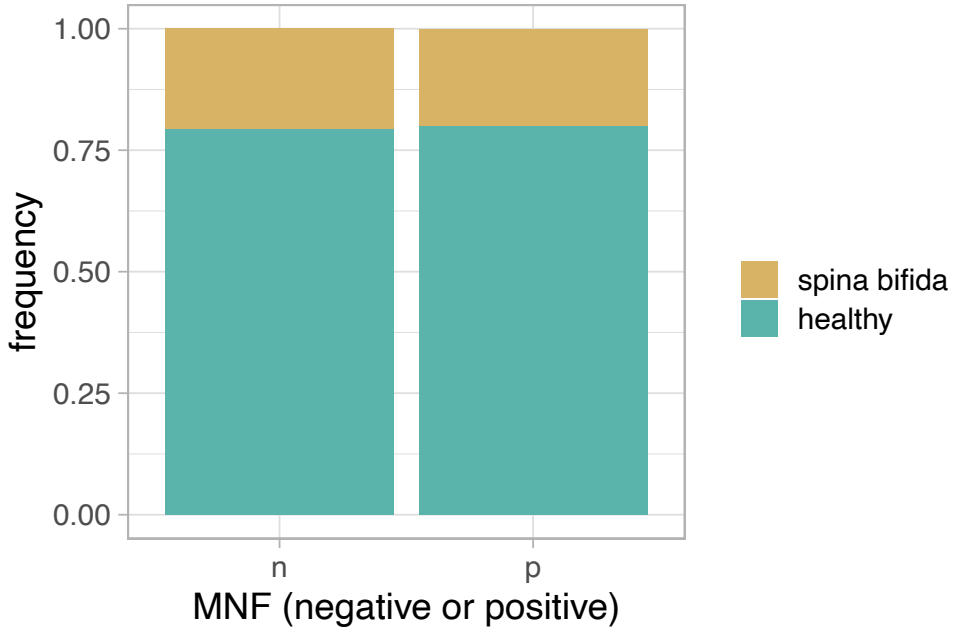

Figure 49: Relation of MNF Values in Healthy versus Affected Patients n=negative, p=positive

The following logistic regression model was used to determine the usefulness of MNF in evaluating the risk of spina bifida. MNF was therefore encoded as a binary variable, with zero for negative and 1 for positive:

$$\log\left(\frac{\pi}{1-\pi}\right) = \text{intercept} + \beta_{MNF}\text{MNF} \tag{5}$$

.

where π is the probability of the outcome being spina bifida, given the measurement of MNF.

Based on this logistic regression model, it can be said that MNF alone is not useful in screening for spina bifida, as shown in table 9 and fig.50.

Table 9: Logistic Regression Model Coefficients for MNF

	estimate	std.error	statistic	p.value	2.5 %	97.5 %
(Intercept)	1.28	0.20	6.40	0.00	0.90	1.69
β_{MNF}	-0.02	0.29	-0.06	0.95	-0.58	0.55

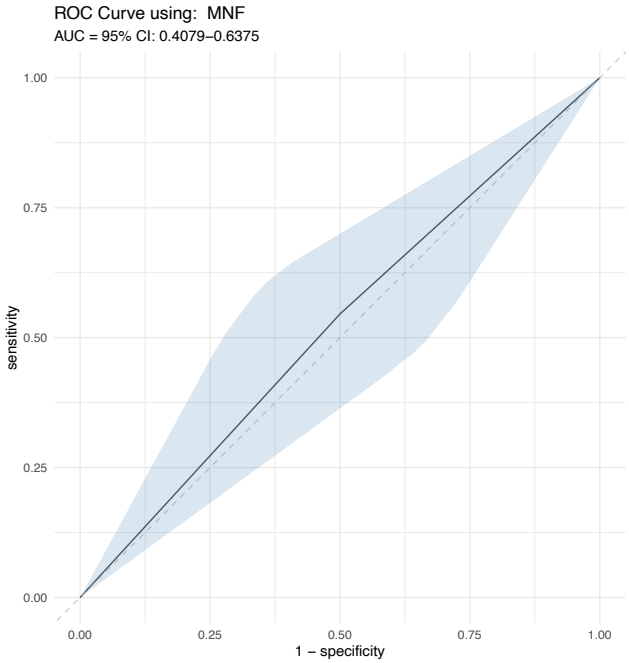

Figure 50: ROC Curve with 95% Confidence Interval for the Area under the Curve for MNF

3.8 Combined Prediction Models

The next step in our analysis was to apply a logistic regression model to a combination of parameters, as previously described. To identify the most predictive and, at the same time, most parsimonious model, we built a series of nested models using the sets of predictors shown in table 10.

Table 10: Nested Logistic Regression Models and their Sets of Predictors

Model Index	Predictors
1	FMF Angle
2	FMF Angle + PFSR
3	FMF Angle + PFSR + PFS
4	FMF Angle + PFSR + PFS + CCM + MNF

The models were compared using likelihood ratio tests, Akaike information criterion (AIC) and Bayesian information criterion (BIC). Both BIC and the likelihood ratio test suggest that the two-parameter model using the FMF angle is the most parsimonious, as the reduction in negative log-likelihood is not statistically significant (likelihood ratio tests) or offset by the complexity penalty (BIC), as seen in tables 11, 12, 13 and 14 and figures 52, 53 and 54.

Table 11: Detection Rates for Fixed False Positive Rates for the Combined Models

Model	DR at 10% FPR	DR at 20% FPR	DR at 30% FPR	DR at 40% FPR
1	32%	46%	54%	74%
2	30%	43%	52%	74%
3	28%	43%	51%	73%
4	27%	39%	45%	73%

DR=detection rate, FPR=false positive rate

Table 12: Area Under the ROC Curve and 95% Confidence Intervals for the Combined Models

Model	AUC	95% CI
1	0.67	0.55 - 0.79
2	0.66	0.54 - 0.78
3	0.65	0.53 - 0.78
4	0.66	0.53 - 0.78

AUC=area under the ROC curve , CI=confidence interval

Table 13: Model Comparison Using the Likelihood Ratio Test

Model	#Df	Log-Likelihood	Δ Df	chi square	p-value
1	2	-136.28			
2	3	-134.82	1	2.92	0.0876
3	4	-133.70	1	2.23	0.1351
4	6	-132.95	2	1.50	0.4719

Df=degrees of freedom

Table 14: Model Comparison Using Information Criteria

Models	#Df	Akaike information criterion	Bayesian information criterion
1	2	276.6	283.8
2	3	275.6	286.6
3	4	275.4	290.0
4	6	277.9	299.8

Df=degrees of freedom

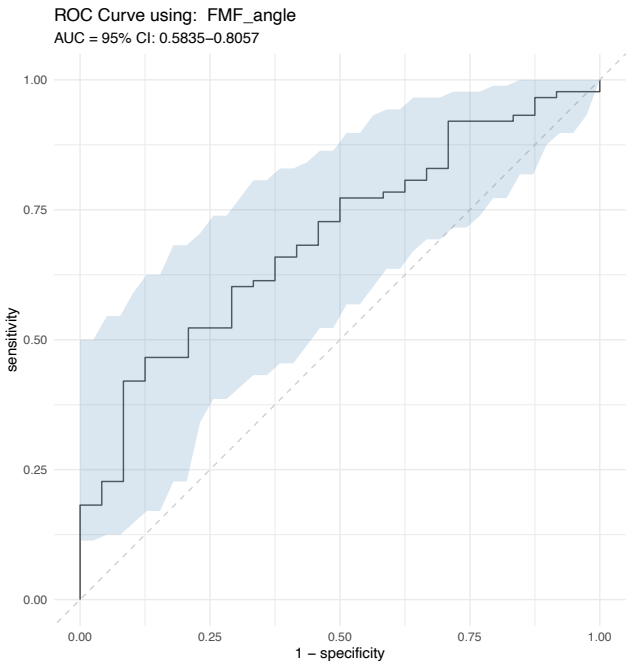

Figure 51: ROC Curve Analysis of Prediction Model 1

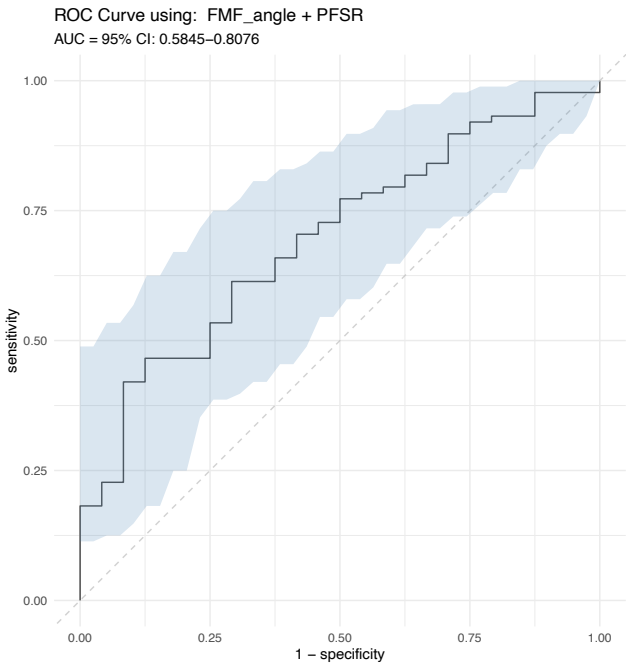

Figure 52: ROC Curve Analysis of Combined Prediction Model 2

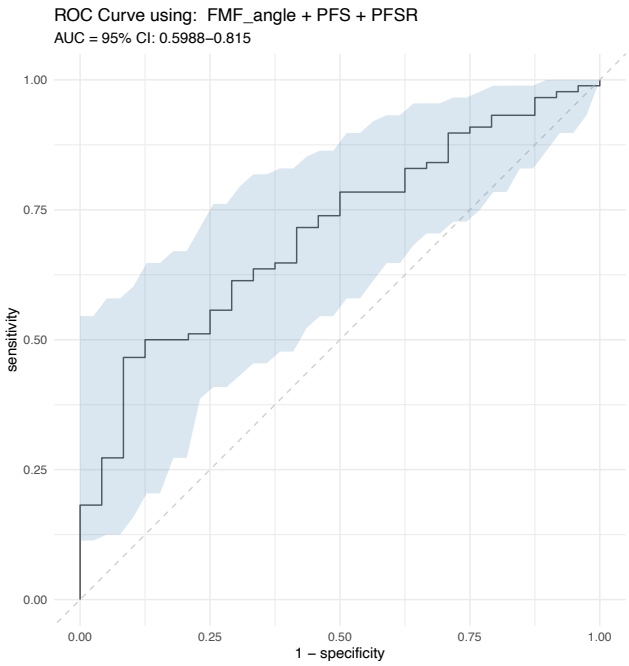

Figure 53: ROC Curve Analysis of Combined Prediction Model 3

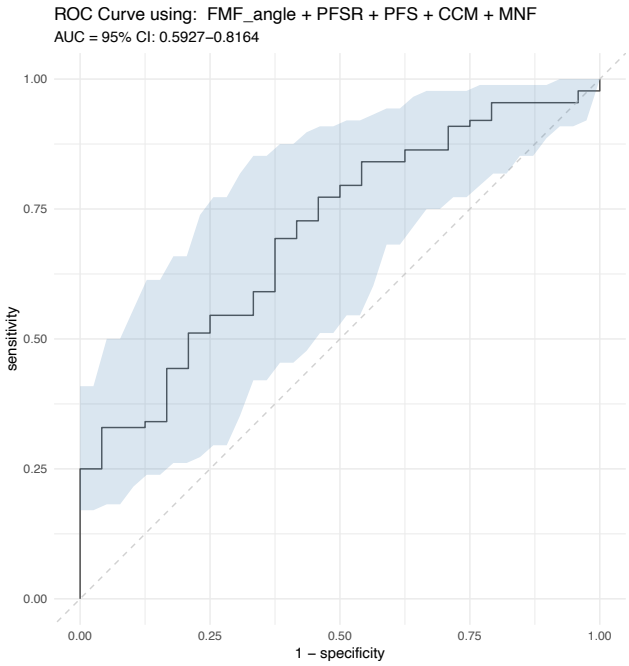

Figure 54: ROC Curve Analysis of Combined Prediction Model 4

4 Discussion

4.1 Diagnostic Challenges of Spina Bifida

The present research attempted to describe second and third trimester ultrasound parameters of fetal profile which might be used as screening tools and help improve the detection of spina bifida.

We focused on the detection of spina bifida because it is one of the most prevalent fetal non-lethal abnormalities compatible with long term survival. We believe that a timely diagnosis would be beneficial for the parents of affected fetuses in their decision making. Patients, their families and their caregivers would also be aided in the counselling, discussion of therapeutic options and planning of treatment by an early and appropriate diagnosis.

Although the diagnostic of spina bifida has improved much over the years, there are still situations where the diagnosis is made late in pregnancy or after birth. For instance an Italian study found that in a cohort analysed retrospectively over 10 years little over 80% of cases of spina bifida were diagnosed prenatally and only around 73% were diagnosed before 23 weeks of pregnancy (Ghi et al. 2015). The vast majority of the prenatally diagnosed cases were terminated.

Ultrasound is currently the method of choice in the prenatal diagnosis of spina bifida, as for many other fetal defects. This is a relatively inexpensive diagnostic method and is used by doctors, midwives or medical technicians on a large scale to assist in prenatal care. It is in the most part standardised and amenable to continuous training and improvement. Currently, the ultrasound diagnosis of spina bifida is based on the second and third trimester sonographical assessment. If the fetal position and technical conditions, including the BMI and abdominal scars, are favourable, the diagnosis of the defect can be made directly and the level of the defect can be assessed easily. Commonly, however, the suspicion of spina bifida relies on the indirect cranial and cerebral signs, such as microcephaly, ventriculomegaly, frontal scalloping (lemon sign), the collapse of the cisterna magna and a small and misshapen cerebellum (banana sign), which are presumably caused by the Arnold-Chiari complex typical for open spina bifida. Some of these markers were observed and characterised more than three decades ago (Nicolaides & Campbell 1989) (Van den Hof et al. 1990). Since then numerous studies demonstrated their efficacy in the prediction of spina bifida. Among them, a German study showed

that in the fetuses between 18+0 and 22+0 weeks of pregnancy and a normal karyotype, the lemon sign was detected by experienced sonographers in over 88% of the cases, the banana sign in approximately 97% and the collapse of the cistern magna in over 96% (Bahlmann et al. 2015). We can therefore conclude that these markers do improve the detection rate of open spina bifida in the second trimester in the hands of experienced sonographers, however they still do not enable a diagnosis in all cases.

As with most of prenatal diagnosis the focus has shifted from the second trimester to the first trimester of pregnancy. Ultrasound abnormalities caused by the same pathological processes pertaining to the Arnold-Chiari malformation are visible from the time of the nuchal scan. The collapse of posterior fossa, the so-called absence of intracranial translucency, is well defined and has a good diagnostic yield, as shown in the studies of Chaoui et al. (Chaoui et al. 2011). The fetal facial angle or the FMF angle was investigated as a potentially useful parameter as well and the study of Lachmann et al. indicated that the facial angle was about 10° smaller in fetuses with an open spine defect than in the normal population and that 90% of fetuses with spina bifida will have a measurements below the 5th centile of the reference range (Lachmann et al. 2010). Using all these ultrasound parameters the first trimester ultrasound detection of spina bifida improved over the years, as indicated by two extensive retrospective studies conducted in the United Kingdom. The first one (Syngelaki et al. 2011) was conducted between 2006 and 2009 and had a detection rate for spina bifida of 14%, whereas the second one between 2009 and 2018 showed a 2.5 fold increase to 35% detection rate (Syngelaki et al. 2019). It has to be mentioned however, that the first trimester diagnosis of spina bifida is challenging, time consuming and implies extensive training, therefore probably it cannot be implemented on a large scale as a screening method.

Thus, despite these advances, if we are to offer to all affected families the possibility of counselling and sufficient time for appropriate decision-making regarding the management, the prenatal diagnosis of spina bifida should be further improved.

4.2 Prevention of Spina bifida. Folic Acid

The influence of nutrient and vitamin deficiency on the risk for NTDs was long known and was observed in postwar populations, as well as in women

of lower socioeconomic status. In the 1980s several studies also established that a deficit in folic acid, either dietary in pregnant women or induced by folic acid antagonist in animal model studies, was strongly associated with the risk for NTDs (Milunsky et al. 1989).

The hypothesis that vitamin supplementation will reduce the likelihood of NTDs in the offspring was tested in thousands of women in prospective studies (Czeizel & Dudás 1992).

Three public health approaches regarding folic acid based prevention had been devised and later on tested: fortification of foods, dietary advice and supplementation (Mulinare & Erickson 1997).

In many countries throughout the world, with the exception of European countries, food fortification to improve dietary intake of folate is mandatory. After the practice was implemented on a large scale in the United States of America, the prevalence of the disease has declined by 19%, however other factors than food fortification might have had a role in this reduction (Honein et al. 2001). Research has shown however, that even after food fortification, the daily folic acid intake of pregnant women does not meet the requirements of regulatory bodies (Martiniak et al. 2015).

A randomised trial conducted in the United Kingdom showed that acid folic oral supplementation with 400 µg daily prevents approximately 72% of NTDs in patients with a previous affected pregnancy (Wald et al. 1991). All over the world the implementation of periconceptional folic acid supplementation has reduced the incidence of NTDs (Berry et al. 1999) (Liu et al. 2018). Although it difficult to known precisely how many cases of NTDs will be avoided by adequate folic acid intake in the overall pregnant population, it is estimated that approximately 50% of them could be averted (Mulinare & Erickson 1997). Thus the intake of 400 µg folic acid daily for all women reproductive age remains an important public health recommendation.

It must be noted however that for the folic acid supplementation to be effective in reducing the incidence of NTDs, most of the pregnancies need to be planned and pregnant women need to have access to counselling, health care services and effective dietary interventions, which, especially in low-income countries, are only available on a limited scale (Blencowe et al. 2010).

Additional strategies are therefore needed for the prevention and appropriate diagnosis of such defects. The timely ultrasound diagnostic has further reduced the prevalence in live births, as some of the parents of these babies will decide to terminate the pregnancy (Botto et al. 1999). Furthermore, the ultrasound monitoring of spina bifida fetuses allows for appropriate coun-

selling of the parents and planning of therapeutic interventions.

4.3 Spina bifida Therapy. Intrauterine and post-natal Surgery

Individuals affected by NTDs, specifically spina bifida, need elaborate and combined surgical interventions, as well as medical supportive management (Mitchell et al. 2004).

The surgical repair of spina bifida defects and associated malformations is normally undertaken within a short period of time, usually 48 hours, after birth and consists in the surgical closure of the defect (Adzick 2013). If ventriculomegaly is diagnosed before birth or the baby has manifest symptoms of hydrocephalus or brainstem compression, a ventriculo-peritoneal shunt will be placed after birth as well (Adzick 2010).

However after the two-hit hypothesis was developed based on observation of pathological specimens and animal models, new in utero operative techniques to reduce the secondary damage were explored (Moldenhauer & Adzick 2017). A randomised trial which investigated the prenatal versus postnatal corrective surgery for myelomeningocele, the so-called "Management of Myelomeningocele Study" or MOMS trial, was completed a decade ago and showed that prenatal repair before 26 weeks of gestation was beneficial regarding the need for postnatal shunting, as well as neuromotor function and mental development compared to postnatal surgery (Adzick et al. 2011). The reduction of the need of ventriculoperitoneal shunt placement at one year of age after fetal surgery was around 40%. At 30 months of age over 40% of the children who underwent a fetal repair showed independent ambulation, compared to only 21% in the postnatal surgery group, which indicates a remarkable preservation of the neuromotor function after in utero surgery. More recent analyses indicated that even after 5 to 10 years, the neuromotor advantage is maintained in the children of the initial MOMS trial group (Houtrow et al. 2021). Similarly, the hindbrain herniation showed a substantial improvement in the fetal surgery group compared to postnatal surgery group: 15% less cases of severe herniation and 32% more children with no herniation. These outstanding outcomes came at the expense of a higher rate of complications, especially a significant increase in the rate of preterm delivery by 64%. In the fetal surgery group the average gestational age at delivery was 34.1 pregnancy weeks compared to 37.3 pregnancy weeks in the postnatal surgery group, and 13% of the children in the fetal surgery group were

born before 30 weeks. The rate of maternal complications was also slightly increased, with approximately 25% showing evidence of thinning of the uterotomy scar, but fortunately none had a complete uterine rupture.

Following this groundbreaking research, the prenatal intervention is currently offered in specialised centres as a standard therapeutic option for patients who fulfil certain criteria. The operation is performed between 20 and 26 weeks, by a large uterotomy. The fetus is manipulated in a favourable dorso-anterior position, so that the spinal defect is directly accessible through the uterotomy. The spinal defect is closed using a technique similar to that used in the post-natal surgery, after which the uterotomy and the abdominal incisions are sutured. A prerequisite for the intervention is the lack of associated malformations or chromosomal defects. The risks of an extensive surgery with a significant impact on the current and subsequent pregnancies need to be thoroughly explained to the parents beforehand and the benefit of the operation for the unborn child weighed against the likelihood of premature delivery.

Additional fetoscopic repair techniques were explored with partial success (Pedreira et al. 2016) (Carrabba et al. 2019). They circumvent the need of a large uterotomy by using the "key-hole" surgery technique, which represents an advantage, as it significantly reduces the risk of premature delivery. The repair involves closing the defect by suturing a synthetic patch over it, and while technically elegant, it involves the risk of adhesions and the need to later remove the patch, as it will not be sufficiently large anymore for the growing structures of the spine. Despite this theoretical limitations, recent research showed that the results of the open versus fetoscopic in utero spina bifida repair are comparable (Cortes et al. 2021), and the authors suggest that the fetoscopic approach might be advantageous in respect to future pregnancies and obstetric outcomes, due to the reduced uterine scar.

The early ultrasound detection of spina bifida is essential in this respect, as it will allow parents and their caregivers to opt for the adequate method of corrective surgery.

4.4 Prognosis and Long-Term Outcome of Patients with Spina bifida

Spina bifida remains a devastating disease and affected individuals and families must expect life long care needs and high costs. Although advances

in the multidisciplinary surgical and medical care over the past 50 years led to longer lives and improved quality of life, there is still a relative high mortality and morbidity associated with the disease itself, as well as complications associated with the operations.

Around 14% of the affected children will die in the first 5 years of life, and the mortality of those with brainstem compression is around 2.5 fold higher (Bowman et al. 2001) (Adzick 2013). The survival into adulthood is dependent on the level of the lesion, as expected. Thus, a study of 40 years follow-up of spina bifida patients in the United Kingdom found that almost two thirds of patients with a lesion below the 3rd lumbar vertebra, but only 17% with cervical and thoracic defects had survived later on (Copp et al. 2015).

The leading cause of death in the children with spina bifida and Arnold Chiari malformation is the hindbrain herniation through foramen magnum, which causes cerebellar dysfunction, compression of the respiratory center in the medulla, dysfunction of cranial nerves and hydrocephalus (Bowman et al. 2001). This can be treated by placing a ventricular shunt to insure the decompression of intracranial structures, but some children will require extensive neurosurgical interventions.

At least a quarter of affected children will have neurodevelopmental impairment and this appears to be dependent on the presence of hydrocephalus (Inversetti et al. 2019). Children with spina bifida who do not develop significant ventriculomegaly appear be unaffected neurodevelopmentally (Iddon et al. 2004). It is known, however, that a significant proportion of children, around 60 to 80 %, will present a symptomatic ventriculomegaly or will develop hydrocephalus secondary to Arnold-Chiari malformation and will need placements of shunts (McCarthy et al. 2019). Around half of these patients will have complications of shunts within one year after operation (Adzick 2013).

Another significant issue is the tethering of the cord, due to adhesions between the previously exposed neural structures and adjacent tissue. This is in some cases diagnosed after successful repair and leads to a deterioration of an initially satisfactory neural function (Adzick 2013). Urine and fecal incontinence affect approximately half of the spina bifida patients into adulthood, require surgery and affect the quality of life (Verhoef et al. 2005) (Freeman et al. 2017). Orthopaedic problems related to joint deformities and contractures, such as scoliosis and kyphosis, hip dislocation, talipes, are common and need surgical of conservative management and physiotherapy. Independent ambulation is a function of joint malfunction and neurological deterioration

and is achieved in only around 37% of those treated prenatally and in around 19% of those with postnatal surgery (Inversetti et al. 2019).

In utero surgery was able to improve some deficiencies. The MOMS trial showed a 2-fold improvement regarding independent ambulation at 30 months of age, the need for ventricular shunting was halved and hindbrain herniation was significantly reduced (Adzick et al. 2011). However, these gains were achieved at the expense of a significantly higher rate of preterm deliveries (79% versus 15%), of which 13% were born before 30 weeks of gestation (Adzick 2013). These complications appear to be circumvented by the fetoscopic repair, while maintaining the same beneficial results, as previously noticed. However, these initial positive outcomes for the fetoscopic repair lack extensive follow-up reports at the moment.

Due to the extensive need for care, numerous deficiencies and various complication and despite progress made in the multidisciplinary approach, around 50% of patients will not be able to live independently in adulthood and the life-time medical and indirect costs, derived from special needs education and loss of employment, is staggering (Copp et al. 2015).

4.5 Present Research

We designed the present research with the aim of defining easy to use second and third trimester ultrasound parameters and with the scope of improving the detection of this afflicting disease. We used five parameters of fetal facial profile in the second and third trimester of pregnancy and compared them in fetuses with spina bifida and in healthy controls.

Two of these parameters (PFSR and FMF angle) were defined and and investigated by other research groups, as well as our own, as part of research projects conducted in different areas of interest, namely screening for chromosomal abnormalities. The other three parameters were described here for the first time.

All these parameters were measured in the midsagittal fetal profile plane. The parameters themselves are easily investigated and showed a good reproducibility. The midsagittal facial plane is assessed routinely as a part of second trimester screening for fetal abnormalities, as well as being almost always easily accessible in the third trimester, especially in the early and mid third trimester. Later in the pregnancy however this plane might not be straightforward, because of fetal position.

Our study shows that, similar to the first trimester assessment, the changes in fetal profile are present in the second and third trimesters as well, but the differences are less evident. We found that of these parameters, the FMF angle was significantly smaller in fetuses with spina bifida compared to healthy controls. We have also seen that the PFS and CCM display a statistically significant difference between the two groups. However, these differences were not marked enough, despite statistical significance. The other two parameters, PFSR and MNF, do not show significant differences between the affected group compared with controls. We then developed a logistic regression model based on all these parameters, which also showed no improvement in the detection of spina bifida.

We can conclude therefore that assessing the fetal profile is at present not useful in the detection of spina bifida and cannot be recommended as a part of screening programmes for this defect in the second and third trimesters of pregnancy.

5 Summary

The objective of this project was to determine whether, in the second and third trimesters of pregnancy, the prefrontal space ratio, the frontomaxillary facial angle, the prefrontal space, the distance between corpus callosum and the mandibulomaxillary axis, as well as the mandibulo-fronto-nasal axis can be helpful in screening for open spinal defects by ultrasound.

These parameters were measured in 71 spina bifida fetuses in the second and third trimester of pregnancy according to standardised protocols. The normal values for the PFSR were previously published by our group and were derived from 279 normal control cases. To determine the normal values for the FMF angle, PFS, CCM and MNF in the second and third trimesters of pregnancy we used the same stored images from the above-mentioned study.

In fetuses with spina bifida, the frontomaxillary angle was significantly smaller than in the normal population. However, only in about a fifth of the affected fetuses, the measurement was below the 5th centile. The PFSR, PFS, CCM and MFN were similar in both groups.

Thus, the frontomaxillary angle is smaller in second and third trimester fetuses with open spina bifida than in controls, however the difference is not pronounced enough to implement this marker in current screening programs.

6 Zusammenfassung

Das Ziel dieser Arbeit war, gewissen sonographischen Parameter des fetalen Gesichtsprofil zu definieren, deren Normalwerte zu etablieren und zu untersuchen, ob sie in der Detektion der Feten mit Spina bifida im zweiten und dritten Trimenon hilfreich sein können. Diese Parameter sind wie folgt: das präfrontale Raumverhältnis (PFSR), der frontomaxilläre Gesichtswinkel (FMF angle), der präfrontale Raum (PSR), der Abstand zwischen Corpus callosum und der maxillo-mandibulären Linie (CCM), sowie die mandibulo-naso-frontalen Linie (MNF).

Diese Parameter wurden bei 71 Feten mit Spina bifida im zweiten und dritten Trimester nach zuvor definiertem Protokoll gemessen. Die Normalwerte für das PFSR wurden bereits von unserer Arbeitsgruppe nach der Untersuchung von 279 normalen Feten veröffentlicht. Zur Bestimmung der Normalwerte für den FMF-Winkel, PFS, CCM und MNF haben wir die gleichen Bilder aus der oben genannten Studie verwendet.

Bei Feten mit Spina bifida war der FMF angle signifikant geringer als in der Kontrollgruppe. Allerdings lag der Messwert nur bei etwa einem Fünftel der betroffenen Föten unterhalb der 5. Perzentile. Die Werte der PFSR, PFS, CCM und MFN zeigten in beiden Gruppen kein signifikanter Unterschied.

Unsere Schlussfolgerung ist daher, dass der FMF Angle im zweiten und dritten Trimenon ist bei Feten mit offener Spina bifida geringer als bei normalen Feten, jedoch ist der Unterschied nicht relevant genug, um diesen Marker in aktuellen Screeningprogrammen zu implementieren.

References

Adé-Damilano, M., Schöni-Affolter, F., Dubuis-Grieder, C. & Strauch, E. (2008), 'Embryology.ch', http://www.embryology.ch/indexde.html.

Adzick, N. S. (2010), 'Fetal myelomeningocele: Natural history, pathophysiology, and in-utero intervention', *Seminars in Fetal and Neonatal Medicine* **15**(1), 9–14.

Adzick, N. S. (2013), 'Fetal surgery for spina bifida: Past, present, future', *Seminars in Pediatric Surgery* **22**(1), 10–17.

Adzick, N. S., Thom, E. A., Spong, C. Y., Brock, J. W., Burrows, P. K., Johnson, M. P., Howell, L. J., Farrell, J. A., Dabrowiak, M. E., Sutton, L. N., Gupta, N., Tulipan, N. B., D'Alton, M. E. & Farmer, D. L. (2011), 'A Randomized Trial of Prenatal versus Postnatal Repair of Myelomeningocele', *New England Journal of Medicine* **364**(11), 993–1004.

Agopian, A. J., Tinker, S. C., Lupo, P. J., Canfield, M. A. & Mitchell, L. E. (2013), 'Proportion of Neural Tube Defects Attributable to Known Risk Factors', *Birth defects research. Part A, Clinical and molecular teratology* **97**(1), 42–46.

Au, K. S., Ashley-Koch, A. & Northrup, H. (2010), 'Epidemiologic and genetic aspects of spina bifida and other neural tube defects', *Developmental Disabilities Research Reviews* **16**(1), 6–15.

Bahlmann, F., Reinhard, I., Schramm, T., Geipel, A., Gembruch, U., von Kaisenberg, C. S., Schmitz, R., Stupin, J., Chaoui, R., Karl, K., Kalache, K., Faschingbauer, F., Ponnath, M., Rempen, A. & Kozlowski, P. (2015), 'Cranial and cerebral signs in the diagnosis of spina bifida between 18 and 22 weeks of gestation: A German multicentre study', *Prenatal Diagnosis* **35**(3), 228–235.

Bartsch, O., Kirmes, I., Thiede, A., Lechno, S., Gocan, H., Florian, I. S., Haaf, T., Zechner, U., Sabova, L. & Horn, F. (2012), 'Novel VANGL1 Gene Mutations in 144 Slovakian, Romanian and German Patients with Neural Tube Defects', *Molecular Syndromology* **3**(2), 76–81.

Basaloglu, H. K., Celik, S., Dogan Kilic, K., Cavusoglu, T., Yigitturk, G., Bilge, O., Uyanikgil, Y. & Turgut, M. (2017), 'Spina Bifida: Morphological Features, Molecular Regulations and Signal Pathways', *Journal of Spine* **06**(01).

Bassuk, A. G. & Kibar, Z. (2009), 'Genetic Basis of Neural Tube Defects', *Seminars in Pediatric Neurology* **16**(3), 101–110.

Ben Miled, S., Laurence, L., Jean-Paul, D. V. H., Bettina, B., Amel, S., Brigitte, L., Julia, T., Homa, A.-B., Houria, S., Maryse, B.-D., Aude (, T., Jelena, M., Yoann, S., Frédéric, C., Julie, B., Syril, J., Yves, V., Tania, A.-B., Ferechte, E.-R. & Julien, S. (2020), 'Severe and progressive neuronal loss in myelomeningocele begins before 16 weeks of pregnancy.', *American Journal of Obstetrics & Gynecology* **0**(0).

Berry, R. J., Li, Z., Erickson, J. D., Li, S., Moore, C. A., Wang, H., Mulinare, J., Zhao, P., Wong, L. Y., Gindler, J., Hong, S. X. & Correa, A. (1999), 'Prevention of neural-tube defects with folic acid in China. China-U.S. Collaborative Project for Neural Tube Defect Prevention', *The New England Journal of Medicine* **341**(20), 1485–1490.

Bican, O., Minagar, A. & Pruitt, A. A. (2013), 'The Spinal Cord: A Review of Functional Neuroanatomy', *Neurologic Clinics* **31**(1), 1–18.

Blencowe, H., Cousens, S., Modell, B. & Lawn, J. (2010), 'Folic acid to reduce neonatal mortality from neural tube disorders', *International Journal of Epidemiology* **39**(Suppl 1), i110–i121.

Blom, H. J., Shaw, G. M., den Heijer, M. & Finnell, R. H. (2006), 'Neural tube defects and folate: Case far from closed', *Nature Reviews Neuroscience* **7**(9), 724–731.

Borenstein, M., Persico, N., Kagan, K. O., Gazzoni, A. & Nicolaides, K. H. (2008), 'Frontomaxillary facial angle in screening for trisomy 21 at 11 + 0 to 13 + 6 weeks', *Ultrasound in Obstetrics and Gynecology* **32**(1), 5–11.

Bosoi, C. M., Capra, V., Allache, R., Trinh, V. Q.-H., De Marco, P., Merello, E., Drapeau, P., Bassuk, A. G. & Kibar, Z. (2011), 'Identification and characterization of novel rare mutations in the planar cell polarity gene PRICKLE1 in human neural tube defects', *Human mutation* **32**(12), 1371–1375.

Botto, L. D., Moore, C. A., Khoury, M. J. & Erickson, J. D. (1999), 'Neural-Tube Defects', *New England Journal of Medicine* **341**(20), 1509–1519.

Bowman, R. M., McLone, D. G., Grant, J. A., Tomita, T. & Ito, J. A. (2001), 'Spina bifida outcome: A 25-year prospective', *Pediatric Neurosurgery* **34**(3), 114–120.

Bredaki, F. E., Poon, L. C., Birdir, C., Escalante, D. & Nicolaides, K. H. (2012), 'First-Trimester Screening for Neural Tube Defects Using Alpha-Fetoprotein', *Fetal Diagnosis and Therapy* **31**(2), 109–114.

Brock, D. J. H. (1976), 'Alphafetoprotein and neural tube defects', *Journal of Clinical Pathology. Supplement (Royal College of Pathologists).* **10**, 157–164.

Cabrera, R. M., Shaw, G. M., Ballard, J. L., Carmichael, S. L., Yang, W., Lammer, E. J. & Finnell, R. H. (2008), 'Autoantibodies to folate receptor during pregnancy and neural tube defect risk', *Journal of reproductive immunology* **79**(1), 85–92.

Campbell, J., Gilbert, W., Nicolaides, K. H. & Campbell, S. (1987), 'Ultrasound screening for spina bifida: Cranial and cerebellar signs in a high-risk population', https://pubmed.ncbi.nlm.nih.gov/3299184/.

Carlson, L. M. & Vora, N. L. (2017), 'Prenatal Diagnosis', *Obstetrics and gynecology clinics of North America* **44**(2), 245–256.

Carmichael, S. L., Rasmussen, S. A. & Shaw, G. M. (2010), 'Prepregnancy obesity: A complex risk factor for selected birth defects', *Birth Defects Research Part A: Clinical and Molecular Teratology* **88**(10), 804–810.

Carrabba, G., Macchini, F., Fabietti, I., Schisano, L., Meccariello, G., Campanella, R., Bertani, G., Locatelli, M., Boito, S., Porro, G. A., Gabetta, L., Picciolini, O., Cinnante, C., Triulzi, F., Ciralli, F., Mosca, F., Lapa, D. A., Leva, E., Rampini, P. & Persico, N. (2019), 'Minimally invasive fetal surgery for myelomeningocele: Preliminary report from a single center', *Neurosurgical Focus* **47**(4), E12.

Chaoui, R., Benoit, B., Heling, K. S., Kagan, K. O., Pietzsch, V., Lopez, A. S., Tekesin, I. & Karl, K. (2011), 'Prospective detection of open spina bifida at 11–13 weeks by assessing intracranial translucency and posterior brain', *Ultrasound in Obstetrics & Gynecology* **38**(6), 722–726.

Chaoui, R., Benoit, B., Mitkowska-Wozniak, H., Heling, K. S. & Nicolaides, K. H. (2009), 'Assessment of intracranial translucency (IT) in the detection of spina bifida at the 11–13-week scan', *Ultrasound in Obstetrics & Gynecology* **34**(3), 249–252.

Chaoui, R. & Nicolaides, K. H. (2010), 'From nuchal translucency to intracranial translucency: Towards the early detection of spina bifida', *Ultrasound in Obstetrics & Gynecology* **35**(2), 133–138.

Chaoui, R. & Nicolaides, K. H. (2011), 'Detecting open spina bifida at the 11–13-week scan by assessing intracranial translucency and the posterior brain region: Mid-sagittal or axial plane?', *Ultrasound in Obstetrics & Gynecology* **38**(6), 609–612.

Chen, C.-P. (2008), 'Syndromes, Disorders and Maternal Risk Factors Associated With Neural Tube Defects (VII)', *Taiwanese Journal of Obstetrics and Gynecology* **47**(3), 276–282.

Cho, T. A. (2015), 'Spinal Cord Functional Anatomy', *CONTINUUM: Lifelong Learning in Neurology* **21**(1), 13.

Copp, A. J., Adzick, N. S., Chitty, L. S., Fletcher, J. M., Holmbeck, G. N. & Shaw, G. M. (2015), 'Spina Bifida', *Nature reviews. Disease primers* **1**, 15007.

Copp, A. J. & Greene, N. D. E. (2010), 'Genetics and development of neural tube defects', *The Journal of pathology* **220**(2), 217–230.

Copp, A. J. & Greene, N. D. E. (2013), 'Neural tube defects–disorders of neurulation and related embryonic processes', *Wiley Interdisciplinary Reviews. Developmental Biology* **2**(2), 213–227.

Cortes, M. S., Chmait, R. H., Lapa, D. A., Belfort, M. A., Carreras, E., Miller, J. L., biskupski Samaha, R. B., Gonzalez, G. S., Gielchinsky, Y., Yamamoto, M., Persico, N., Santorum, M., Otaño, L., Nicolaou, E., Yinon, Y., Faig-leite, F., Brandt, R., Whitehead, W., Maiz, N., Baschat, A., Kosinski, P., Nieto-sanjuanero, A., Chu, J., Kershenovich, A. & Nicolaides, K. H. (2021), 'Experience of 300 cases of prenatal fetoscopic open spina bifida repair: Report of the International Fetoscopic Neural Tube Defect Repair Consortium.', *American Journal of Obstetrics & Gynecology* **0**(0).

Czeizel, A. E. & Dudás, I. (1992), 'Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation', *The New England Journal of Medicine* **327**(26), 1832–1835.

D'Addario, V., Rossi, A. C., Pinto, V., Pintucci, A. & Di Cagno, L. (2008), 'Comparison of six sonographic signs in the prenatal diagnosis of spina bifida', *Journal of Perinatal Medicine* **36**(4), 330–334.

Denny, K. J., Jeanes, A., Fathe, K., Finnell, R. H., Taylor, S. M. & Woodruff, T. M. (2013), 'Neural Tube Defects, Folate, and Immune Modulation', *Birth defects research. Part A, Clinical and molecular teratology* **97**(9), 602–609.

Deora, H., Srinivas, D., Shukla, D., Devi, B. I., Mishra, A., Beniwal, M., Kannepalli, N. R. & Somanna, S. (2019), 'Multiple-site neural tube defects: Embryogenesis with complete review of existing literature', *Neurosurgical Focus* **47**(4), E18.

Detrait, E., George, T. M., Etchevers, H., Gilbert, J., Vekemans, M. & Speer, M. C. (2005), 'Human Neural Tube Defects: Developmental Biology, Epidemiology, and Genetics', *Neurotoxicology and teratology* **27**(3), 515–524.

Dolk, H., Loane, M. & Garne, E. (2010), The Prevalence of Congenital Anomalies in Europe, *in* M. Posada de la Paz & S. C. Groft, eds, 'Rare Diseases Epidemiology', Advances in Experimental Medicine and Biology, Springer Netherlands, Dordrecht, pp. 349–364.

Doudney, K., Moore, G. E., Stanier, P., Ybot-Gonzalez, P., Paternotte, C., Greene, N. D. E., Copp, A. J. & Stevenson, R. E. (2005), 'Analysis of the planar cell polarity gene Vangl2 and its co-expressed paralogue Vangl1 in neural tube defect patients', *American Journal of Medical Genetics Part A* **136A**(1), 90–92.

Eibach, S. & Pang, D. (2020), 'Junctional Neural Tube Defect', *Journal of Korean Neurosurgical Society* .

EUROCAT (2021), 'European Registration of Congenital Anomalies and Twins Prevalence Charts and Tables', https://eu-rd-platform.jrc.ec.europa.eu.

Freeman, K. A., Castillo, H., Castillo, J., Liu, T., Schechter, M., Wiener, J. S., Thibadeau, J., Ward, E. & Brei, T. (2017), 'Variation in bowel and bladder continence across US spina bifida programs: A descriptive study', *Journal of pediatric rehabilitation medicine* **10**(3-4), 231–241.

Galea, G. L., Nychyk, O., Mole, M. A., Moulding, D., Savery, D., Nikolopoulou, E., Henderson, D. J., Greene, N. D. E. & Copp, A. J. (2018), 'Vangl2 disruption alters the biomechanics of late spinal neurulation leading to spina bifida in mouse embryos', *Disease Models & Mechanisms* **11**(3).

Ghi, T., Cocchi, G., Conti, L., Pacella, G., Youssef, A., Rizzo, N. & Pilu, G. (2015), 'Prenatal Diagnosis of Open Spina Bifida in Emilia-Romagna', *Fetal Diagnosis and Therapy* **37**(4), 301–304.

Ghi, T., Pilu, G., Falco, P., Segata, M., Carletti, A., Cocchi, G., Santini, D., Bonasoni, P., Tani, G. & Rizzo, N. (2006), 'Prenatal diagnosis of open and closed spina bifida', *Ultrasound in Obstetrics & Gynecology* **28**(7), 899–903.

Hernández-Díaz, S. & Mitchell, A. A. (2000), 'Folic Acid Antagonists during Pregnancy and the Risk of Birth Defects', *The New England Journal of Medicine* p. 7.

Honein, M. A., Paulozzi, L. J., Mathews, T. J., Erickson, J. D. & Wong, L.-Y. C. (2001), 'Impact of Folic Acid Fortification of the US Food Supply on the Occurrence of Neural Tube Defects', *JAMA* **285**(23), 2981–2986.

Houtrow, A. J., MacPherson, C., Jackson-Coty, J., Rivera, M., Flynn, L., Burrows, P. K., Adzick, N. S., Fletcher, J., Gupta, N., Howell, L. J., Brock, J. W., Lee, H., Walker, W. O. & Thom, E. A. (2021), 'Prenatal Repair and Physical Functioning Among Children With Myelomeningocele: A Secondary Analysis of a Randomized Clinical Trial', *JAMA pediatrics* **175**(4), e205674.

Iddon, J., Morgan, D., Loveday, C., Sahakian, B. & Pickard, J. (2004), 'Neuropsychological profile of young adults with spina bifida with or without hydrocephalus', *Journal of Neurology, Neurosurgery, and Psychiatry* **75**(8), 1112–1118.

Inversetti, A., der Veeken, L. V., Thompson, D., Jansen, K., Calenbergh, F. V., Joyeux, L., Bosteels, J. & Deprest, J. (2019), 'Neurodevelopmental outcome of children with spina bifida aperta repaired prenatally vs postnatally: Systematic review and meta-analysis', *Ultrasound in Obstetrics & Gynecology* **53**(3), 293–301.

ISUOG Practice Guidelines: Performance of First-Trimester Fetal Ultrasound Scan: ISUOG Guidelines (2013), *Ultrasound in Obstetrics & Gynecology* **41**(1), 102–113.

Kaplan, K. M., Spivak, J. M. & Bendo, J. A. (2005), 'Embryology of the spine and associated congenital abnormalities', *The Spine Journal* **5**(5), 564–576.

Karl, K., Benoit, B., Entezami, M., Heling, K. S. & Chaoui, R. (2012), 'Small biparietal diameter in fetuses with spina bifida on 11–13-week and mid-gestation ultrasound', *Ultrasound in Obstetrics & Gynecology* **40**(2), 140–144.

Khalil, A., Caric, V., Papageorghiou, A., Bhide, A., Akolekar, R. & Thilaganathan, B. (2014), 'Prenatal prediction of need for ventriculoperitoneal

shunt in open spina bifida', *Ultrasound in Obstetrics & Gynecology* **43**(2), 159–164.

Kibar, Z., Salem, S., Bosoi, C. M., Pauwels, E., De Marco, P., Merello, E., Bassuk, A. G., Capra, V. & Gros, P. (2011), 'Contribution of VANGL2 mutations to isolated neural tube defects', *Clinical genetics* **80**(1), 76–82.

Lachmann, R., Chaoui, R., Moratalla, J., Picciarelli, G. & Nicolaides, K. H. (2011), 'Posterior brain in fetuses with open spina bifida at 11 to 13 weeks', *Prenatal Diagnosis* **31**(1), 103–106.

Lachmann, R., Picciarelli, G., Moratalla, J., Greene, N. & Nicolaides, K. H. (2010), 'Frontomaxillary facial angle in fetuses with spina bifida at 11-13 weeks' gestation', *Ultrasound in Obstetrics and Gynecology* **36**(3), 268–271.

Lawson, L. Y. & Harfe, B. D. (2017), 'Developmental mechanisms of intervertebral disc and vertebral column formation', *WIREs Developmental Biology* **6**(6), e283.

Liu, J., Li, Z., Ye, R., Liu, J. & Ren, A. (2018), 'Periconceptional folic acid supplementation and sex difference in prevention of neural tube defects and their subtypes in China: Results from a large prospective cohort study', *Nutrition Journal* **17**.

Martiniak, Y., Heuer, T. & Hoffmann, I. (2015), 'Intake of dietary folate and folic acid in Germany based on different scenarios for food fortification with folic acid', *European Journal of Nutrition* **54**(7), 1045–1054.

McCarthy, D. J., Sheinberg, D. L., Luther, E. & McCrea, H. J. (2019), 'Myelomeningocele-associated hydrocephalus: Nationwide analysis and systematic review', *Neurosurgical Focus* **47**(4), E5.

Melvin, E. C., George, T. M., Worley, G., Franklin, A., Mackey, J., Viles, K., Shah, N., Drake, C. R., Enterline, D. S., McLone, D., Nye, J., Oakes, W. J., McLaughlin, C., Walker, M. L., Peterson, P., Brei, T., Buran, C., Aben, J., Ohm, B., Bermans, I., Qumsiyeh, M., Vance, J., Pericak-Vance, M. A. & Speer, M. C. (2000), 'Genetic Studies in Neural Tube Defects', *Pediatric Neurosurgery* **32**(1), 1–9.

Merz, E., Eichhorn, K.-H., von Kaisenberg, C., Schramm, T. & Arbeitsgruppe der DEGUM-Stufe III (2012), 'Aktualisierte Qualitaetsanforderungen an die weiterfuehrende differenzierte Ultraschalluntersuchung in der paenatalen Diagnostik (= DEGUM-Stufe II) im Zeitraum von 18 + 0 bis 21 +

6 Schwangerschaftswochen', *Ultraschall in der Medizin - European Journal of Ultrasound* **33**(06), 593–596.

Meuli, M. & Moehrlen, U. (2014), 'Fetal surgery for myelomeningocele is effective: A critical look at the whys', *Pediatric Surgery International* **30**(7), 689–697.

Miller, J. L. & Huisman, T. A. G. M. (2019), 'Spinal Dysraphia, Chiari 2 Malformation, Unified Theory, and Advances in Fetoscopic Repair', *Neuroimaging Clinics of North America* **29**(3), 357–366.

Milunsky, A., Jick, H., Jick, S. S., Bruell, C. L., MacLaughlin, D. S., Rothman, K. J. & Willett, W. (1989), 'Multivitamin/Folic Acid Supplementation in Early Pregnancy Reduces the Prevalence of Neural Tube Defects', *JAMA* **262**(20), 2847–2852.

Mitchell, L. E., Adzick, N. S., Melchionne, J., Pasquariello, P. S., Sutton, L. N. & Whitehead, A. S. (2004), 'Spina bifida', *Lancet (London, England)* **364**(9448), 1885–1895.

Moldenhauer, J. S. & Adzick, N. S. (2017), 'Fetal surgery for myelomeningocele: After the Management of Myelomeningocele Study (MOMS)', *Seminars in Fetal and Neonatal Medicine* **22**(6), 360–366.

Mowla, S., Gissler, M., Räisänen, S. & Kancherla, V. (2020), 'Association between maternal pregestational diabetes mellitus and spina bifida: A population-based case–control study, Finland, 2000–2014', *Birth Defects Research* **112**(2), 186–195.

Mulinare, J. & Erickson, J. D. (1997), 'Prevention of neural tube defects', *Teratology* **56**(1-2), 17–18.

Nicolaides, K. & Campbell, J. (1989), 'Neural tube abnormalities', *Clinics in diagnostic ultrasound* **Volume 25**.

Nicolaides, K. H., Gabbe, S. G., Campbell, S. & Guidetti, R. (1980), 'Ultrasound Screening for Spina Bifida: Cranial and Cerebellar Signs', *The Lancet* p. 3.

Nolting, D., Hansen, B. F., Keeling, J. & odont Kjær, I. D. (1998), 'Prenatal Development of the Normal Human Vertebral Corpora in Different Segments of the Spine', *Spine* **23**(21), 2265–2271.

Nyberg, D. A., Mack, L. A., Hirsch, J. & Mahony, B. S. (1988), 'Abnormalities of fetal cranial contour in sonographic detection of spina bifida: Evaluation of the "lemon" sign.', *Radiology* **167**(2), 387–392.

O'Rahilly, R. & Müller, F. (1999), 'Minireview: Summary of the initial development of the human nervous system', *Teratology* **60**(1), 39–41.

O'Rahilly, R. & Müller, F. (2006), *The Embryonic Human Brain An Atlas of Developmental Stages*, third edition edn, Wiley-Liss.

O'Rahilly, R. & Müller, F. (2007), 'The development of the neural crest in the human', *Journal of Anatomy* **211**(3), 335–351.

O'Rahilly, R. & Müller, F. (2010), 'Developmental stages in human embryos: Revised and new measurements', *Cells, Tissues, Organs* **192**(2), 73–84.

Ossipova, O., Kim, K. & Sokol, S. Y. (2015), 'Planar polarization of Vangl2 in the vertebrate neural plate is controlled by Wnt and Myosin II signaling', *Biology Open* **4**(6), 722–730.

Pedreira, D. A. L., Zanon, N., Nishikuni, K., de Sá, R. A. M., Acacio, G. L., Chmait, R. H., Kontopoulos, E. V. & Quintero, R. A. (2016), 'Endoscopic surgery for the antenatal treatment of myelomeningocele: The CECAM trial', *American Journal of Obstetrics & Gynecology* **214**(1), 111.e1–111.e11.

Plasencia, W., Dagklis, T., Sotiriadis, A., Borenstein, M. & Nicolaides, K. H. (2007), 'Frontomaxillary facial angle at 11 + 0 to 13 + 6 weeks' gestation—reproducibility of measurements', *Ultrasound in Obstetrics and Gynecology* **29**(1), 18–21.

Prodan, N., Hoopmann, M., Sonek, J., Oettling, C., Abele, H., Wagner, P. & Kagan, K. O. (2020), 'Fetal profile in fetuses with open spina bifida', *Archives of Gynecology and Obstetrics* **301**(5), 1167–1171.

R: A Language and Environment for Statistical Computing (2021), R Foundation for Statistical Computing.

Reghunath, A., Ghasi, R. G. & Aggarwal, A. (2019), 'Unveiling the tale of the tail: An illustration of spinal dysraphisms', *Neurosurgical Review* .

Sadler, T. W., Sadler-Redmond, S. L., Tosney, K., Byrne, J. & Imseis, H. (2019), *Langman's Medical Embryology*.

Salbaum, J. M. & Kappen, C. (2010), 'Neural Tube Defect Genes and Maternal Diabetes during Pregnancy', *Birth defects research. Part A, Clinical and molecular teratology* **88**(8), 601–611.

Salomon, L. J., Alfirevic, Z., Berghella, V., Bilardo, C., Hernandez-Andrade, E., Johnsen, S. L., Kalache, K., Leung, K.-Y., Malinger, G., Munoz, H., Prefumo, F., Toi, A., Lee, W. & Committee, o. b. o. t. I. C. S. (2011), 'Practice guidelines for performance of the routine mid-trimester fetal ultrasound scan', *Ultrasound in Obstetrics & Gynecology* **37**(1), 116–126.

Scaal, M. (2016), 'Early development of the vertebral column', *Seminars in Cell & Developmental Biology* **49**, 83–91.

Sebire, N. J., Noble, P. L., Thorpe-Beeston, J. G., Snijders, R. J. M. & Nicolaides, K. H. (1997), 'Presence of the 'lemon' sign in fetuses with spina bifida at the 10–14-week scan', *Ultrasound in Obstetrics & Gynecology* **10**(6), 403–405.

Smithells, R. W., Sheppard, S. & Schorah, C. J. (1976), 'Vitamin deficiencies and neural tube defects', *Archives of Disease in Childhood* p. 7.

Sonek, J., Borenstein, M., Dagklis, T., Persico, N. & Nicolaides, K. H. (2007), 'Frontomaxillary facial angle in fetuses with trisomy 21 at 11-13+6 weeks', *American Journal of Obstetrics & Gynecology* **196**(3), 271.e1–271.e4.

Sonek, J., Borenstein, M., Downing, C., McKenna, D., Neiger, R., Croom, C., Genrich, T. & Nicolaides, K. H. (2007), 'Frontomaxillary facial angles in screening for trisomy 21 at 14-23 weeks' gestation', *American Journal of Obstetrics & Gynecology* **197**(2), 160.e1–160.e5.

Sonek, J., Molina, F., Hiett, A. K., Glover, M., McKenna, D. & Nicolaides, K. H. (2012), 'Prefrontal space ratio: Comparison between trisomy 21 and euploid fetuses in the second trimester: Prefrontal space ratio: Trisomy 21 euploid fetuses', *Ultrasound in Obstetrics & Gynecology* **40**(3), 293–296.

Stiefel, D., Copp, A. J. & Meuli, M. (2007), 'Fetal spina bifida: Loss of neural function in utero', *Journal of neurosurgery* **106**(3 0), 213–221.

Stothard, K. J., Tennant, P. W. G., Bell, R. & Rankin, J. (2009), 'Maternal overweight and obesity and the risk of congenital anomalies: A systematic review and meta-analysis', *JAMA* **301**(6), 636–650.

Syngelaki, A., Chelemen, T., Dagklis, T., Allan, L. & Nicolaides, K. H. (2011), 'Challenges in the diagnosis of fetal non-chromosomal abnormalities at 11–13 weeks', *Prenatal Diagnosis* **31**(1), 90–102.

Syngelaki, A., Hammami, A., Bower, S., Zidere, V., Akolekar, R. & Nicolaides, K. H. (2019), 'Diagnosis of fetal non-chromosomal abnormalities on routine ultrasound examination at 11–13 weeks' gestation', *Ultrasound in Obstetrics & Gynecology* **54**(4), 468–476.

Tada, M. & Heisenberg, C.-P. (2012), 'Convergent extension: Using collective cell migration and cell intercalation to shape embryos', *Development* **139**(21), 3897–3904.

Thiagarajah, S., Henke, J., Hogge, W. A., Abbitt, P. L., Breeden, N. & Ferguson, J. E. (1990), 'Early diagnosis of spina bifida: The value of cranial ultrasound markers', *Obstetrics and Gynecology* **76**(1), 54–57.

Van den Hof, M. C., Nicolaides, K. H., Campbell, J. & Campbell, S. (1990), 'Evaluation of the lemon and banana signs in one hundred thirty fetuses with open spina bifida', *American Journal of Obstetrics and Gynecology* **162**(2), 322–327.

van der Linden, I. J. M., Afman, L. A., Heil, S. G. & Blom, H. J. (2006), 'Genetic variation in genes of folate metabolism and neural-tube defect risk*', *Proceedings of the Nutrition Society* **65**(2), 204–215.

Verhoef, M., Lurvink, M., Barf, H. A., Post, M. W. M., van Asbeck, F. W. A., Gooskens, R. H. J. M. & Prevo, A. J. H. (2005), 'High prevalence of incontinence among young adults with spina bifida: Description, prediction and problem perception', *Spinal Cord* **43**(6), 331–340.

von Kaisenberg, C., Chaoui, R., Häusler, M., Kagan, K. O., Kozlowski, P., Merz, E., Rempen, A., Steiner, H., Tercanli, S., Wisser, J. & Heling, K.-S. (2016), 'Quality Requirements for the early Fetal Ultrasound Assessment at 11-13+6 Weeks of Gestation (DEGUM Levels II and III)', *Ultraschall in Der Medizin (Stuttgart, Germany: 1980)* **37**(3), 297–302.

Wald, N. J., Cuckle, H., Brock, J. H., Peto, R., Polani, P. E. & Woodford, F. P. (1977), 'Maternal serum-alpha-fetoprotein measurement in antenatal screening for anencephaly and spina bifida in early pregnancy. Report of U.K. collaborative study on alpha-fetoprotein in relation to neural-tube defects', *Lancet (London, England)* **1**(8026), 1323–1332.

Wald, N., Sneddon, J., Densem, J., Frost, C. & Stone, R. (1991), 'Prevention of neural tube defects: Results of the Medical Research Council Vitamin Study', *The Lancet* **338**(8760), 131–137.

Wallingford, J. B. & Harland, R. M. (2002), 'Neural tube closure requires Dishevelled-dependent convergent extension of the midline', *Development* **129**(24), 5815–5825.

Watkins, M. L., Rasmussen, S. A., Honein, M. A., Botto, L. D. & Moore, C. A. (2003), 'Maternal obesity and risk for birth defects', *Pediatrics* **111**(5 Pt 2), 1152–1158.

Yazdi, B., Sonek, J., Oettling, C., Hoopmann, M., Abele, H., Schaelike, M. & Kagan, K. O. (2013), 'Prefrontal space ratio in second- and third-trimester screening for trisomy 21', *Ultrasound in Obstetrics & Gynecology* **41**(3), 262–266.

Declaration of Personal Contribution

The thesis "Facial profile in fetuses with spina bifida" was designed, developed and carried out at Tübingen University Hospital for Obstetrics and Gynaecology in the Department of prenatal Diagnostic under the supervision of Prof. Dr. med. Karl Oliver Kagan.

A part of the thesis, regarding the performance of the FMF angle, was previously published (Prodan et al. 2020). The present work builds upon and expands the publication, by describing additional facial profile parameters and analysing their performance in screening for spina bifida. Additionally, for the purpose of this dissertation, several combined prediction models were computed and analysed.

The data collection was performed by myself. The statistical analysis was carried out by me according to the instructions of Prof. Dr. med. Karl Oliver Kagan.

I attest that I wrote the manuscript independently following the guidance of Prof. Dr. med. Karl Oliver Kagan and that I have used no other sources than those specified by me.

Publication

Prodan et. al - Fetal Profile in Fetuses With Open Spina Bifida *Arch Gynecol Obstet. 2020 May;301(5):1167-1171. doi: 10.1007/s00404-020-05503-8*

Acknowledgements

I would like to express my gratitude to Prof. Dr. med. Karl Oliver Kagan for his support and guidance in my scientific and clinical work. Special acknowledgements go to Prof. Dr. med. Markus Hoopmann from the Prenatal Diagnosis Department of our clinic for his assistance and advice, as well as to my colleague Dr. med. Christoph Oettling for his help in obtaining the parameter measurements.

I am very grateful for the backing and encouragement of my parents, Ilona and Vasile, throughout my life and career, as this was paramount for all my achievements. Many thanks to my cousin, Mali, for her diligent proofreading of this thesis and to my friends, Madalina and Adrian, for their assistance in formatting it.

Last but not least, my partner, Radu, has all my gratitude for his unwavering support, care, trust, friendship and advice.