Case Report

Open Spina Bifida: The Role of Ultrasound Markers in the First Trimester and Morphopathology Correlation

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ABSTRACT: Objectives-To evaluate ultrasound markers during a first-trimester (FT) routine ultrasound examination for an early detection of open spina bifida (OSB) and to correlate the sonographic findings with the morpho-histological ones. Materials and Methods: This retrospective research was performed using data from foetuses that underwent FT anatomy scans (FTAS) with a gestational age between 11 weeks and 13 weeks and 6 days in the Prenatal Diagnostic Unit of the Clinical Emergency County Hospital Craiova from October 2022 until September 2023. Results: The study included 648 FT singleton pregnancies and 5 OSB cases were detected. In the OSB group, we found abnormal aspects of the fourth ventricle, also named intracranial translucency (IT) in 4 out of 5 cases of OSB (80%), a brain stem anteroposterior diameter, and brain stem to occipital bone ratio abnormal in all 5 cases (greater than 1) (100%), the crash sign was present in 80% (4 out of 5 cases) and the spinal defect was visualized in 4 out of 5 patients (80%). Medical termination of pregnancy (MTOP) was the preferred option in all cases of OSB. This allowed us to include an extended histological study to confirm the ultrasound diagnosis. Conclusions: A combined detailed FTAS that includes both cranial ultrasound markers of the posterior fossa and also a good visualization of the foetal spine offers an early optimal detection rate of spine abnormalities.

KEYWORDS: *Prenatal diagnosis, ultrasound, anomaly scan, spina bifida.*

Introduction

Among the most frequent foetal congenital malformations are brain and spine abnormalities, with a prevalence of 0.5-0.8 per 1000 births in Europe [1] and 20 times higher in China, with an average of 10 per 1000 births.

The central nervous system (CNS) abnormalities are represented by acrania, exencephaly, anencephaly, holoprosencephaly, cephalocele, and spina bifida.

All of them can be diagnosed at the FTAS [2-5].

The development of the CNS is a dynamic, continuous process, which implies significant anatomic changes that heavily impact the ultrasound appearance during different gestational periods.

The most common anomaly of the spine is spina bifida, an anomaly frequently associated with abnormal morphometry of the posterior brain.

Spina bifida stands for the failure of the foetal spine or spinal cord closure, which takes place in the fourth week post-fertilization, during the embryo neural tube closure that usually occurs by the sixth week of gestation.

The presentation of this anomaly may be as a closed neural tube or an open embryonic neural plate.

In closed spina bifida, there is no cerebrospinal fluid loss because the defect is fully covered by skin [6-8].

These patients are usually asymptomatic at delivery, while neurologic symptoms can develop later as sequelae of a tethered cord (attachment of the filum terminal in the spinal canal) [9,10].

The most common is open spina bifida with three subgroups: myelomeningocele, meningocele and myeloschisis, based on the position of the spinal cord [9-11].

The taxonomy applied in spina bifida classification is inconsistent with the terminology regarding the correct spinal cord phenotype.

This issue is also found in histological findings of spina bifida, where coinciding or ambiguous terminology is used [3,9,12].

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The prognosis of the foetus with CNS congenital malformations is generally poor, with developmental sequelae, cognitive issues, and limited therapeutic solutions.

For this reason, early detection of major CNS anomalies offers the possibility for an easier termination of pregnancy following proper counselling.

This is crucial from an economic, psychological, and social point of view for an optimal obstetrical outcome [2-4].

Spina bifida can occur as an isolated event but also in the context of some aneuploidies, such as trisomy 18, triploidy and others [13].

The standard ultrasound anomaly scan during the genetic screening at 11-13 weeks+6 days increased the interest in assessing the anatomy of the CNS in the FT.

The evaluation of the foetal CNS at this gestational age includes the demonstration of the foetal skull integrity to exclude anencephaly and the foetal spine to detect open spina bifida [14,15].

Because of the difficulty of directly evaluating the foetal spine at the FTAS, intracranial markers were proposed for early spina bifida detection, followed by a remarkable interest in understanding neuroembryology and foetal anatomy in the FT [16-21].

The early assessment of the fetal CNS (11-14 weeks) is continued by fetal neurosonographic evaluation in the second trimester of pregnancy, as recommended by guidelines [22].

However, there is an essential interest in decreasing the gestational age for significant anomaly detection as much as possible.

The 3D ultrasound is frequently only considered a method of achieving a portrait of the foetus.

However, depending on the ultrasound equipment and the examiner's experience, it can offer much more than this.

In the first trimester of pregnancy, it can be used with increased accuracy in plane reconstruction and visualization of the structures inaccessible to conventional ultrasound.

Given that in the FT of pregnancy, the foetus rarely stays in that particular ideal position for assessing anatomical elements, purchasing the 3D volumes with a multiplanar display of reconstructed planes can be beneficial at this time of gestation, especially during the transvaginal examination.

Getting this information is very useful, as the location of the lesion in the spine correlates [23] with the severity of the motor and neurofunctional impairment.

Consequently, the couple counselling practitioners and the paediatric neurosurgery specialists will consider these aspects.

This study aimed to investigate the optimal detection of OSB by the FTAS using cranial sonographic markers, with particular attention to the posterior brain, previously proposed by the specialized literature together with a good visualization of the integrity of the fetal spine.

We included the fourth ventricle measurement [18], also referred to as intracranial translucency (IT), the brain stem anteroposterior diameter [21] and brain stem to brain stem-occipital bone ratio (BS/BSOB) [21] (abnormal when greater than 1), "crash sign" (the posterior displacement of the mesencephalon and deformation against the occipital bone in the axial view) [24] and the direct evaluation of the fetal spine.

A secondary objective was to obtain histological specimens of the spinal cord from the affected foetuses to precisely describe and classify SB anomaly in the FT as part of the foetal autopsy audit.

Materials and Methods

This retrospective study was performed in the Obstetrics-Gynecology Prenatal Diagnostic Unit of the Clinical Emergency County Hospital Craiova.

All eligible patients signed an informed consent for the use of data and were enrolled from October 2022 until September 2023.

The University of Medicine and Pharmacy Craiova Ethics Committee approved our study.

We searched for all cases of open spina bifida with a gestational age between 11-13 weeks and 5 days detected in the respective period.

Voluson E10 and E8 systems (GE Healthcare) were used to perform all scans. Transducers for transabdominal (3-9MHz) and transvaginal (5-9MHz) were used for examinations.

The transabdominal approach was preferred in most cases in the first trimester.

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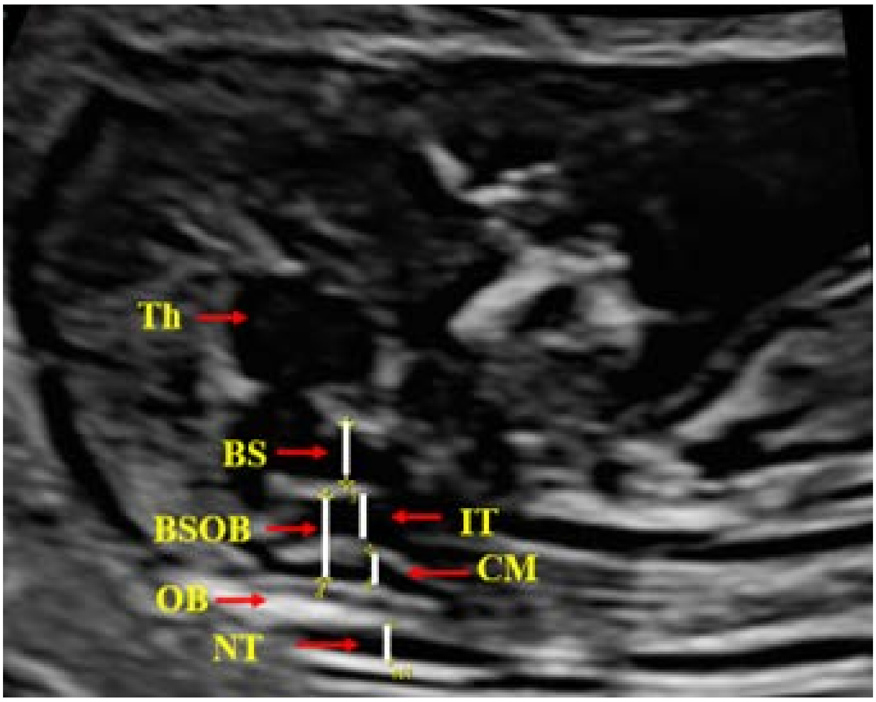

Figure 1. Sagittal view of the first trimester brain: showing the nuchal translucency (NT); cisterna magna (CM); the fourth ventricle, also named intracranial translucency (IT); brainstem (BS); occipital bone (OB); brain stem to occipital bone diameter distance (BSOB); thalamus (Th).

At the same time, transvaginal sonography was reserved only in selected cases (high body mass index (BMI), direct proper visualization of the spine or central nervous system in first-trimester foetuses with unfavourable positions, maternal abdominal scars, or fibroids) to obtain good visualization of the foetal CNS anatomy.

For early evaluation of the CNS in the first trimester, we adopted the standard protocol corresponding to the guidelines for the first-trimester screening [22] and a detailed protocol for CNS evaluation that included:

- the sagittal plane of the brain (Figure 1), with evaluation of the 4th cerebral ventricle (intracranial translucency), BS/BSOB ratio
- the longitudinal view of the fetal spine in both 2D and 3D imaging (Figure 2 A,B)
- transverse views of the brain showing the contour and shape of the foetal skull, the choroid plexus and the filling of lateral cerebral ventricles, the presence of the third ventricle and aqueduct of Sylvius and the position of the thalamus in relation to occiput bone (Figure 3).

In assessing the anatomical integrity of the fetal FT brain, the described planes that we mentioned offer a correct evaluation of the markers that we proposed for OSB early detection.

In OSB cases, the fourth ventricle and cisterna magna are obliterated because the cerebrospinal fluid displacement causes the hindbrain to migrate posteriorly and downwards [25,26].

This anatomic deviation leads further to the thickening of the brainstem and the shortening of the distance between the brainstem and the occipital bone.

Thus, the ratio between the sagittal brainstem diameter and the distance between the brainstem and the occipital bone exceeds 1.

We considered the cut-off for BS/BSOB ratio 1, as suggested by the literature [21].

In the midsagittal plane, in normal foetuses, the fourth ventricle appears as an intracranial translucency (IT) parallel to nuchal translucency (NT), delimited by two echogenic lines (Figure 1).

The anterior one delineates the posterior portion of the brainstem, and the posterior one represents the choroid plexus of the fourth ventricle.

In some OSB cases, the ‘crash’ sign is present and described as a posterior and caudal displacement of the mesencephalon and a subjective evaluation of the midbrain relative to the occiput [24].

A longitudinal spine assessment is mandatory because it can highlight other malformations, such as vertebral anomalies, sacral agenesis or teratoma [27].

All 5 OSB cases from the study group were detected during the FTAS.

The couples opted for MTOP following detailed counselling.

The foetuses were sent to the Pathology Department, where a macroscopic and microscopic evaluation was performed.

Fortunately, because of the medical TOP, an autopsy was possible and performed to confirm the diagnosis in all cases of SB in the Pathology Department of the Clinical Emergency Hospital Craiova.

Therefore, it is important to sample pathology/histological material and always classify the SB according to its most accurate phenotype.

From a histopathological point of view, using the classic Hematoxylin-Eosin (HE) staining, the neural tube defect at the spinal level is highlighted to improve the structural aspects of ultrasound assessment.

The parts included in the paraffin were sectioned with the help of the HMB450 microtome at a thickness of 4 microns, applied to glass slides, dried, deparaffinized with Xylene, dehydrated in successive alcohol baths with decreasing concentrations of 100%, 90%, 70%, rehydrated with distilled water and coloured with the classic HE staining.

These were scanned utilizing a Nikon Eclipse.

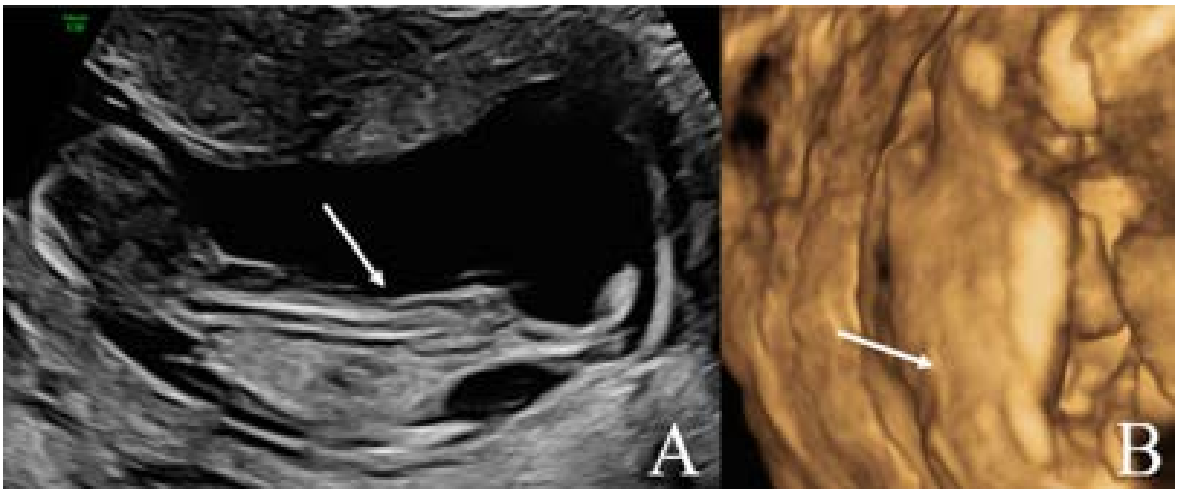

Figure 2. Longitudinal view of the fetal spine showing spine regularity and underlying skin (arrow) in 2D imaging (A) and 3D imaging (B).

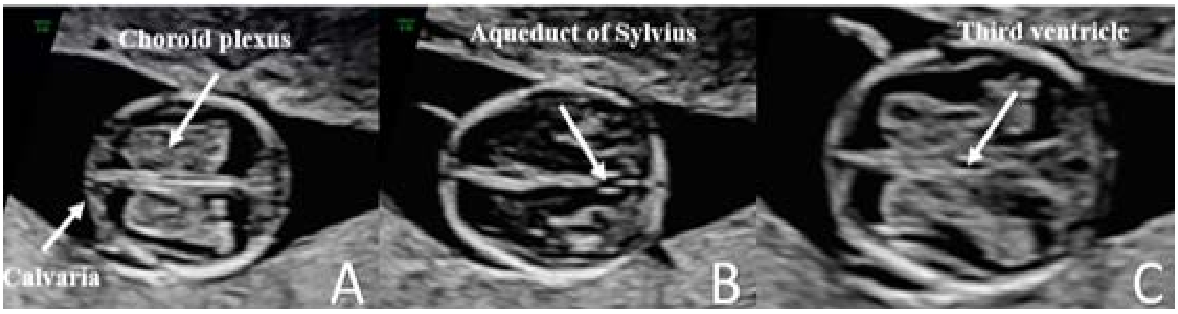

Figure 3. Transverse view of the foetal head showing: A. the contour and shape of the foetal skull (calvaria) and choroid plexus, B. the third ventricle, C. the posterior fossa of the brain with the aqueduct of Sylvius.

Results

Our database included 648 singleton pregnancies morphologically assessed from the first trimester to delivery and has identified 5 cases of OSB, all detected in the FT of pregnancy.

In the affected group, the maternal age ranged from 27 to 42 years.

The gestational age at diagnosis varied from 11w+3d to 13w+5d.

Ultrasound findings found in OSB cases in the FT are shown in Table 1.

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Table 1. The evaluation of the first-trimester ultrasound markers used for foetal CNS evaluation in the 5 OSB cases.

Cases/markers	Intracranial translucency	BS/BSOB	Direct fetal spine evaluation	Crash sign
Case 1	<5th percentile	1.43	abnormal	+
Case 2	Absent-not measurable	1.74	normal	+
Case 3	Absent-not measurable	2.12	abnormal	+
Case 4	Absent-not measurable	1.82	abnormal	+
Case 5	normal	1.1	abnormal	-
Detection rate	80%	100%	80%	80%

In 60% of cases (3/5 cases), the intracranial translucency was declared absent as it was impossible to measure in the sagittal plane.

In all 643 cases with no OSB-named control group, we noted normal (measurable) values of the IT between 1.51 and 2.63mm, with a mean value of 2.023, according to the published CRL-depended nomograms.

In the OSB group-the affected group, the IT was declared absent because no measurement could be made in 3 out of 5 cases (60%) and was found abnormally thin-less than the 5th percentile in 1 case of OSB (20%).

The mean BS/BSOB ratio value was 0.75 in the control group and 1.618 in the OSB group. In the affected foetuses, the BS/BSOB varied from 1.1 to 2.1.

Considering a cut-off of 1 for BS/BSOB ratio, the measurement proved a 100% detection rate for OSB cases respectively 5 out of 5 cases of spine defect.

Direct evaluation of the foetal spine was marked as normal or abnormal.

Initial 2D direct visualization of the spine suggested a normal aspect for 3 out of the 5 OSB cases.

After adding a second examination in suspected cases of OSB, the direct foetal spine visualization was noted abnormal by the sonographer both in 2D and also in 3D planes in 4 out of 5 cases of spine defect (80%).

In 80% of cases of OSB (4/5 cases), the ‘crash sign’ was marked present when examining the posterior foetal brain in transverse view.

In the control group, all 643 cases examined were noted without the ‘crash sign’.

Case Series

Case 1

A 42-year-old woman with a BMI of 28.3 was referred to our prenatal unit for an FTAS.

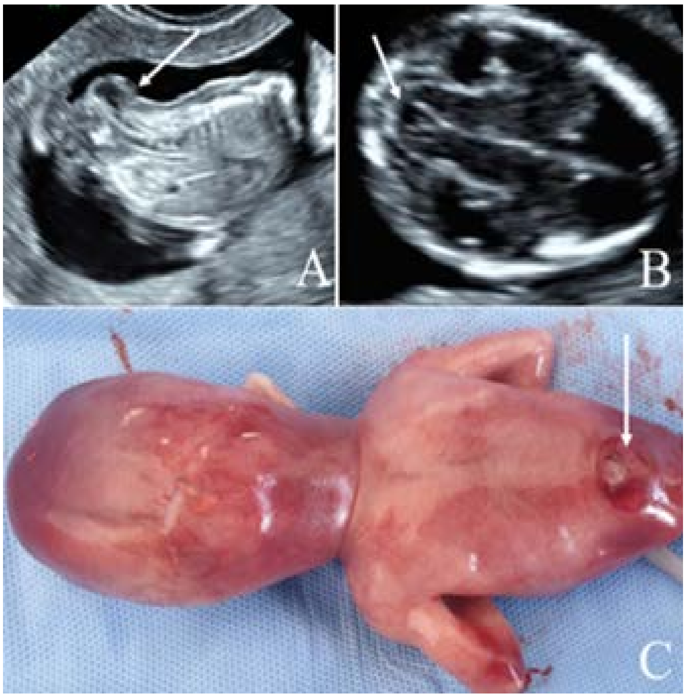

Figure 4. A case of OSB diagnosed in the first trimester (case 1): A: in the longitudinal plane, by transvaginal approach in 2D imaging, the spinal defect was identified (arrow); B: in transverse view, the “crash sign“ was noted (arrow); C: the aspect of the specimen with OSB after MTOP.

The foetus presented a crown-rump length (CRL) of 57.7mm, a gestational age of 12 weeks+2 days, and a NT of 1.2mm.

The posterior brain evaluation revealed increased BS/BOSB above the 95 percentile and a thickened brainstem.

The "crash sign" was present (Figure 4B).

Intracranian translucency was noted as less than the 5th percentile.

A bidimensional (2D) examination of the spine (Figure 4A), confirmed by three-dimensional (3D) images, revealed a low lumbosacral OSB (Figure 4C).

The patient opted for medical TOP, and the macroscopical examination of the specimen confirmed the suspected spinal defect (Figure 4C).

Case 2

In a 38-year-old patient with a BMI of 31.0, we examined a foetus with a CRL of 52.6mm, corresponding to a gestational age of 11 weeks+6 days.

The FTAS showed a normal evaluation of the foetal spine using 2D and 3D imaging, but absent IT, BS/BSOB above the 95 percentile, and an obvious "crash sign" (Figure 5 A,B,C,D).

The examination was repeated a week later and the diagnosis of OSB was made.

The spinal defect was confirmed by autopsy following TOP (Figure 5E).

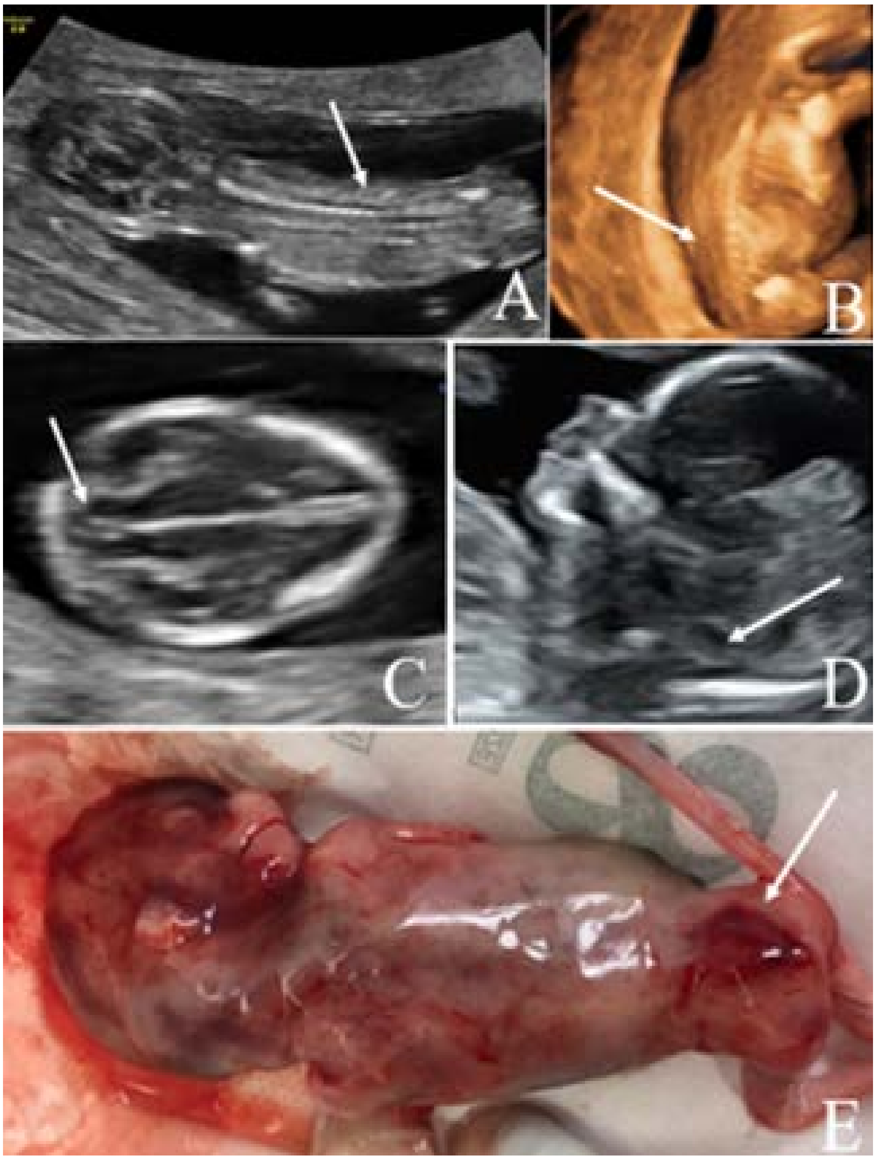

Figure 5. A case of OSB diagnosed in the first trimester (case 2): A: in the longitudinal plane, normal aspect of the integrity of the foetal spine in 2D imaging (arrow); B: foetal spine examination in 3D (arrow); C: in transverse view, the "crash sign" was noted (arrow); D: mid-sagittal view of the foetal brain with absent IT (arrow); E: the aspect of the specimen with a spinal defect (arrow) after MTOP.

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Case 3

A 27-year-old woman with a BMI of 25.1 came for a first-trimester screening at a gestational age of 13 weeks and 1 day carrying a fetus with a CRL of 69.8mm.

Absent IT, a thickened brainstem, the presence of the "crash sign" (Figure 6 A,B), a reduced BSOB distance, and an increased BS/BSOB ratio of 2.12 were noted.

Direct evaluation of the foetal spine showed a defect at the lumbosacral level both in 2D and 3D imaging (Figure 6 C,D).

The spinal defect seemed to be covered by the foetal skin during the FTAS, but the anatomopathological report showed skin discontinuity.

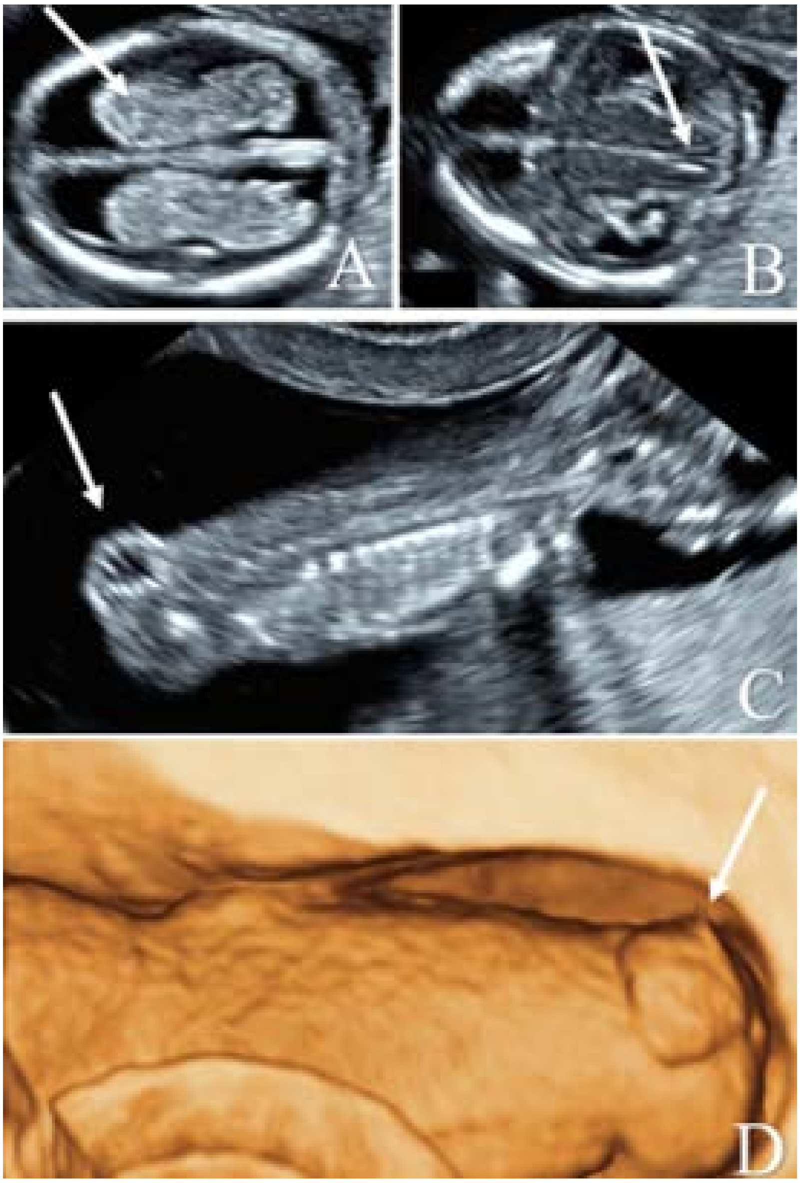

Figure 6. A case of OSB diagnosed in the first trimester (case 3):
A: in the transverse plane, the enlarged choroid plexus (arrow) and absence of the third ventricle were noted;
B: in transverse view, the "crash sign" (arrow) was also noted;
C: the spinal defect was diagnosed using 2D imaging; D: the 3D assessment confirmed OSB.

Case 4

A 36-year-old woman with a BMI of 23.1 was referred to our unit as a tertiary centre to confirm a suspected spinal defect and proper counselling.

We evaluated a 13 weeks+5 days foetus that measured 76mm in CRL and presented an absent IT, abnormal evaluation of the foetal spine in 2D imaging, a BS/BSOB of 1.82 and a present "crash sign".

The anatomopathological report confirmed the ultrasound findings.

Case 5

A 29-year-old patient with a BMI of 26.2 presented for the FTAS. We noted a CRL of 46.2mm of the foetus, corresponding to a gestational age of 11 weeks+3 days.

The examiner identified a normal IT, a BS/BSOB ratio of 1.1 and a declared normal first evaluation of the spine (Figure 7 A,B).

The “Crash sign” was absent, as the thalamus was not impacted in the occipital bone (Figure 7 C).

The same aspects were seen 10 days later during a second examination.

More than that, a diagnosis of OSB was highly suspected because the direct visualization of the integrity and skin coverage of the spine in 2D and 3D imaging showed abnormal aspects, missed by the first exam.

Later, pathology confirmed the diagnosis after MTOP.

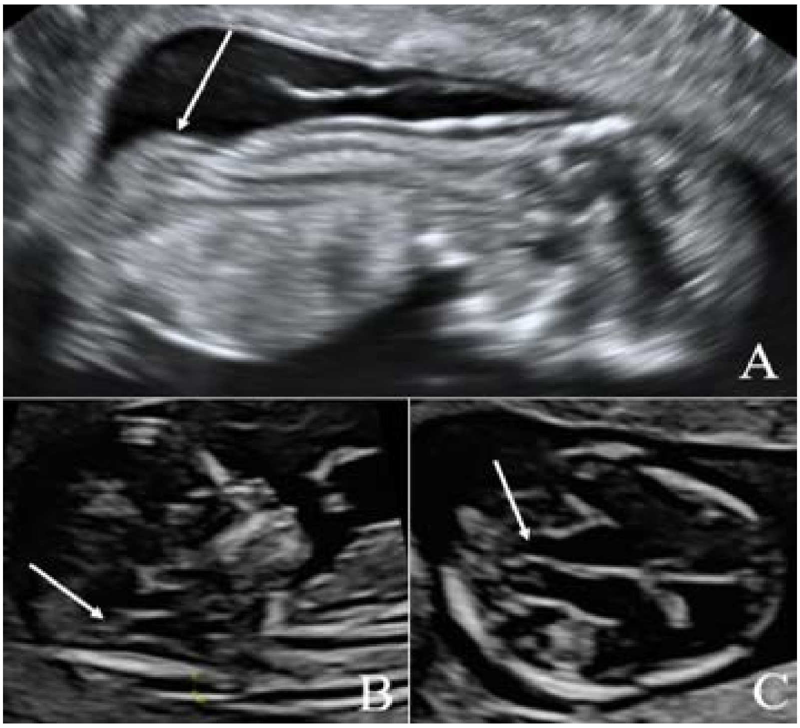

Figure 7. A case of OSB diagnosed in the first trimester (case 5):
A: in longitudinal plane, by transvaginal approach, the abnormal aspect of the foetal spine (arrow);
B: in sagittal view, a normal IT was noted (arrow);
C: in transverse view, the absence of the “crash sign“ (arrow) was also noted.

Histological Study for Ultrasound Confirmation

In the department of Histology, we evaluated all 5 foetuses with OSB and sections from the spinal defect were studied.

Histopathological examination aids in establishing a final complete diagnosis and also in the classification of OSB phenotype.

Thus, it can serve as an audit for prenatal ultrasound evaluation.

There are several types of spina bifida, classified according to the presence of the skin that covers the defect or its absence: spina bifida occulta, spina bifida aperta with meningocele, myelomeningocele and myeloschisis (Figure 8 A).

Appearances vary from a closed neural tube to an open embryonic neural plate.

Meningocele is defined by the presence of the spinal cord in the medullary canal and only the dura mater, arachnoid, and pia mater herniate through the open vertebrae.

Myelomeningocele is characterized by the herniation of the spinal cord together with the dura mater, arachnoid and pia mater.

Myeloschisis or rachischisis is similar to the aspect of the myelomeningocele, but the skin no longer covers the spinal defect (Figure 8 A,C).

In contrast to these phenotypes with complete neurulation, the spinal cord was present as an open medullary plate in myeloschisis and at this level, neurulation had not occurred and the neural tissue was embedded in the skin surface (Figure 8 B).

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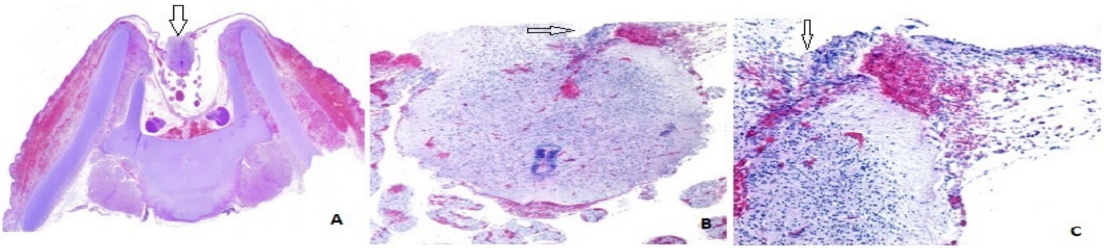

Figure 8. A cross-section through the area of the spinal defect in classic Hematoxylin-eosin staining showing the defect-a myeloschisis (A) and the herniation of the spinal cord (B,C) without coverage of the skin.

Discussions

Prenatal diagnosis performed by ultrasound to examine foetal anatomy in the first trimester of pregnancy is nowadays performed worldwide.

Although ultrasound equipment has significantly improved and examiners have perfected their technique, some abnormalities can be missed [28-31].

Here is where pathology comes in as an audit because some sonographic findings can be imprecise, and the post-mortem examination following TOP accurately describes the defect [32,33].

Our present report found discordance in one out of 5-cases of OSB after a complete histopathological assessment.

We considered the importance of comparison between histological and sonographic findings because the definitive OSB diagnosis and type can be accurately highlighted by microscopy [34-40].

Our study reported a 100% (5/5 cases) detection rate of OSB by the FTAS using the mentioned protocol of CNS evaluation combining intracranial markers with direct examination of the spine.

In 2009, Chaoui described for the first time the “intracranial translucency” as a screening marker used in the FT of pregnancy for detecting OSB.

In Orlandi et al. [41] IT was one of the studied features of the literature review, which included 11 small-size studies.

IT appearance varied from not measurable in all OSB cases in the studies of Chaoui et al. [16], Adiego et al. [17] and Iliescu et al. [18], to normal appearance in all OSB cases of Scheier et al. [19] and Solt et al. [20] (Table 2).

In our present study, IT was not visualized in 60% of cases (3/5 cases), while in the 20% of cases (1/5 cases), the IT was measured under the 5th percentile. 20% of cases of OSB (1/5cases) presented a normal IT.

We also noted that most studies reviewed are retrospective.

Lachman et al. [21] conducted a study that included 1000 unaffected foetuses and 30 OSB cases at a gestational age of 11 weeks-13 weeks+6 days.

The search showed that BS/BSOB was less than 1 in all cases of normal foetuses and always greater than 1 in the ones affected.

Iuculano et al. [42] also reported BS/BSOB greater than 1 in all OSB cases.

In our study, the BS/BSOB ratio was more than 1 in all 5 OSB cases, proving to be a reliable marker with 100% accuracy in detecting OSB in the FT.

Since the direct visualization of the spine lesions at 11-14 weeks of gestation could not be possible in all cases (only 80% of the cases in our study), we concluded that the direct US examination of the spine in the FT by herself was not a trustworthy marker for a correct OSB diagnosis.

We agreed that the screening for OSB should be based on the intracranial markers, as mentioned by previous studies [18,43,44] combined with the visualization of the spine integrity and skin coverage in 2D and 3D imaging.

A relatively new marker was described in the foetal brain axial plane, named the "crash sign."

The “crash sign” is due to a posterior shift and distortion of the midbrain toward the occipital bone.

In the same plane, narrowing or absence of the Sylvius Aqueduct may present in OSB cases [45,46].

Ushakov et al. [24] reported a detection rate of 90.6% for the crash sign in OSB cases between 11 weeks and 13 weeks+6 days.

The study of Xia Zhu et al. [44] indicated a detection rate of 85.7% and uncovered a particular situation in which the cases with OSB that had the crash sign present also presented a 3-line view.

In the one case of OSB with an absent crash sign, the 4-line view was noted.

On the midsagittal view, the posterior brain markers included 3 anechoic bands and 4 lines, which gives the 4-line view.

The 4 lines are from bottom to top: the occipital bone, the fourth ventricle choroid plexus, the fourth ventricle anterior border and the sphenoid bone posterior border as previously described [44].

Even in the negative “crash sign” case, the BS/BSOB ratio was greater than 1.

Our study reports a detection rate of OSB by using the crash sign slightly smaller (80%) than in these previous papers [24,41].

As opposed to direct visualization of the spine lesion, searching these intracranial markers includes taking measurements of the posterior cerebral structures that can add time to the examination [51,52].

Still, IT, BS diameter or BS/BSOB ratio can be evaluated in the sagittal plane commonly used for the NT measurement and nasal bone assessment.

Our protocol included all these intracranial markers with no additional time reported.

Table 2 Studies regarding the identification of spina bifida using intracranial translucency.

Study authors	Study design	Number of cases		Application	Main outcome
		Total no. of cases	Affected foetuses		
Mangione et. al [47]	Retrospective	260	52	Spina bifida	CM sensitivity > IT sensitivity (DR=29-48%)
Garcia-Posada. [48]	Retrospective	95	5	Spina bifida	IT n.v.in 2/5 cases (DR=40%)
Fong et al. [49]	Retrospective	199	8	Spina bifida	Sensitivity of absent IT=50%
Chaoui et al. [43, 50]	Retrospective	204	4	Spina bifida	n.v. IT sensitivity is 100%
Iliescu et al. [18]	Retrospective	1824	2	Spina bifida	IT sensitivity is 100%
Adiego et al. [17].	Prospective	999	1	Spina bifida	IT sensitivity is 100%
Orlandi et al, [41]	Prospective	3	1	Spina bifida	IT n.v. in 1/3cases

IT, intracranial translucency; OSB, open spina bifida; CM, cisterna magna

Conclusions

This study confirmed that OSB is associated with cerebral abnormalities detected by the FTAS.

Combined intracranial ultrasound markers, such as abnormal IT, the presence of "crash sign" and especially BS/BSOB ratio greater than 1 offer a high accuracy in diagnosing OSB in FT.

Meanwhile, the direct visualization of the spinal defect can sometimes escape a FT evaluation.

Therefore, we encourage health providers to use both midsagittal and axial planes in the FT in order to search for OSB intracranial sonographic markers and also for OSB direct features (spinal defect and meningocele).

The histopathological examination offers additional information for the final complete diagnosis and also aids in the correct classification of OSB phenotype.

Moreover, it can also serve as an audit for prenatal ultrasound evaluation.

Microscopic images have been acquired in the Research Centre for Microscopic Morphology and Immunology, University of Medicine and Pharmacy of Craiova, Romania (Manager: Laurențiu Mogoantă, Professor, MD, PhD).

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Author’s contribution

Delia Roxana Ungureanu and Roxana-Cristina Dragusin equally contributed to this article.

Conflict of interests

The authors report no conflicts of interest.

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