Ultrasound Obstet Gynecol 2016; **48**: 16–25
Published online in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.15926

Editorial

Diagnostic imaging tools to elucidate decreased cephalic biometry and fetal microcephaly: a systematic analysis of the central nervous system

L. GUIBAUD* and A. LACALM

Université Claude Bernard Lyon I, Imagerie Pédiatrique et Fœtale, Centre Pluridisciplinaire de Diagnostic Prénatal, Hôpital Femme Mère Enfant, Lyon-Bron, France
**Correspondence. (e-mail: laurent.guibaud@chu-lyon.fr)*

Introduction

Although the most important feature warranting investigation of central nervous system (CNS) disorders during prenatal examination is ventriculomegaly, anomalies of cephalic biometry are also an important clue to the presence of fetal cerebral pathology. In routine practice, any decrease in cephalic biometry to $< 5^{th}$ percentile raises suspicion of underlying microcephaly. However, as discussed below, distinction must be made between suspicion of microcephaly and true microcephaly. True fetal microcephaly (as defined in Tool 2) is associated, in many prenatal cases, with morphological anomalies[1–3]. It may be considered as part of a complex disorder, such as syndromes with or without chromosomal anomalies, or it may be associated exclusively with cerebral anomalies[1,3], being related either to primary cerebral organization disorders affecting all steps of CNS development, or to clastic events due to ischemohemorrhagic or infectious conditions; the latter is of particular interest currently, in light of the recent emergence of microcephaly related to Zika virus infection[1–5]. Primary cerebral organization disorders include disorders of neural tube development (neural tube defect), prosencephalon cleavage (holoprosencephaly), precursor cell proliferation (microcephaly with simplified gyral pattern), neuroblast migration (lissencephaly) and corticogenesis (polymicrogyria). In all these cases, microcephaly is associated with structural CNS anomalies. However, in some cases, microcephaly can be truly isolated, i.e. with no associated structural anomalies, often being related to familial disorders and transmitted mostly as an autosomal recessive trait[1]. In such cases, prenatal cephalic biometry is usually within the normal or subnormal limits and microcephaly develops after birth, with dramatic worsening of cephalic biometry during the first years of postnatal life[6,7]. Such isolated familial microcephaly is rarely of concern when facing isolated microcephaly in the prenatal period.

As published previously for ventriculomegaly[8], we outline here our systematic approach to the management of patients referred to our institution due to an unexplained decrease in cephalic biometry. Our approach is based on a set of diagnostic tools to diagnose and elucidate a decrease in fetal cephalic biometry, which may lead to suspicion of microcephaly with underlying CNS structural anomalies. It should be noted that its application should always be guided according to the clinical context. These tools are tailored for ultrasound examination, as recommended by Persutte[9] and Kurtz *et al.*[10], but can also be used for magnetic resonance imaging (MRI) investigation. Indeed, this etiological approach to the diagnosis of microcephaly is a perfect illustration of the potential contribution of MRI to the investigation of CNS anomalies, as ultrasound is often limited in more severe cases as a result of poor acoustic windows due to the narrowing of the sutures. In such cases, MRI undoubtedly offers more information than does ultrasound for cerebral anatomical analysis, especially regarding complete gyration and pericerebral space examination during the third trimester of pregnancy[11]. Finally, we should emphasize that this article is intended not as an epidemiological study, but as a proposal to conduct a systematic analysis of the CNS when facing unexplained decreased cephalic biometry, based on a selection of pedagogical cases.

Tool 1: Context

As for unexplained ventriculomegaly, the clinical context should always be considered when facing an unexplained decrease in cephalic biometry. For the etiological work-up the context is crucial, as factors underlying microcephaly can be environmental, genetic or of unknown origin[1,3]. Clinical data such as unexplained fever during pregnancy, drug use, alcohol consumption, risk factors for vascular clastic lesions (e.g. monochorionic twin pregnancy) and maternal phenylketonuria should be scrutinized and integrated into the work-up in order to diagnose environmental microcephaly. Consanguinity and cephalic biometry of both siblings and parents may also provide important clues in the diagnosis of microcephaly as well as of variants in cephalic biometry of genetic origin.

The context should also include a complete analysis of extracephalic biometry. This may help to differentiate between a predominant or exclusive decrease in cephalic biometry related to an encephaloclastic mechanism, such as that encountered in cytomegalovirus (CMV) infection or lissencephaly Type 3, and global reduction of fetal biometry, such as in severe global intrauterine growth restriction.

Tool 2: Determine severity of decreased cephalic biometry and follow up longitudinally

Unlike ventriculomegaly, for which there is consensus on its prenatal definition, there is no absolute threshold for the definition of prenatal microcephaly. However, as has been stated by Pilu *et al.*[3] and Deloison *et al.*[12], the smaller the head dimension, the greater the probability of microcephaly with underlying pathology. For pediatricians, 'true' microcephaly refers to cephalic biometry > 3 SD below the mean for age and sex, as also reported in the prenatal period by Chervenak *et al.*[2]. However, the issues regarding a positive diagnosis of microcephaly are completely different between the pre- and postnatal periods. In the postnatal period, the positive diagnosis based on 3 SD below the mean is a clinical threshold that leads to investigation of the underlying etiology by adequate work-up, this being otherwise indicated according to the clinical status and family history of the patient if the threshold is not reached. In the prenatal period, any decrease in cephalic biometry should be investigated in order to diagnose underlying CNS pathology as early as possible, even before reaching 3 SD below the mean, which is more likely to occur late in pregnancy at a time when termination of pregnancy is not permissible in many countries or the obstetric risks are greater.

The use of percentiles for routine prenatal sonography leads to suspicion of fetal microcephaly when cephalic biometry is below either the 5th or 3rd percentile, depending on the reference being used (the choice of appropriate reference is beyond the scope of this article). It should be noted that the 3rd percentile corresponds to approximately 2 SD, while 3 SD corresponds to 0.2 percentile. Thus, defining microcephaly as cephalic biometry < 3rd percentile leads to significant overdiagnosis of fetal microcephaly, including 3% of the general population, whereas only 0.2% of the general population are included using a limit of 3 SD[12,13]. This accounts for the fact that prenatal head circumference (HC) between 2 SD and 3 SD below the gestational mean is not a particular risk factor for later abnormal neuropsychological development, as has been shown by Stoler-Poria *et al.*[14]. Thus, we propose that one should differentiate in the prenatal period between two different HC thresholds, i.e. a cut-off for 'diagnosis of microcephaly', defined as fetal HC ≥ 3 SD below the mean for gestational age, and a cut-off for 'prompting investigation', defined as fetal HC ≥ 2 SD below the mean, which should lead to prompt neurosonographic examination, as outlined in this article.

It should be emphasized also that the use of percentiles is not suitable for evaluation of the severity of decrease in cephalic biometry as it is based on the distance to the mean, thus, use of either SD or *Z*-scores is required[12]. In routine practice, the distance to the mean can also be expressed using the growth delay (in number of gestational weeks) between the theoretical gestational age and the gestational age corresponding to the 50th percentile of the observed HC. For example, for a gestational age of *X* weeks, if the HC corresponds to the 50th percentile of *X* – 3 weeks, the growth delay is 3 weeks. The correlation between growth delay (in number of weeks) and SD of HC has been presented by Hadlock *et al.*[15] (Table 1). Correct expression of the severity of the decrease in cephalic biometry is an important issue as, in some particularly severe cases, it can be a clue to etiology.

Table 1 Correlation between head circumference ± 2SD from the mean and growth delay according to gestational age (from Hadlock *et al.*[15])

Gestational age (weeks)	*Growth delay (weeks)*
12–18	± 1.3
18–24	± 1.6
24–30	± 2.3
30–36	± 2.7
36–42	± 3.4

As mentioned above, it should be noted that decrease in cephalic biometry can be a progressive process. In some cases, microcephaly can be detected in the second trimester, especially in cases of spina bifida and in some syndromic entities, particularly chromosomal anomalies[12]. However, microcephaly cannot be excluded by normal biometry during the second trimester, as shown by Bromley and Benacerraf[16], and can be identified more easily or exclusively during the third trimester, in cases of underlying late developmental or clastic anomalies, respectively (Figure S1). Because of this potential for late manifestation of microcephaly, particular care should be taken when cephalic biometry is close to the 3rd percentile during the second trimester, especially if there is a discrepancy between small HC and extracephalic biometry. In such cases, close follow-up of the cephalic biometry and careful cerebral sonographic investigation using the following tools may help in diagnosing or excluding any underlying CNS anomalies, which may be more obvious during late pregnancy.

Tool 3: Investigate the pericerebral space

As we have previously maintained[8], the pericerebral space, which is often overlooked, should in fact be included in a systematic approach to patients with an unexplained decrease in cephalic biometry, even though, to date, this remains subjective. Investigation of the pericerebral space is useful for accurate evaluation of the severity of any decrease in cephalic biometry and even, in some cases, for positive diagnosis of microcephaly, as well as for understanding the underlying mechanism. As diagnosis of microcephaly is based on sonographic measurement of the HC, by means of an ellipse drawn on or around the calvarium (depending on the reference being used), it follows that a decrease in this sonographic measurement should lead to a diagnosis of 'microcrania'. In fact, 'microcephaly' refers to reduction of the cerebral volume (or microencephaly), as defined by neuropathologists. In most cases, reduction of the sonographic cephalic measurement, or microcrania, based on the circumference of the calvarium, reflects underlying microencephaly. However, as

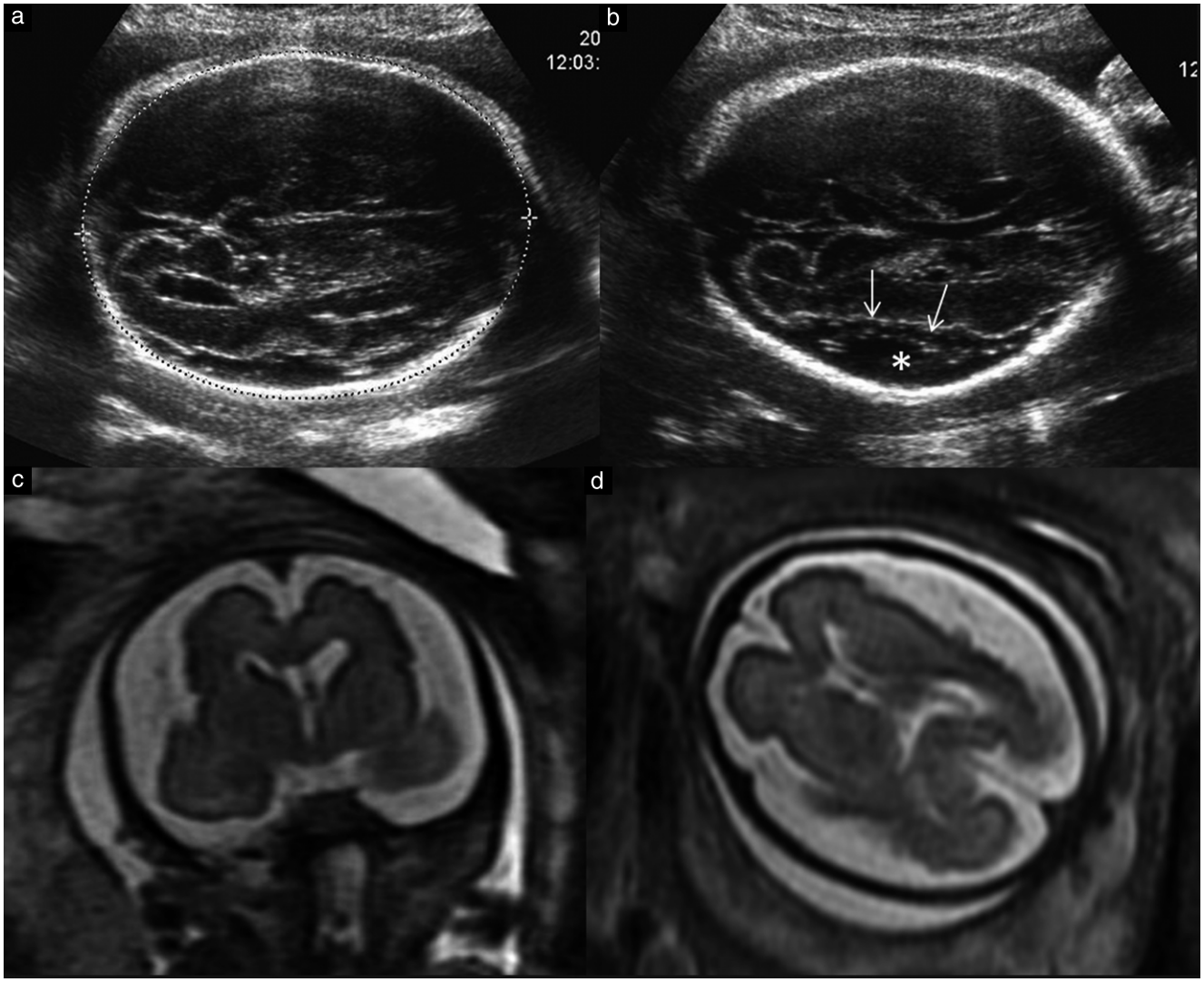

Figure 1 Fetal imaging in a patient referred at 27 gestational weeks due to unexplained enlargement of pericerebral space. Cephalic biometry was close to 50th percentile (head circumference, 270 mm). Axial sonographic images (a,b) confirming enlargement of the pericerebral space, which was more pronounced close to the corona radiata (*), associated with abnormal smooth cortical surface (arrows). Coronal (c) and axial (d) T2-weighted fetal magnetic resonance images (MRI) demonstrating both a major diffuse increase in pericerebral space and diffuse anomalies of the cortical ribbon, which show lack of gyration and an undulated cortical surface suggestive of diffuse polymicrogyria. Cephalic biometry at MRI gave biparietal and fronto-occipital diameters of −3 SD and −5 SD, respectively, according to the atlas of Garel[33]. Pathological examination confirmed both microcephaly, as suggested by a cerebral weight of 140 g (< 5th percentile), and diffuse polymicrogyria, associated with neuronal and leptomeningeal cortical heterotopia, suggestive of a diffuse clastic mechanism (not shown).

stated clearly by Pilu *et al.*[3], measurement of the calvarium represents, at best, a rough estimate of cerebral development. Depending on the size of the pericerebral space, the sonographic HC does not always reflect the underlying microencephaly, and, indeed, we have encountered cases in which a normal or subnormal sonographic HC is associated with severe underlying microencephaly due to markedly enlarged pericerebral space, as illustrated in Figure 1. In such cases, evaluation of the cerebral volume using MRI or, at least, use of MRI cerebral measurements (biparietal and fronto-occipital diameters) is more accurate than is sonographic cephalic measurement (Figure 1).

Investigation of the pericerebral space may also provide an etiological clue to diagnosis of the underlying cerebral pathology. In most severe encephaloclastic lesions, the pericerebral space is enlarged, as illustrated by the more severe cases of CMV infection (Figure 2) or diffuse vascular ischemic lesions, as reported by Pilu *et al.*[3]. Such enlargement of the pericerebral space is also typical in lissencephaly Type 3, a developmental condition in which microcephaly is associated with akinesia, due to neuroapoptosis that leads to a marked neuronal depletion of the entire CNS with subsequent severe cerebral atrophy[17] (see Figure S6). In some vascular clastic events, the enlargement of the pericerebral space can be more limited and localized, adjacent to the focal clastic lesion.

Tool 4: Look for the Sylvian fissure as the main marker of gyration

As the Sylvian fissure represents the main landmark for gyration, its analysis should be integrated into the imaging

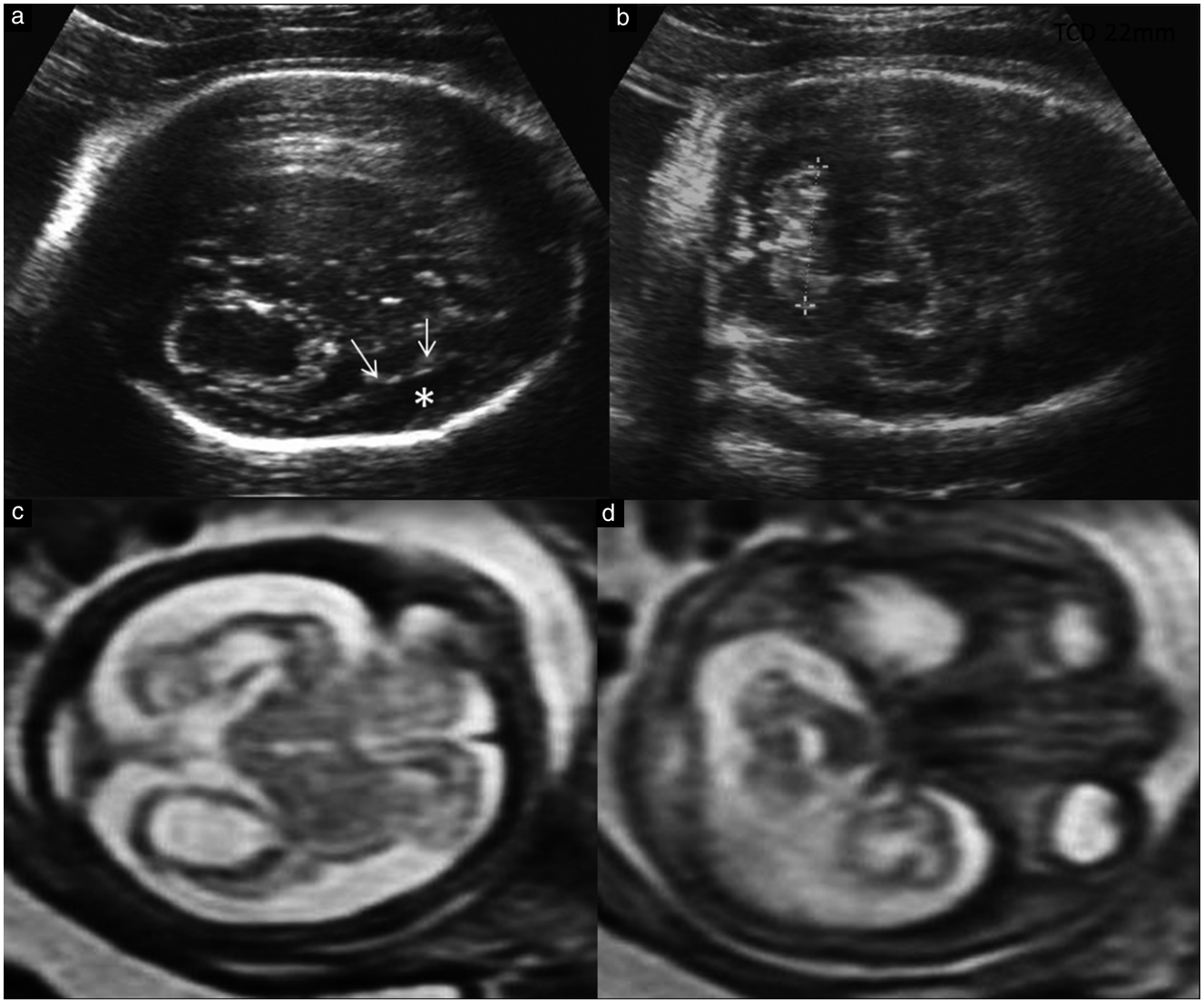

Figure 2 Fetal imaging in a patient referred at 29 gestational weeks due to unexplained ventriculomegaly, associated with significant reduction in cephalic biometry (head circumference corresponded to the 50th percentile at 25 weeks, equivalent to a decrease of between −3 SD and −4 SD). (a) Axial supratentorial sonographic image showing an echogenic periventricular band, echogenic foci suggestive of calcifications, an abnormal largely open Sylvian fissure (arrows) and increased pericerebral space (*). (c) Global increased pericerebral space was confirmed on T2-weighted magnetic resonance imaging in the axial plane, which also showed thinning of the parenchymal cerebral mantle more clearly. Axial sonographic (b) and T2-weighted magnetic resonance infratentorial (d) images demonstrating a major decrease in transverse cerebellar diameter (22 mm), suggestive of severe cerebellar hypoplasia. The combination of an 'infectious clastic pattern' with increased pericerebral space, diffuse cortical dysplasia (polymicrogyria) and cerebellar hypoplasia was highly suggestive of encephaloclastic cytomegalovirus fetopathy, which was confirmed by the biological work-up.

work-up of the fetal brain. Gyration should be scrutinized carefully when there is any decrease in cephalic biometry or true microcephaly, for both diagnostic and etiological purposes. This requires knowledge regarding the shape of the Sylvian fissure in the axial plane, according to gestational age. We published previously a reliable subjective method with which to assess the shape of the Sylvian fissure between 22 and 32 weeks of gestation using a simple score-based evaluation of operculization of the posterior part of the fissure in a standardized view on an axial cerebral plane[18,19] (Figure S2). Using this method in routine prenatal imaging can help in the early diagnosis of anomalies of operculization. These anomalies can reflect underlying cortical developmental or clastic lesions, such as diffuse polymicrogyria (Figures 2 and S1) or lissencephaly Type 1 (Figure 3)[19], which are, in most cases, associated with microcephaly, but can also reflect extracortical developmental anomalies, especially abnormal cerebral volume. We have shown that lack of development of the anterior margin of the Sylvian fissure may be a clue to frontal hypoplasia and therefore be diagnostic of microcephaly, as also shown by Goldstein *et al.*[20] and Persutte *et al.*[21] (Figure 4c and d).

Tool 5: Do not assume that a smooth cerebral surface indicates lissencephaly

It should be noted that a smooth cerebral surface with complete absence of folding or with reduced gyri and abnormal shallow sulci on sonographic examination does

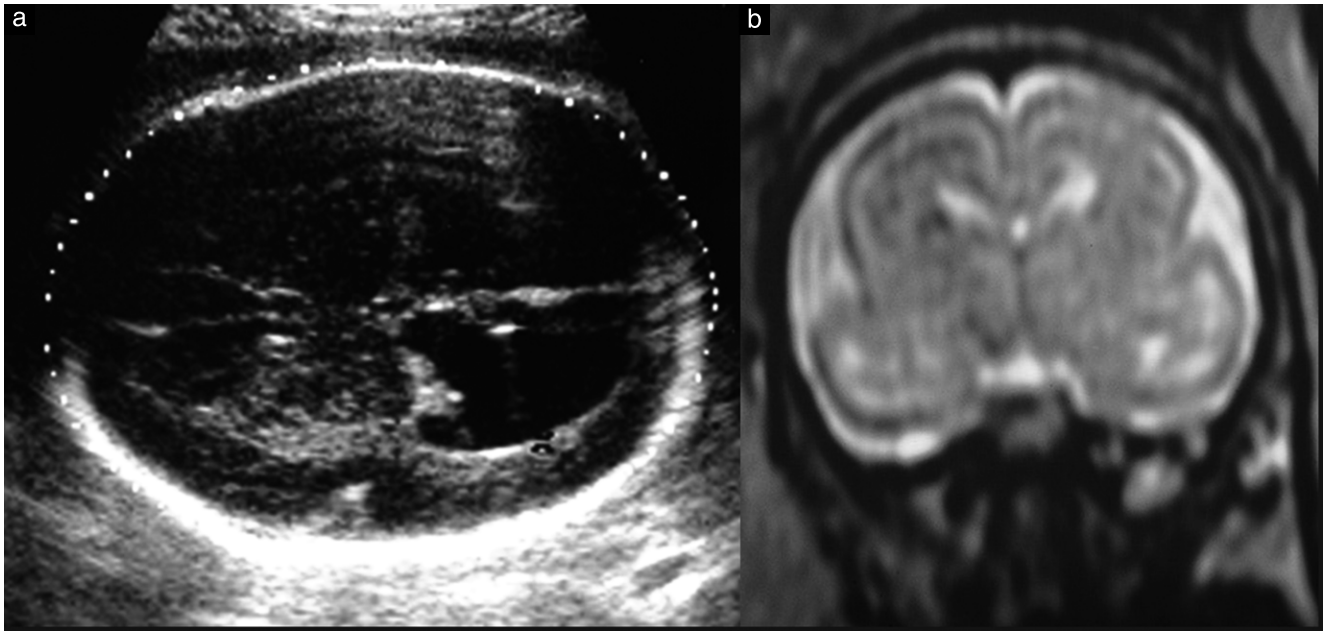

Figure 3 Fetal imaging in a patient referred at 28 gestational weeks due to ventriculomegaly and severe reduction in cephalic biometry. (a) Axial sonographic image showing an echogenic periventricular area and abnormal operculization of the Sylvian fissure, demonstrating rudimentary and moderate ventriculomegaly. Cephalic biometry corresponded to the 50th percentile at 24.5 weeks, equivalent to a decrease close to −3 SD. (b) Coronal T2-weighted magnetic resonance image (MRI) confirming suspicion of gyral anomalies, demonstrating a typical figure-of-eight-shaped appearance of the brain, with a thick cortex and smooth surface suggestive of lissencephaly Type 1, which was confirmed on postnatal MRI. Note that the echogenic periventricular area on sonographic examination was associated with abnormal waves of neuronal migration, as shown by MRI.

not necessarily indicate a diagnosis of lissencephaly. Although the term 'lissencephaly' means 'smooth brain' and refers to a paucity of gyral and sulcal development on the surface of the brain, it in fact has a particular etiology and histological characteristics[22]. Classic lissencephaly (or lissencephaly Type 1), the most common form, refers to a smooth brain associated with a thick or double cortex, with a specific histological layered structure; it is related, in two-thirds of cases, to either *LIS1* (Figure 3) or *DCX* gene mutation, or, in a few cases, to a mutation in one of the tubulin genes, mostly *TUBA1A*[23]. However, the sonographic appearance of 'smooth brain' is also encountered in microcephaly with a simplified gyral pattern, which displays a normal or thin cortical ribbon (Figures 4 and S3), or with diffuse polymicrogyria (Figure 5)[4]. In these two entities, related to deficient precursor cell proliferation and cortical organization disorders, respectively, the putative genes involved differ completely from those involved in classic lissencephaly. Therefore, when faced with a smooth brain on sonographic examination, one should avoid using the term 'lissencephaly', because, in the absence of any confirmation by neuropathological examination, doing so may lead to inadequate genetic testing (for example, testing for *LIS1* or *DCX* gene mutation in a case of microcephaly with simplified gyral pattern, related in fact to one of the 12 currently known genes for this entity, such as *MCPH1* or *ASPM*). In such cases, fetal MRI can be of help, enabling differentiation between classic lissencephaly and microcephaly with simplified gyral pattern or diffuse polymicrogyria on the basis of an analysis of cortical thickness (cortical ribbon) that cannot be achieved using ultrasound[4,11].

Tool 6: Look for clastic events

Diffuse cerebral clastic events, of either primitive vascular or infectious origin, result in cerebral atrophy and subsequently in both increased pericerebral space and ventriculomegaly. Therefore, as stated for the imaging work-up of unexplained ventriculomegaly[8], one should look systematically for a clastic pattern when faced with an unexplained decrease in cephalic biometry. The clastic pattern associated with infection is similar to that described for ventriculomegaly. This 'infectious clastic pattern' can include a periventricular echogenic halo in the subependymal zone, which may be associated with germinolysis cysts, periventricular or parenchymal calcification, or pathognomonic cystic changes posterior to the occipital horn or anterior to the temporal horn[8]. This pattern can also be associated with an abnormal Sylvian fissure, suggestive of polymicrogyria, and, as mentioned previously, with enlarged pericerebral space adjacent to the abnormal cortical surface (Figure 2). Such a clastic pattern is typically encountered in severe CMV infection[24]; it has also been described very recently, in Brazil, in Zika viral intrauterine infection, a mosquito-borne disease related closely to yellow fever, dengue fever and West Nile and Japanese encephalitis[5]. In the two cases reported by Oliveira Melo *et al.*[5], the vertical intrauterine transmission of this viral infection led to

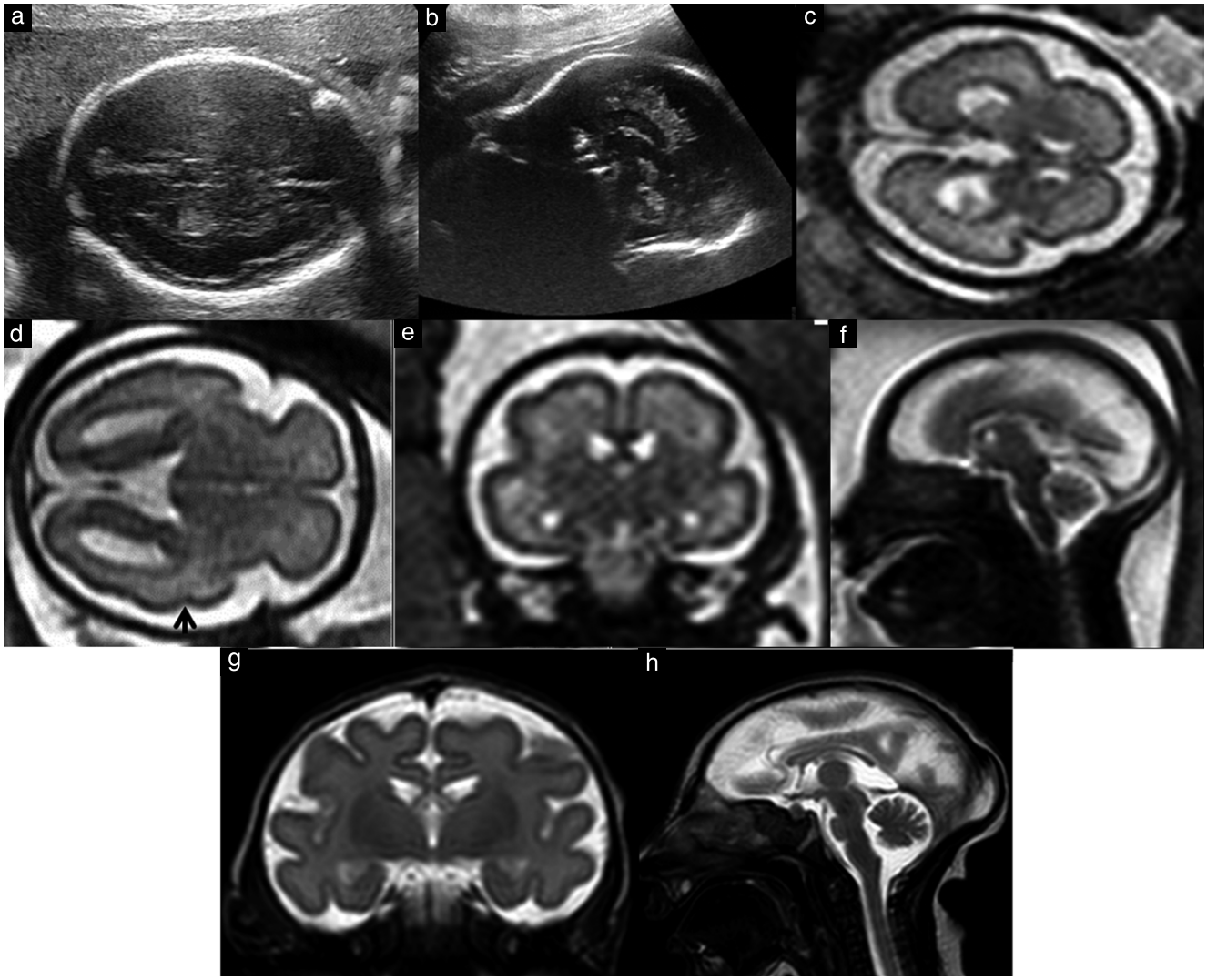

Figure 4 Fetal and postnatal imaging in a patient referred at 26 gestational weeks due to both a decrease in cephalic biometry and non-identification of the corpus callosum, with no context of consanguinity. At first sonographic examination, microcephaly was confirmed, with head circumference (HC) corresponding to the 50th percentile at 23 weeks, equivalent to a decrease of 2.5 SD, associated with normal extracephalic biometry. Axial (a) and mid-sagittal (b) sonographic images showing both smooth cortical surface with a lack of gyral pattern (a) and presence of thin complete corpus callosum (b). Sonographic follow-up at 28.3 weeks demonstrated severe, non-progressive microcephaly (delayed by 3 weeks according to cephalic biometry) without any changes in gyral pattern (smooth cortical surface) on axial sonographic image (not shown). These findings were confirmed on axial (c) and coronal (e) T2-weighted fetal magnetic resonance imaging (MRI), which demonstrated smooth cortical surface, very few sulci and normal thickness of cortical ribbon. Note lack of operculization of Sylvian fissure and absence of superior temporal sulcus (c) compared with control at 28 weeks on axial T2-weighted fetal MRI (arrow) (d), in particular an absence of any anterior margin of the Sylvian fissure related to marked frontal hypoplasia, as stated in Guibaud *et al.*[19]. Sagittal T2-weighted fetal MRI (f) confirmed presence of a thin corpus callosum and revealed suspicion of a slight decrease in volume of the pons. Prenatal imaging data were suggestive of microcephaly with a highly simplified gyral pattern. After prenatal counseling, the parents elected to continue the pregnancy despite high probability of poor cognitive outcome. (g,h) Postnatal MRI at 8 days confirmed the diagnosis of microcephaly with a highly simplified gyral pattern (HC, 28.5 cm at term) as well as pontine hypoplasia.

fetal microcephaly and brain damage, including calcifications, suggestive of an underlying infectious condition, but also severe damage of the cerebellum, brainstem and thalami. In one case, bilateral cataracts, intraocular calcifications and unilateral microphthalmia were demonstrated.

The vascular clastic pattern differs slightly from that described for unexplained ventriculomegaly. For the latter, we underlined the hemorrhagic changes of both ventricular wall and lumen, which lead to the diagnosis of the underlying ischemohemorrhagic event[8]. In the case of a decrease in cephalic biometry, the clastic vascular pattern includes mainly late anatomical changes related to diffuse ischemic events. Even if both hemispheres are involved, this includes asymmetrical parenchymal changes, such as abnormal parenchymal echogenicity, cavitations, parenchymal cleft, calcifications, asymmetrical ventriculomegaly and enlargement of the subarachnoid space (Figures 6 and S4), as well as diffuse and asymmetrical cortical anomalies[25]. This pattern is typically encountered in severe brain damage following fetal death in monochorionic twin pregnancy.

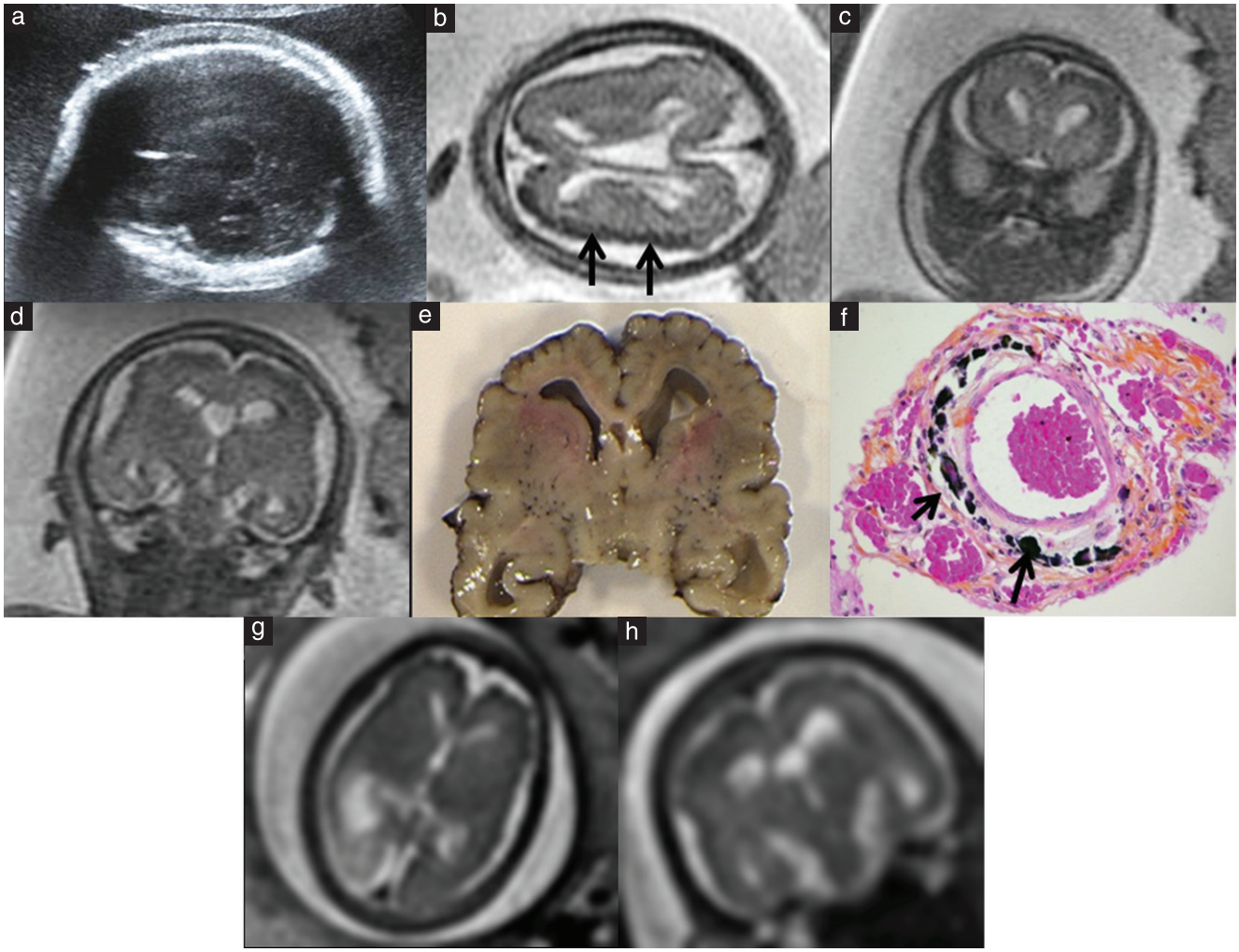

Figure 5 Fetal imaging in a patient referred at 29 gestational weeks due to a major decrease in cephalic biometry within the context of consanguinity, associated with medical history of neonatal death of the mother's first baby, with a prenatal imaging diagnosis of significant reduction in cephalic biometry related to 'lissencephaly'. This latter diagnosis was not confirmed by pathological examination because the parents declined autopsy. All genetic testing for classic lissencephaly was negative. Sonographic examination performed in our department confirmed major microcephaly with head circumference corresponding to the 50th percentile at 25 weeks, equivalent to a decrease to between −3 SD and −4 SD, associated with normal extracephalic biometry. (a) Despite poor acoustic windows due to narrowing of sutures related to microcephaly, axial sonographic examination showed a smooth cortical surface as well as a very rudimentary Sylvian fissure. Axial (b) and coronal (c,d) T2-weighted fetal magnetic resonance (MR) images demonstrated abnormal gyration with undulated and mildly thickened cortical ribbon, suggestive of diffuse polymicrogyria. After prenatal counseling, the parents elected for termination of pregnancy due to high probability of severe postnatal outcome. Neuropathological examination confirmed diffuse polymicrogyria at macroscopy (e) and showed parietal arterial calcifications destroying the media (f, arrows), which was replaced by fibroblastic proliferation, leading to reduction of vascular lumen on microscopy. These findings were suggestive of diffuse polymicrogyria related to vascular insult, most likely due to underlying genetic vasculopathy, in keeping with the medical history. Interestingly, retrospective review of axial (g) and coronal (h) T2-weighted fetal MR images from previous pregnancy showed similar gyral anomalies, suggestive of diffuse polymicrogyria, which had been misinterpreted as lissencephaly.

Tool 7: Evaluate the midline

In the case of a decrease in cephalic biometry, the midline should be examined carefully to investigate etiology. It should be borne in mind that the appearance of the commissures, especially the corpus callosum, can be affected anatomically by severe microcephaly, regardless of underlying etiology.

One should assess the anterior complex on routine axial images[26]. Holoprosencephaly, especially the most severe forms (alobar and semilobar), is usually associated with decreased cephalic biometry, so diagnosis relies on the absence of a normal anterior complex, including non-identification of the cavum septi pellucidi, distortion of interhemispheric fissure and abnormal or absent frontal horns (Figure S5)[26]. Analysis of the corpus callosum should be performed by an expert in fetal CNS imaging (neurosonogram or fetal MRI). It should be performed in the mid-sagittal plane. Absence of its anterior part can also be a clue to the diagnosis of semilobar holoprosencephaly, although microcephaly associated with corpus callosal agenesis/dysgenesis, including hypoplasia, has also been reported in some syndromes. However, it should be noted that, in severe microcephaly, corpus callosal hypoplasia

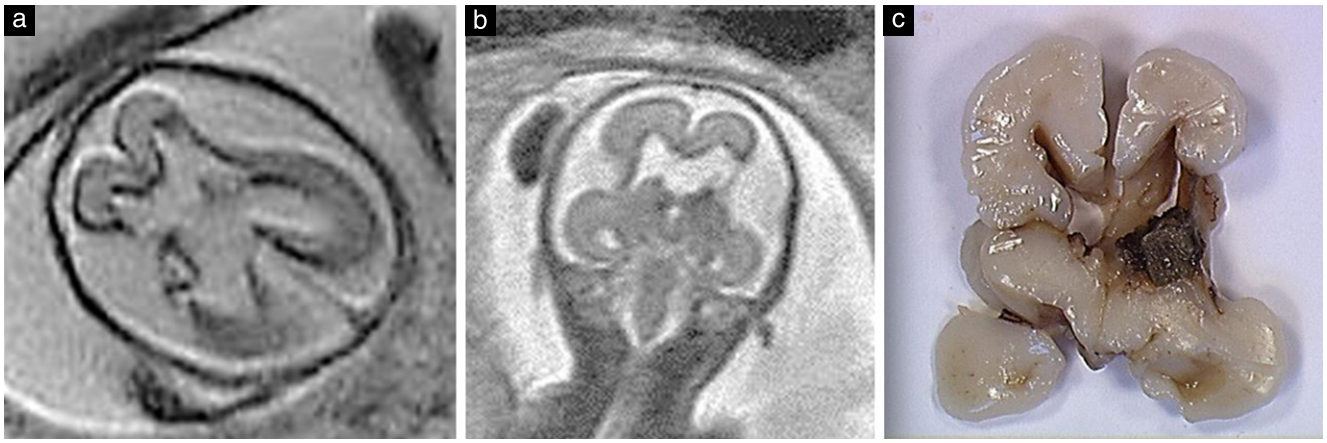

Figure 6 Fetal imaging in a patient referred at 22 gestational weeks due to enlarged pericerebral space with mild decrease in cephalic biometry (5^{th} percentile). Axial (a) and coronal (b) T2-weighted fetal magnetic resonance images demonstrating major amputation of cerebral parenchyma adjacent to the insula, more pronounced on the left hemisphere, associated with no evidence of the septum pellucidum. Major increase of the pericerebral space adjacent to the clastic changes was also noted. (c) Macroscopic pathological examination confirmed clastic lesions adjacent to the insula with both porencephalic lesions and bilateral pseudoschizencephalies related to an ischemohemorrhagic phenomenon.

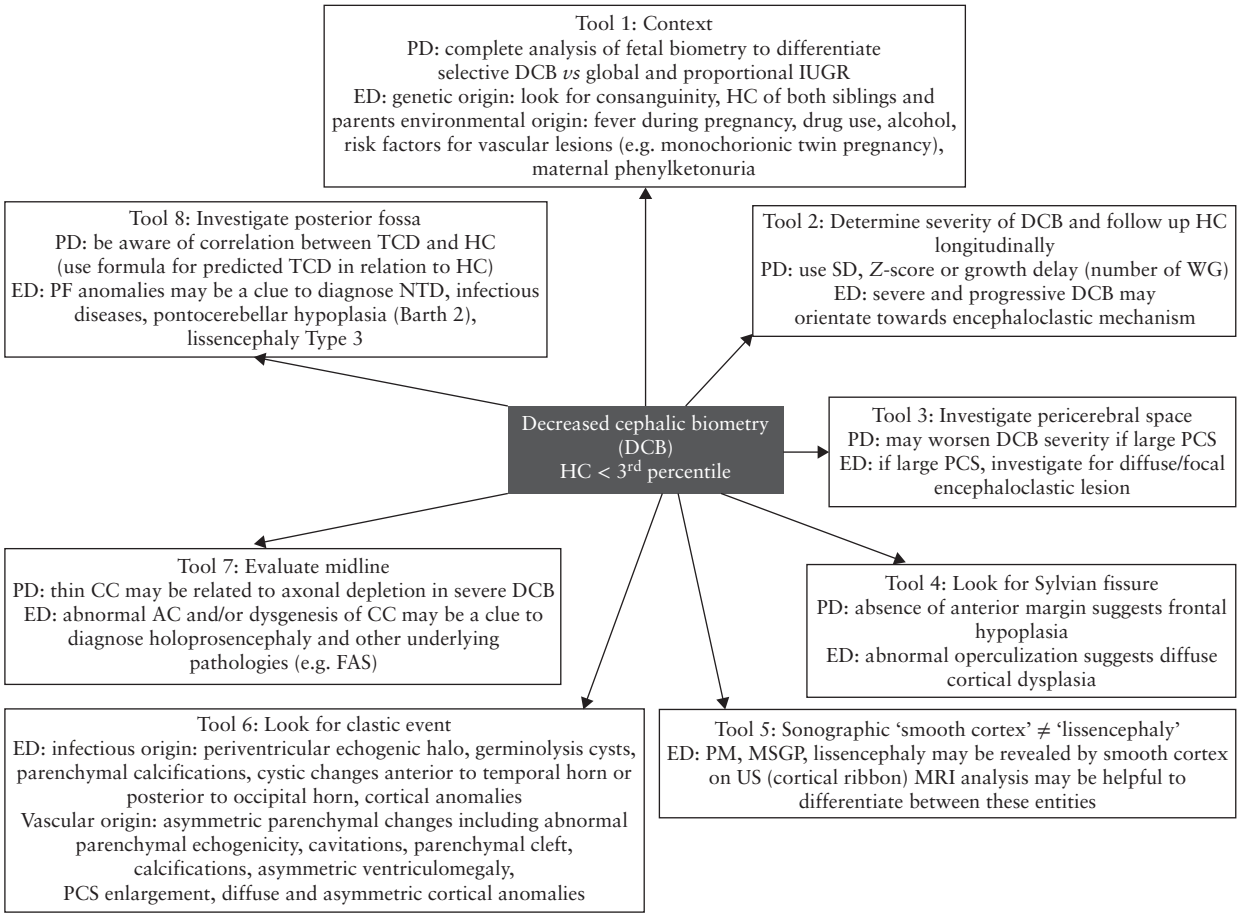

Figure 7 Diagnostic imaging tools to elucidate decreased cephalic biometry using a systematic analysis of the central nervous system. AC, anterior complex; CC, corpus callosum; ED, etiological diagnosis; FAS, fetal alcohol syndrome; HC, head circumference; IUGR, intrauterine growth restriction; MRI, magnetic resonance imaging; MSGP, microcephaly with simplified gyral pattern; NTD, neural tube defect; PCS, pericerebral space; PD, positive diagnosis; PF, posterior fossa; PM, polymicrogyria; TCD, transverse cerebellar diameter; US, ultrasound; WG, weeks' gestation.

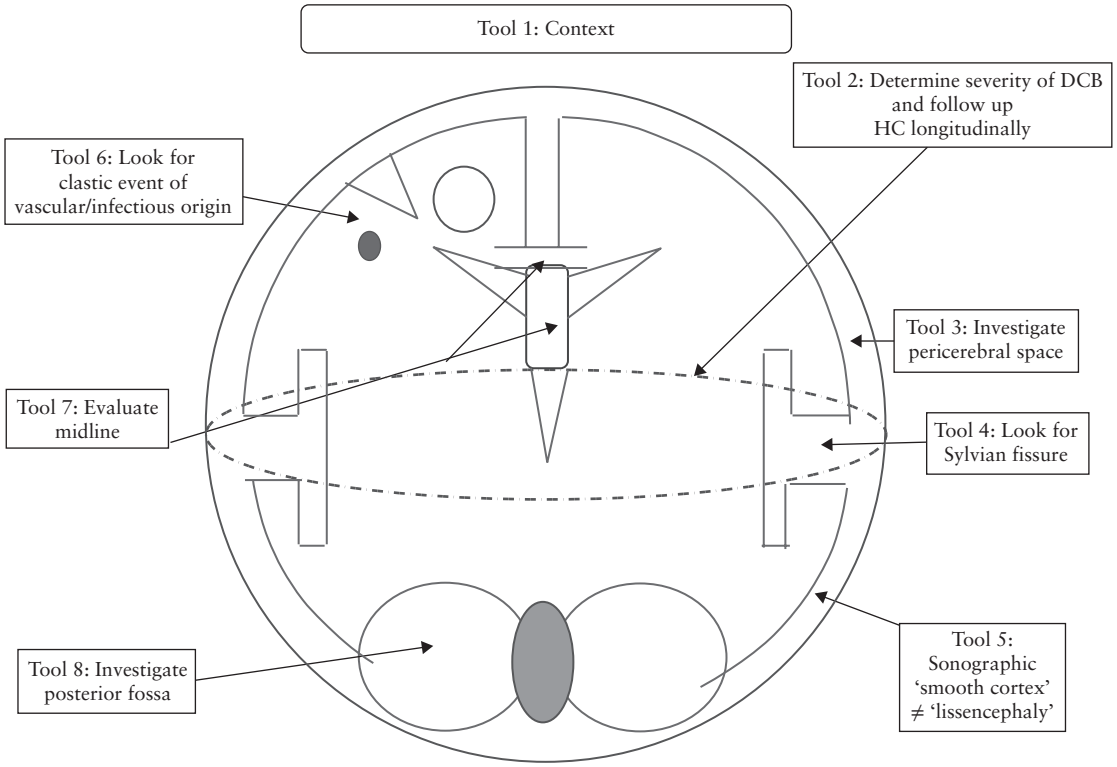

Figure 8 Summary of diagnostic imaging tools for use when investigating decreased cephalic biometry (DCB). HC, head circumference.

can be related exclusively to a major decrease in neurons and commissural axons, as illustrated in Figures 4 and S4. In such cases, corpus callosal hypoplasia should be considered as a consequence of the microcephaly and not as an etiological clue that may be helpful in investigating the underlying condition. In some cases, association between microcephaly and corpus callosal dysgenesis/agenesis can orientate the etiological diagnosis depending on either clinical or imaging patterns. For example, in a suggestive clinical context and even in the absence of apparent alcohol abuse, the association between microcephaly and hypoplastic corpus callosum, commonly associated with a characteristic abnormal profile (long upper lip), can be diagnostic of fetal alcohol syndrome[27].

Tool 8: Investigate the posterior fossa

As for the midline, investigation of the posterior fossa is important in the etiological work-up for any decrease in cephalic biometry, but one should be aware that reduction of supratentorial cephalic biometry can be associated with a decrease in the transverse cerebellar diameter (TCD). Indeed, these two parameters are correlated; we have shown recently that cerebellar growth occurs exponentially, relative to the increase in HC at a given gestational age[28].

As widely accepted, microcephaly can be a biometric clue in the diagnosis of neural tube defect, which can affect cephalic biometry as early as in the first trimester[29]. In the series of Deloison *et al.*[12], spina bifida was diagnosed in five fetuses among 16 with small HC on second-trimester ultrasound. Therefore, microcephaly should lead to the investigation of both the lumbosacral spine to detect myelomeningocele and the posterior fossa to detect Chiari-II malformation, which represent direct and indirect findings of spina bifida, respectively. Hypoplasia of the cerebellum and/or brainstem can also be an important clue for etiological diagnosis other than neural tube defect. Lissencephaly Type 3, a condition characterized by diffuse neuroapoptosis, results in cerebral atrophy leading to increased pericerebral space (see Tool 3), but also to severe pontocerebellar hypoplasia (Figure S6)[17]. Brainstem and cerebellar hypoplasia have also been reported in the so-called 'lethal microcephalies with simplified gyral pattern', such as in Amish lethal microcephaly related to *SLC2A19* gene mutation[4], as well as in a novel entity described recently by Rajab *et al.*[30]. Finally, cerebellar hypoplasia can be encountered in clastic events, such as CMV (Figure 2) and Zika virus infection, as mentioned previously.

One should be aware that a mild decrease in cephalic biometry can be associated with moderate reduction in infratentorial biometry, especially TCD. As previously suggested by Goldstein *et al.*[31] and Guihard-Costa & Larroche[32], we have shown recently a correlation between TCD and HC, based on a prospective cohort study of 65 250 pregnant women[28]. Considering this correlation, we might consider using the predicted TCD in relation to the HC at a given gestational age (according to the following formula: expected TCD = 0.7355*exp (0.059*HC)) for biometric analysis of the posterior fossa when investigating decreased cephalic

biometry. This is of importance in cases of moderate reduction in cephalic biometry, close to −2 SD, associated with decreased TCD around the 3rd percentile. In such cases, the mild decrease in cephalic biometry may be considered to be associated with cerebellar hypoplasia. However, if TCD is corrected according to gestational age, the TCD may increase above the 5th percentile, leading to an assumption that the decrease in cephalic biometry is 'isolated'; this may affect prenatal counseling.

Conclusion

Systematic use of the tools described above should assist in elucidating cases of unexplained isolated decrease in cephalic biometry. Summarized in Figures 7 and 8, these tools can be divided into two groups. The first focuses on the positive diagnosis of a decrease in cephalic biometry or microcephaly, including direct and indirect imaging findings related to decreased biometry, and the second focuses on etiological diagnosis. Some tools can be used both for positive diagnosis and as a clue to orientate towards the underlying cause. For example, a major increase in pericerebral space associated with a decrease in cephalic biometry indicates and reinforces the severity of the microcephaly but will also orientate the etiological work-up towards an encephaloclastic mechanism, which can be either clastic (ischemic or infectious) or genetic (such as lissencephaly Type 3). In routine practice, these tools can be useful to ensure that a moderate decrease in cephalic biometry (to between −2 SD and −3 SD) is truly isolated, which is important for prenatal counseling. Indeed, a moderate, isolated and non-progressive decrease in cephalic biometry is, in most cases, associated with favorable outcome when the appropriate biological work-up (including cytogenetic and infectious testing) is also negative. This clinical message is of great importance for patients and medical teams facing a decrease in cephalic biometry, particularly in light of the recent exponential surge in Zika virus infection.

ACKNOWLEDGMENTS

We would like to thank the Department of Pathology and especially Dr Annie Buenerd for her major input regarding fetal CNS pathologies and her constant effort in correlating prenatal imaging findings with pathological data.

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SUPPORTING INFORMATION ON THE INTERNET

Figures S1–S6 may be found in the online version of this article.

