Corpus callosum morphology in children on mid-sagittal MR imaging

Lauren Raubenheimer

RBNLAU002

University of Cape Town

MMed Diagnostic Radiology

Supervisors: Savvas Andronikou MBBCh, FCRad, FRCR, PhD (Professor of Radiology)

Tracy Kilborn MBChB, FRCR

Table of contents

Declaration:

This research is based on independent work and neither the whole work nor any part of it is being, or is to be, submitted for another degree to any other university. This work has not been reported or published prior to registration for the abovementioned degree.

Signed by candidate

01/08/18

Abstract

Background: There is little published research on the wide variation of corpus callosum (CC) morphology in children, the assessment of which is made difficult by the complex alteration of its appearance in childhood.

Objective: The purpose of our study was to assess the morphology of the CC on mid-sagittal T1-weighted magnetic resonance imaging (MRI) in a large number of children and correlate the findings with demographic and clinical criteria.

Materials and methods: We reviewed all brain mid-sagittal T1-weighted MRI's performed from July to December 2015 and obtained relevant demographic and clinical information from the accompanying report and laboratory system. The CC morphology was analysed by three radiologists and compared using cross tabulation with the chi-square test and ANOVA. Interobserver correlation was assessed using Kappa coefficient of conformance.

Results: 257 patients with mean age 72±60 months were included, 142 were male (55%). In abnormal MRI's the CC was less likely to have an identifiable isthmus and was more likely to be convex, thin and have separation of the fornix insertion (all $p<0.01$). In young children (< 5 years) the CC was also less likely to have an identifiable isthmus ($p=0.01$) and was more likely to be convex ($p=0.04$) but the fornix was more likely to insert normally ($p<0.01$). Children with tuberous sclerosis had significantly thinner splenia ($p=0.02$).

Conclusion: There is a distinct pathological appearance of the CC. The immature appearance of the corpus callosum can mirror this but is distinguished by normal insertion of the fornix and normal quantitative measurements. Splenial thinning in children with tuberous sclerosis warrants further investigation.

Acknowledgements and Contributions:

First author: LR collected and collated the data and was responsible for protocol and final thesis write up.

Supervisor 1: SA edited protocol and thesis; provided direction of research question; contributed line drawings

Supervisor 2: TK reviewed protocol and provided direction of research question

Abbreviations

CC	Corpus callosum
HIV	Human Immunodeficiency Virus
MRI	Magnetic Resonance Imaging
NHLS	National Health Laboratory System
PI	Principal investigator
RCWMCH	Red Cross War Memorial Children's Hospital
FI	Fractional anisotropy
PACS	Picture Archiving and Communication System

Chapter 1 introduction and literature review

1. Rationale

There is a wide variation of what is considered to be normal corpus callosum (CC) morphology in children but there is very little published research about the distribution of this range and if these variants are in fact normal. Most of the available research utilises sophisticated software tools, not available to or practical for the working radiologist, to analyse quantitative CC morphological properties. The complex development of the CC's shape during childhood adds to the wide discrepancies of the accepted normal paediatric range of CC morphology. This study intends to demonstrate a practical morphological approach to analysing the paediatric CC, analyse the reproducibility of such an approach and determine if any particular morphological variants are related to any specific clinical or demographic factors. CC abnormalities may be gross but subtle morphological variations, now considered normal, may in actuality be pathological.

2. Introduction

The CC is the major commissure that links the right and left cerebral hemispheres (1, 2) and is responsible for integrating functional connections between the two (3). Mid-sagittal views in magnetic resonance imaging (MRI) are useful for assessing the CC and the changes to it that occur during childhood development (4). The rostrum, genu, body and splenium are readily identifiable segments of the CC (1).

Conventionally the CC has been assessed either by calculating the area of the entire CC or by sub-dividing it into segments and measuring each segments' individual area or thickness (3). The rationale for this volumetric emphasis is the assumption that the volume of the CC is directly related to the number of axons it contains and that this is an approximation of connectivity between the two hemispheres (3). The thickness may not only be related to axon number but also to the degree of axon myelination (1) and to hemispheric volume. It can be reduced secondary to Wallerian degeneration (1) and the thickness of the CC and its various segments can be an important indicator of abnormalities in the cerebral hemispheres (1, 4). Therefore assessing CC thickness may be of great help in diagnosing pathology.

However there are a few pitfalls associated with the evaluation of the paediatric CC. Firstly, the age of the child must be taken into consideration as significant transformation takes place antenatally and during childhood development (1).

Secondly, there is a wide spectrum of what is considered to be normal CC thickness and shape (1, 2, 5). Acceptable normal variations include the presence or absence of an isthmus and differing splenium shapes (circular and bulbous versus long) (2). The CC is notoriously difficult to analyse, as there are so many morphological variations (6).

2.1. Corpus Callosum function

Axons within the CC link cerebral sensory-motor and associative areas (2). The position, size and degree of axon myelination are dependent on which areas they connect (1-3).

Thick fibres are located in the isthmus and connect sensory-motor and auditory areas (1-3). Thin fibres are found in the genu and connect the prefrontal areas and lateral and medial frontal lobes (1-3, 6). The rostrum also contains fibres from the prefrontal cortex as well as the frontal lobes' orbital surfaces (1-3, 6). Thick and thin fibres that connect parietal and occipital lobes are located in the splenium (1-3). The visual cortex projects thick fibres through the posterior splenium and thin fibres from the prefrontal and temporo-parietal associative areas traverse the anterior splenium (1, 2, 6). Fibres in the body connect extensively with the hemispheres through the corona radiata (1, 2). The posterior body contains fibres from the superior frontal cortex (1-3).

2.2. CC development

The CC develops from part of the dorsal lamina reuniens at 6-8 weeks post-conception (2). Connecting fibres are noted in the genu at 11-12 weeks gestation and the CC continues to develop in an anterior to posterior direction. The rostrum is the last segment to develop at 18-20 weeks gestation and the now complete CC continues to grow (2). At this time there has been no CC modelling and it is a simple tubular structure with no discernable isthmus or genu (1, 2). Insults to the CC before development is complete result in agenesis or hypogenesis while an insult after completion will result in atrophy (4).

Using MRI of 622 children, Garel and colleagues demonstrated that there is rapid growth of the corpus callosum until 3 years of age (2), after which modelling is completed and stabilization of the isthmus-splenial thickness ratio occurs (1). By 7-8 years body and isthmus growth is complete (2).

Postnatal CC maturation principally involves axon myelination that takes place in a posterior to anterior direction (2). Fractional anisotropy (FA) facilitates assessment of this myelination. Initially in neonates there is higher FA demonstrated in the splenium as compared to the genu (2). In the first year of life the FA increases precipitously in the both the genu and splenium with marked genu thickening at 2-3 months and rapid splenial enlargement from 4-5 months (1, 2). By the 7^{th} month the splenium is equal in size to the genu and both continue to grow uniformly (1). By the 9^{th}-10^{th} month the CC has an adult appearance. Myelination stabilises in the splenium by 2 years of age and in the genu, where myelination is slower, by three years of age (2). The CC body grows evenly throughout childhood (1).

2.3. Factors affecting CC size and morphology

Morphological variations of the CC may be diffuse or focal and have been linked to gender, age, handedness and many diseases (4).

2.3.1. Gender

The literature contains conflicting findings of the effect of gender on CC morphology.
A study from 1982 reported that some females had a larger posterior fifth of the corpus callosum but no difference in total callosal area (7). Byne et al, using MRI of the callosum in living subjects, did not confirm that the splenium was larger and more bulbous in women (5). Whitelson et al showed that when controlling for cerebral weight, sex differences disappeared (8).

Garel et al showed a statistically significant increased fronto-occiptal diameter in boys from the age of 1 year but other parameters, including segmental thickness, did not show any sex effects (2). Ng and colleagues also noted no significant sex difference in the thickness of any part of the corpus callosum (9).

2.3.2. Handedness

The total CC area and the areas of its anterior and posterior portions were found to be significantly larger in ambidextrous as compared to right-handed subjects (10). In the same study the size of the posterior 5th of the CC did not show significant size differences between groups (10).

In a later study Witelson et al again found that non-consistent right-handed subjects had a larger total callosal area than consistent right-handed subjects (8). The greatest difference was found in the posterior CC, most notably the isthmus (8). This association was found only in males (8).

2.3.3. Prematurity

In a 2011 study of adolescents born very prematurely (less than 33 weeks gestational age) and adolescents born at term the total CC area was significantly smaller in the former group (2). The most notable decrease in size was seen in the posterior quarter of the CC (2). While the total CC area was smaller in size in the ex-premature group the anterior quarter was larger (2).

2.3.4. Diseases

Much of the literature dedicated to corpus callosum morphology concerns its relation to disease and developmental abnormalities. The related conditions can be subdivided into (a) primary abnormalities of CC development, (b) primary abnormalities of CC growth and maturation, (c) diseases or insults which directly cause CC abnormalities, (d) diseases that are associated with CC abnormalities and (e) morphological variants that may predict future pathology.

(a) Primary abnormalities of CC development

Complete absence of the CC is termed agenesis, incomplete development hypogenesis and defective development dysgenesis. The posterior body, splenium and rostrum of the CC are typically absent in hypogenesis as these are the last segments to form during antenatal development (1, 11). Hypogenesis may been seen in children with mucolipidosis type IV (1). Simplified sulcation like classic lissencephaly is associated with CC abnormalities such as hypoplasia due to the reduced white matter volume (1). In holoprosencephaly callosal dysgenesis is seen with paucity of anterior and middle CC sections (1, 11). Antenatal exposure to alcohol has been linked to localised thinning of the CC, predominantly the anterior third (1). Microcephaly from various causes can be related to CC anomalies (1).

While large CC's can be incidental in normal subjects they can be related to conditions associated with megalencephaly including the syndrome of megalencephaly, mega corpus callosum and polymicogyria; macrocephaly-capillary malformation; syndrome of mega corpus callosum and caudate nuclei with bilateral hippocampal malformation and, lastly, the syndrome of megalencephaly, mega corpus callosum and complete lack of motor development (1). Thick CC's have been reported in neurofibromatosis type 1, the syndrome of hypertrichosis, hyperkeratosis, abnormal corpus callosum and mental retardation and Cohen syndrome (1).

(b) Primary abnormalities of CC maturation and growth

Diseases associated with failed myelination result in diffuse thinning of the CC (1). Causes include hypomyelinating leukoencephalopathies e.g. Pelizaeus-Merzbacher disease, Cockayne syndrome type II, Tay syndrome and metabolic disorders that affect white matter such as mucolipidosis type IV (1).

(c) Diseases or insults which cause CC abnormalities

Diffuse thinning of the CC may represent Wallerian degeneration from global cerebral atrophy. This is seen in severe hypoxic-ischaemic encephalopathy, HIV encephalopathy and hydrocephalus (1). Dysmyelinating and demyelinating conditions such as metachromic leukodystophy, adrenoleukodystrophy and multiple sclerosis can also cause diffuse CC thinning (1).

Focal CC thinning may be a result of Wallerian degeneration secondary to focal cortical atrophy or of direct damage to the CC (1). Urea cycle disorders and hereditary spastic paraplegia both cause focal CC thinning as a consequence of cerebral atrophy, in hereditary spastic paraplegia the genu and body are typically involved (1). Disproportionate thinning of the posterior CC is seen in late imaging of premature neonates with periventricular leukomalacia and in term infants with pre- or perinatal brain injury who have predominantly parietal and occipital white matter volume loss (1). If the volume loss is predominantly perirolandic then mid-segment CC thinning is seen (1). Children with HIV-associated atrophy have anterior CC thinning when the frontal lobe cortices are mainly involved and middle segment thinning with sensorimotor cortical volume loss. In cerebral infarction atrophy of the connecting CC segment can occur (1). Radiation or surgical removal of a cerebral hemisphere can result in subsequent CC focal thinning (1).

Pathological processes that directly affect the CC include neonatal hypoglycaemia which can cause localised splenial thinning if untreated (1). Inherited metabolic disorders can cause focal CC abnormalities such as X-linked adrenoleukodystrophy which results in splenial thinning (1). Colabain deficiency causes marked CC body volume loss (1). While CC infarction is rare anterior cerebral artery vasculitis as seen in tuberculosis meningitis can cause discrete CC infarcts (1). Diffuse axonal injury may result in splenial insults. Surgery can also result in focal CC defects (1).

Acute inflammatory thickening of the CC due to ischaemia, trauma, infection or demyelination has been seen and is often antecedent to CC thinning (1).

(d) Diseases which are associated with CC abnormalities but have no clear pathophysiological link

Thinning of the CC has been noted in patients with Bipolar Mood Disorder with markedly reduced splenial thickness (12). Children with attention deficit hyperactivity disorder have also shown splenium thinning (13).

Shorter, less curved CC's with posterior thinning, especially of the isthmus, were found in William's Syndrome patients (14).

(e) Morphological variants that may predict future pathology

In a 2002 study Narr et al found that upward bowing of the CC might be associated with a genetic predisposition to schizophrenia (15)

2.4. Why CC morphology is important

There are many factors contributing to the morphological appearance of the CC (2). The thickness of the CC is determined by the number of axons it contains, their degree of myelination and the perivascular fluid (2) and thus its shape can be altered by abnormalities in any of the aforementioned.

The CC size is a function of cerebral cortex condition and its assessment can be used as a proxy of hemispheric state (10). In 1988 Byne and colleagues suggested that the wide variation of CC morphology is the result of a complex network of ante- and postnatal factors including genetics, hormones, environment and experience and that its variation may not be significant (5). More recently however a link between cognitive function and CC size and morphology has been demonstrated (13).

Hutchinson et al demonstrated the role of the CC in the procurement of sophisticated cognitive skills during teenage years in a 2008 publication (13). Another study showed that isthmus thickness was positively associated with academic achievement (9).

2.5. Tools that have been used to assess CC size and morphology

There is a wide variation of tools used to assess CC size and morphology in the available literature. Many studies have used the established Witelson segmentation criteria to divide and measure callosal segments (14). Studies have found that there is good intra- and interobserver agreement for measuring the thickness of these segments (2, 9). Poor intra- and interobserver agreement was only demonstrated when measuring isthmus thickness (2).

A recent critique of this method questions the validity of this segmentation as a more precise understanding of axonal tractography is attained (14). While size may be an important indicator of pathology it has also been suggested that quantitative size measurements may not be precise enough to detect subtle morphological changes (12).

2.6. Project in context: Comparison to literature on the topic

The following table summarises the significant findings of CC morphological associations in the literature.

Table 1: Significant morphological CC findings

Study	No. participants	Tools used	Measurements taken	Findings
Byne et al 1988 (5)	33	Mid-sagittal MRI slices with software to calculate areas	Total area Posterior 1/5 area Ratio of posterior 1/5 to total area Anterior 4/5 area Minimum width AP distance Splenial width **Circularity of splenium**	Minimum width smaller in males (p = 0.04)
Erdogan et al 2005 (4)	50	Mid-sagittal T1WI MRI using software to calculate area	CC area Supratentorial-supracallosal area (ST-SC) CC/ST-SC	Males has larger CC and ST-SC areas No sex difference in CC/ST-SC
Garel et al 2011 (2)	622	MRI mid-sagittal T1 and T2WI with direct measurements	Anteroposterior CC diameter True length of CC Genu thickness Body thickness Isthmus thickness Splenium thickness Fronto-occipital diameter Distance splenium/tegmentum	No sex differences
Huang et al 2005 (6)	9 1 stroke 8 controls	MRI mid-sagittal DTI and tractography	CC parcellated and normalised for control subjects – comparison between normalised parcels and stroke pt using complex mathematical tools	Most variability in genu and splenium in normal subjects Loss of CC area in stroke pt which relates to cortical losses
Hutchinson et al 2008 (13)	Meta-analysis of 13 studies 595 participants 284 with ADHD 311 controls	MRI	-	Splenium smaller in girls with ADHD Rostrum smaller in boys with ADHD
Luder et al 2007 (14)	24 12 William's syndrome (WS) 12 controls	MRI mid-sagittal T1WI with mesh-based methods to calculate callosal thickness	Average callosal thickness **Average callosal shape**	**CC shorter, less curved and thinner posterior profile in WS**
Narr 2002 (15)	20 pairs of twins discordant for schizophrenia; mono- and dizygotic	MRI mid-sagittal T1WI	Total area Area of segments (modified Whitelson method)	**Upward bowing found in schizophrenic patients (in both monozygotic twins but not in dizygotic twins without schizophrenia)**
Ng et al 2005 (9)	100	MRI mid-sagittal T2WI	Length Height Total area Segmental thickness (Weiss method)	Thicker measurement at the body: splenial junction related to higher test scores
Rice et al 2005 (16)	20 12 non-mentally retarded autistic children with macrocephaly 8 controls	MRI mid-sagittal T1WI	**Shape** Total area Segmental areas (Whitelson method)	No differences
Walterfang et al 2009 (12)	48 24 BPMD 24 controls	MRI mid-sagittal T1WI	Area Length **Bending angle** Mean callosal thickness Segmental thickness	CC thinner in BPMD Marked thinning of isthmus in BPMD
Witelson et al 1989 (8)	50 32 consistent-right-hand preference CRH 18 non consistent-right-hand preference nCRH	Brains at autopsy	Total area Segmental areas (Witelson method) Length Breadth Thickness of mid-CC	nCRH's has a larger overall area, larger isthmus and larger posterior mid-body than CRH's Genu and part of anterior body larger in males than females
Witelson et al 1985 (10)	N = 42 27 CRH 15 mixed-hand preference	Brains at autopsy	Total area Segmental areas (anterior half, posterior half and posterior 1/5)	Total overall area in mixed-handers (in anterior and posterior halves but not in splenium)

3. Aim

This study aims to evaluate and categorise the corpus callosum shape in a group of children imaged for a variety of clinical indications to determine the morphological range of the CC and if there are pathological associations of shape that may currently be considered normal variants. Correlations of shape with age, gender and HIV status will also be assessed.

4. Study Objectives

1. To determine the morphological shape of the CC on the mid-sagittal T1-weighted MRI sequence and categorise this based on pre-defined morphological categories for a paediatric population imaged for a variety of clinical indications.
2. To determine whether the CC was reported as normal, abnormal, uncertain or not mentioned within the MRI report.
3. To determine any association between shape and age, sex, referral reason, HIV status and diagnostic category.
4. To determine inter-observer variability between three readers for assigning the CC into the different categories.

5. References

1. Andronikou S, Pillay T, Gabuza L, Mahomed N, Naidoo J, Hlabangana LT, et al. Corpus callosum thickness in children: an MR pattern-recognition approach on the midsagittal image. Pediatric radiology. 2014.

2. Garel C, Cont I, Alberti C, Josserand E, Moutard ML, Ducou le Pointe H. Biometry of the corpus callosum in children: MR imaging reference data. AJNR American journal of neuroradiology. 2011;32(8):1436-43.

3. Park HJ, Kim JJ, Lee SK, Seok JH, Chun J, Kim DI, et al. Corpus callosal connection mapping using cortical gray matter parcellation and DT-MRI. Human brain mapping. 2008;29(5):503-16.

4. Erdogan NU, H.; Tuna, I.; Okur A. A novel index to estimate the corpus callosum morphometry in adults: callosal/supratentorial-supracallosal area ratio. Diagn Interv Radiol. 2005;11(4):179-81.

5. Byne WN, R.; Houston, L. . Variations in Human Corpus Callosum Do Not Predict Gender: A Study Using Magnetic Resonance Imaging. Behvaioural Neuroscience. 1988;102(2):222-7.

6. Huang H, Zhang J, Jiang H, Wakana S, Poetscher L, Miller MI, et al. DTI tractography based parcellation of white matter: application to the mid-sagittal morphology of corpus callosum. NeuroImage. 2005;26(1):195-205.

7. DeLacoste-Utamsing C, Holloway R. Sexual dimorphism in the human corpus callosum. Science. 1982;216(4553):1431-2.

8. Witelson SF. Hand and sex differences in the isthmus and genu of the human corpus callosum: a postmortem morphological study. Brain. 1989;112:799-835.

9. Ng WH, Chan YL, Au KS, Yeung KW, Kwan TF, To CY. Morphometry of the corpus callosum in Chinese children: relationship with gender and academic performance. Pediatric radiology. 2005;35(6):565-71.

10. Witelson SF. The brain connection: the corpus callosum is larger in left-handers. Science. 1985;229(665-668).

11. Przbojewski SJ, Andronikou, S. Corpus callosum hypogenesis versus dysgenesis. SA Journal of Radiology. 2006;June.

12. Walterfang MM, G. S.; Wood, A. G.; Reutens, D. C.; Chen, J.; Barton, S.; Yucel, M.; Velakoulis, D.; Pantelis, C. Corpus callosum size and shape in established bipolar affective disorder. Austraian and New Zealand Journal o Psychiatry 2009;43:838-45.

13. Hutchinson AD, Mathias JL, Banich MT. Corpus callosum morphology in children and adolescents with attention deficit hyperactivity disorder: a meta-analytic review. Neuropsychology. 2008;22(3):341-9.

14. Luders E, Di Paola M, Tomaiuolo F, Thompson PM, Toga AW, Vicari S, et al. Callosal morphology in Williams syndrome: a new evaluation of shape and thickness. Neuroreport. 2007;18(3):203-7.

15. Narr KLC, T. D; Woods R. P; Thompson, P. M; Kim. S; Asunction, D; van Erp T. G. M; Poutanen, M; Lonnqvist, J; Standerksjold-Nordenstam, C; Kapro, j; Mazziotta, J. C; Toga, A. W. Genetic Contributions to Altered Callosal Morphology in Schizophrenia. The Journal of Neuroscience. 2002;22(9):3720-9.

16. Rice SAB, E. D.,; Cleavinger HBT, D. F.; Sayer, J; McMahon, W.; Ozonoff, S.; Lu, J., Lainhart, J. E. Macrocephaly, Corpus Callosum Morphology, and Autism. Journal of Child Neurology. 2006;20(1):34-42.

Chapter 2: Publication ready Manuscript (submitted to Paediatric Radiology)

Corpus callosum morphology in children on mid-sagittal MRI imaging

Raubenheimer LA[1], Andronikou S[2], Kilborn T[3]

Corresponding author: LA Raubenheimer laraubs@gmail.com

This research received funding from the University of Cape Town

Keywords: Children, Magnetic Resonance Imaging, Corpus callosum morphology

[1] Department of Radiology, Groote Schuur Hospital, University of Cape Town Medical School, Cape Town, South Africa
[2] University of Bristol, The Bristol Royal Hospital for Children, Bristol United Kingdom and University of Cape Town, Cape Town, South Africa
[3] Department of Paediatric Radiology, Red Cross War Memorial Children's' Hospital, University of Cape Town, Cape Town, South Africa

Abstract

Background: There is little published research on the wide variation of corpus callosum (CC) morphology in children, the assessment of which is made difficult by the complex alteration of its appearance in childhood.

Objective: The purpose of our study was to assess the morphology of the CC on mid-sagittal T1-weighted magnetic resonance imaging (MRI) in a large number of children and correlate the findings with demographic and clinical criteria.

Materials and methods: We reviewed all brain mid-sagittal T1-weighted MRI's performed from July to December 2015 and obtained relevant demographic and clinical information from the accompanying report and laboratory system. The CC morphology was analysed by three radiologists and compared using cross tabulation with the chi-square test and ANOVA. Interobserver correlation was assessed using Kappa coefficient of conformance.

Results: 257 patients with mean age 72±60 months were included, 142 were male (55%). In abnormal MRI's the CC was less likely to have an identifiable isthmus and was more likely to be convex, thin and have separation of the fornix insertion (all $p<0.01$). In young children (< 5 years) the CC was also less likely to have an identifiable isthmus ($p=0.01$) and was more likely to be convex ($p=0.04$) but the fornix was more likely to insert normally ($p<0.01$). Children with tuberous sclerosis had significantly thinner splenia ($p=0.02$).

Conclusion: There is a distinct pathological appearance of the CC. The immature appearance of the corpus callosum can mirror this but is distinguished by normal insertion of the fornix and normal quantitative measurements. Splenial thinning in children with tuberous sclerosis warrants further investigation.

Introduction

There is a wide variation of what is considered normal corpus callosum (CC) morphology in children but very little published research about the distribution of this range and if these variants are in fact normal. The complex development of the CC's shape during childhood adds to the disparity of accepted normal paediatric range of CC morphology.

The CC is the major commissure that links the right and left cerebral hemispheres [1,2] and is responsible for integrating functional connections between the two [3]. Mid-sagittal views in magnetic resonance imaging (MRI) are useful for assessing the CC and the changes to it that occur during childhood [4]. The rostrum, genu, body and splenium are readily identifiable segments of the CC [1].

A wide variety of tools have been used to assess CC size and morphology in the literature. Many studies have used the established Witelson segmentation criteria to divide and measure callosal segments [5] with good intra- and interobserver agreement [2,6]. The rationale for this volumetric emphasis is the assumption that the volume of the CC is directly related to the number of axons it contains and that this is an approximation of connectivity between the two hemispheres [3]. The thickness is probably also related to the degree of axon myelination. Thickness can be reduced secondary to Wallerian degeneration [1] and thus CC size is also a function of cerebral cortex condition and its assessment is a proxy of hemispheric state [7]. Unfortunately these sophisticated software tools are often not available to or practical for the working radiologist.

The wide spectrum of what is considered to be normal CC thickness and shape [1,8,2] includes the presence or absence of an isthmus and differing splenium shapes [2].

The effect of gender, handedness, prematurity and different disease on CC morphology and size have been investigated with complex and sometimes varying results.

This study aimed to evaluate and categorise the corpus callosum shape in a group of children imaged for varying clinical indications to determine the morphological range of the CC (in normal and abnormal MRI's), to assess correlations of shape with age, gender and HIV status and to calculate interobserver variability between three readers for CC morphology assessment.

Materials and methods

This was a retrospective, descriptive cross-sectional study. MRI brain imaging and the attached reports from scans performed at the RCWMCH (Red Cross War Memorial Children's Hospital) from July to December 2015 were accessed. Inclusion criteria were all children ages 0 days – 16 years who had an MR in the specified time-period. Exclusion criteria included all patients whose mid-sagittal T1 image was unavailable or obscured by artefact; patients whose reports were unavailable; children who had suffered traumatic brain injury and follow up imaging in patients who were imaged during the study period.

3D MR T1 weighted (MPRAGE / FSPGR / FFE) images from a Phillips Achieva 1.5T were obtained from the Picture Archiving and Communication System (PACS). A 16-channel neurovascular coil was used in image acquisition. The images were exported in DICOM format and loaded onto the OsiriX platform (OsiriX freeware from Mac downloaded from osirix.viewer.com) for viewing. Each patient's images were anonymised. The CC was assessed on mid-sagittal imaging taking into account the clearest view of the CC and other central structures including the aqueduct of Sylvius, the falx cerebri, the septum pellucidum and the tectum [9] and assigned to predetermined morphological categories by three readers blinded to each other. The readers were the primary investigator (PI), a radiology registrar, and two consultant paediatric radiologists with cumulative experience in paediatric imaging of over 30 years. In the event of interobserver disagreement, a majority decision from the readers was accepted.

After a review of CC abnormalities in the literature six morphological categories were identified:
Completeness in CC development. This was defined as a visible rostrum, genu, body and splenium. Hypogenesis was defined as absence of the posterior body, splenium and/or rostrum and agenesis as no visible CC (**Fig. 1**).
Easily identifiable isthmus. An easily identifiable isthmus was defined as an area of focal thinning readily identified between the posterior body and splenium (**Fig. 2-3**) [6,10,11].
Level of fornix insertion. The length of the CC was obtained by connecting a line from the most anterior to the most posterior aspects of the CC. This line was divided into quarters (areas 1, 2, 3 and 4). The area of the CC where the fornix 'attaches' was recorded (**Fig. 4-5**).
Splenium shape. The splenium was categorised as either predominantly long and tubular in shape or predominantly circular and bulbous (**Fig. 6-7**) [12,13,5].

Thickness of the genu, body and splenium. The thickness of the genu, body and splenium was measured by each reader and categorised as thick, thin or normal based on a table of normal ranges from Garel et al.'s 2011 study [2] (**Fig. 8-9**).
Convexity of CC. The corpus callosum was defined as upwardly bowed, flattened or normal (**Fig. 10**) [5,14].

The PI accessed the MRI reports after the reading and extracted whether the CC was reported as normal, abnormal, uncertain or not commented on; patient age and sex; indication for scan and diagnosis as recorded on the MRI report. Age was recorded to the nearest month.

Indications for the MRI scan were grouped into the following categories: follow up of known abnormality, headaches, seizures, apnoea, developmental delay, tics / movement disorders, dysmorphism, microcephaly, macrocephaly, hemiplegia, behavioural abnormalities, HIV encephalopathy, metabolic, endocrine, cerebral palsy, neoplasm follow up or other.

The children's HIV test results were accessed from the National Health Laboratory System (NHLS) and recorded as 'positive', 'negative' or 'untested' if no result was found.

The MRI diagnoses from the existing radiologist reports were classified under the following broad categories: hypoxia, vascular, infectious, structural, behavioural, metabolic / toxic, neoplastic, endocrine, autoimmune / inflammatory and other. The specific diagnosis was also recorded and up to three possible diagnostic categories could be selected if the child had more than one condition.

Any MRI's considered normal were noted. Children with previous surgical intervention, documented cerebral atrophy or hydrocephalus were flagged as these were considered confounders of CC morphology. These children were excluded from analyses with regards to overall diagnostic category and specific diagnosis, but were included in all other analyses.

All data was anonymised. The key, linking patient details to number, was only available to the PI and was kept under lock and key

Summary statistics were presented in the form of frequency tables and histograms, means and standard deviations. Firstly only the normal MRI's were evaluated and the results of their morphological assessment were presented as bar graphs. The CC morphological measurements of normal MRI's

were assessed with regards to gender using cross tabulation with the chi-square test and age using ANOVA.

MRI measurements between normal studies and all other studies were compared using cross tabulation with the chi-square test.

The broad diagnostic categories (excluding the three identified confounders) were assessed against morphological categories with the chi-square test and where strong correlations were found the specific diagnoses under these broad categories were evaluated, again with the chi-square test.

Interobserver reliability was evaluated using Kappa coefficient of conformance.

Results

257 MRI's met the inclusion and exclusion criteria. The age range was from 0 to16 years with a mean age of 72±60 months (**Fig. 11**). Of the children studied 55% (142/257) were male and 45% (115/257) female. 5% (12/257) of the children were HIV positive, 14% (36/257) HIV negative and 81% (209/257) untested. The indication for MR varied widely as summarised in **Fig. 12**. **Fig. 13** demonstrates the most common diagnoses as established by clinical history and imaging findings.

Fig. 14 summarises the categorisation of the CC in the initial 257 patients as evaluated by the three readers. 6 patients were excluded from morphological evaluation due to CC developmental abnormalities. Of the 251 remaining MRI's evaluated, one third (76/251) were normal. Confounders of CC morphology were surgical intervention, atrophy and hydrocephalus and these were present in 19%, 12% and 9% of MRI's respectively. More than one confounder could be present in a single patient.

The corpus callosum in patients with abnormal MRI scans was less likely to have an identifiable isthmus ($p<0.01$), was more likely to have the fornix insert posteriorly in zone 4 ($p<0.01$) **Fig. 15**; was more likely to be convex ($p<0.01$) and the genu, body and splenium were all more likely to be thin (all $p<0.01$) as compared to normal MRI's. There was no statistically significant difference in splenium shape between normal and abnormal MRI's. As a corollary to the above, a normal CC was more likely to have the fornix insert in zone 3 and the isthmus was more likely to be present.

Gender showed no influence on any of the morphological categories in the normal MRI's. The number of children confirmed as HIV positive was too small for any meaningful analysis of the influence of HIV status.

In children with normal MRI's, age was found to have a significant correlation with morphological shape. Younger children (< 5 years) were less likely to have an identifiable isthmus ($p=0.01$); the fornix was more likely to insert in zone 3 ($p<0.01$); the CC was more likely to be convex ($p=0.04$) and the splenium was more likely to be long and tubular ($p<0.01$) as illustrated in **Fig. 16-20**.

Children with neoplasms were more likely to have a convex CC shape ($p<0.01$) and a thinner CC body ($p=0.02$) and splenium ($p<0.01$) than children without neoplasms. On analysis of specific neoplasms children with tuberous sclerosis (TS) were more likely to have splenial thinning ($p=0.02$).

In general there was fair but not strong interobserver agreement amongst the three readers. Assessment of CC completeness, identifiable isthmus and assessment of genu and body thickness had the strongest agreement with kappa values of 0.65, 0.66, 0.66 and 0.64 respectively. There was fair correlation in assessment of fornix insertion, overall CC shape and splenium thickness with kappa values of 0.57, 0.58 and 0.55. The most variability was found in evaluation of splenium shape with a kappa value of 0.39. There was no marked difference between registrar and consultant agreement.

Discussion

Morphology of the CC as defined in this research relates to completeness of its development, degree of convexity in its shape, its thickness, identification of the isthmus as a distinct region, the position of fornix insertion along the its length and shape of the splenium. Only the thickness measurement of the components represents a truly objective criterion but even this is subjective with regard to the position of measurement. Thus, the results predominantly represent the radiologic visual assessment of the corpus callosum that is current clinical practice.

On analysis of the normal MRI cohort, there was no gender difference found between any of the morphological categories. This is in keeping with current research although the older literature does contain some conflicting evidence of the effect of gender on CC morphology. A study from 1982 reported that some females had a larger posterior fifth of the CC but no difference in total callosal area [15]. Byne et al did not confirm that the splenium was larger and more bulbous in women [8]. Witelson et al showed that when controlling for cerebral weight, sex differences disappeared [11]. Garel et al

showed a statistically significant increased fronto-occiptal diameter in boys from the age of 1 year but other parameters did not show any sex effects [2]. Ng and colleagues also noted no significant sex difference in the thickness of any part of the corpus callosum [6]. Of note, the study that documented sex differences involved post mortem specimens [15] and in the more recent studies which utilised MR imaging sex differences were not found [8,11,2,6].

Our results indicate that children under 5 years of age were more likely to have CC's with a simple configuration (no identifiable isthmus and a long and tubular splenium) and a convex shape. This simple CC morphology found in infants correlates well with the current understanding that at birth the CC is a simple tubular structure with no discernable isthmus or genu and remodelling takes place postnatally [1,2]. Postnatal CC maturation principally involves axon myelination in a posterior to anterior direction and pruning [2,16]. Fractional anisotropy (FA) facilitates assessment of this myelination. Initially in neonates there is higher FA demonstrated in the splenium as compared to the genu [2]. In the first year of life the FA increases precipitously in the both the genu and splenium with marked genu thickening at 2-3 months and rapid splenial enlargement from 4-5 months [1,2]. By the 7^{th} month the splenium is equal in size to the genu and both continue to grow uniformly [1]. By the 9^{th}-10^{th} month the CC has an adult appearance (**Fig. 2**). Myelination stabilises in the splenium by 2 years of age and in the genu by three years of age [2]. The CC body grows evenly throughout childhood [1].

Our results show that a convex, thin (genu, body and splenium) CC with an unidentifiable isthmus and separation of the fornix (insertion at segment 4) indicates pathology.

Similar abnormalities of diffuse or focal CC thinning have been described in various studies. Dysmyelinating and demyelinating conditions can cause diffuse CC thinning [1]. Diffuse thinning of the CC may represent Wallerian degeneration from global cerebral atrophy as seen in severe hypoxic-ischaemic encephalopathy, HIV encephalopathy and hydrocephalus [1]. Focal CC thinning may also be a result of Wallerian degeneration secondary to *focal* cortical atrophy or direct damage to the CC [1]. Urea cycle disorders, hereditary spastic paraplegia, pre- and perinatal brain injury, HIV associated atrophy and cerebral infarction all cause focal CC thinning as a consequence of focal cerebral atrophy [1]. While CC infarction is rare anterior cerebral artery vasculitis in tuberculosis meningitis can cause discrete CC infarcts [1].

In a 2011 study of adolescents born prematurely and adolescents born at term the total CC area, most notably the posterior quarter, was significantly smaller in the former group [2]. Antenatal exposure to

alcohol has been linked to localised thinning of the CC, predominantly the anterior third. A variety of diverse conditions including neonatal hypoglycaemia, inherited metabolic disorders such as X-linked adrenoleukodystrophy, diffuse axonal injury, Bipolar Mood Disorder and attention deficit hyperactivity disorder have all been shown to cause localised splenial thinning [1,12,13].

Children with tuberous sclerosis had significantly thinner splenia in this study as seen in **Fig. 6**. The cause of this thinning is at present uncertain. Possible aetiologies include a direct effect from adjacent subependymal nodules or the white matter abnormalities (radial glial bands) seen in tuberous sclerosis, but the reason for the isolated splenial thinning remains unclear. This appears to be the first documentation of such an association but it should be interpreted with caution as there were only 8 TS patients in this cohort.

In a 2002 study Narr et al found that upward bowing of the CC might be associated with a genetic predisposition to schizophrenia [14]. In particular, our study found that patients with neoplasms were more likely to have convex CC's while children with other diagnoses did not have convex CC's. It may be postulated that conditions in which there is intracranial volume gain result in an upwardly bowed CC, as in our neoplasm patients, while those not associated with volume gain do not have upwardly bowed CC's. Children with atrophy and hydrocephalus were excluded from the analyses of specific diagnoses and this CC deformity must be directly attributed to the primary pathology e.g. tumour with associated volume gain. It is reasonable to postulate that as the CC is only partially myelinated in children it is more vulnerable to morphological deformity.

Our research associated abnormal MRI's with the absence of an identifiable isthmus and more posterior fornix insertion (zone 4) while there was a correlation between an identifiable isthmus and more anterior insertion (zone 3) in normal MRI's. To the authors' knowledge no previous research has investigated the absence of an isthmus nor the level of fornix insertion and its relation to pathology.

We demonstrated that *normal* young children (< 5 years) may have a convex CC with no identifiable isthmus but that the CC is normal in thickness for age and the fornix usually inserts in zone 3. These two latter features can be used to differentiate a normal immature CC from a pathological one in a child less than five years of age.

The paucity of available HIV status data from the NHLS lab system meant that a large number of patients were documented as untested. These children may have had rapid HIV tests but as the clinical

notes were not accessed this data was not available. This has important implications for further research in which clinical notes are not accessed.

Despite the subjective nature of the majority of MRI features evaluated in this study, the reliability of these findings was demonstrated through calculation of interobserver agreement that was fair for most of the categories, except splenium shape. Splenium shape was also found to be an unimportant parameter in this study and thus may not be of practical value.

In this study the fair interobserver agreement for the many of the qualitative categories is similar to the agreement amongst quantitative categories. While this similarity may support the reliability of the qualitative assessment, which is the current practice of many radiologists and the results of which this study suggests can identify pathologic deformity of the CC, it remains concerning that such a high degree of variability exists amongst both the qualitative and quantitative variables. Other studies employing only quantitative analysis techniques show much better interobserver variability [2,6] and more rigorous quantitative methods for CC assessment than those performed in this study may prove superior to both simple measurements and qualitative assessment.

The paucity of research of CC morphological variants in children means that accepted morphological categorisation does not exist and thus several morphological categories were created for this study. These are based on criteria extracted from the reviewed literature but more research is need before these categories can be considered valid.

Limitations of this study include that this is a hospital-based population who were referred for MRI of the head because of suspected or known pathology, and thus may not be representative of the general population in terms of incidence of pathological findings. The inclusion of all-comers referred for brain imaging may be also be a confounder: the fact that some were reported at “normal” does not exclude that the brains (and CC’s) were subclinically abnormal. Accurate assessment of normal CC morphology could only be performed in healthy volunteers and this highlights an important area for future research.

The sample size was relatively small with even fewer specific diagnoses represented. Associations of CC morphology with particular diseases may well have been overlooked with such small numbers. The broad diagnostic categories did not take into account the variable imaging findings within a category that may directly affect CC morphology such as neoplasm type and location. The appearance of the CC in a

patient with a juxta-cortical tumour is almost certainly going to be different to a small more distant tumour.

When assessing CC morphology in specific diseases the findings were compared to other abnormal MRI's without that particular diagnosis rather than normal aged-matched controls. This may skew the results and in future well-planned studies are needed to better assess these associations.

7. Conclusion

Morphology of the corpus callosum differs significantly between children with pathologic MRI scans as compared to normal MRI scans in four distinct ways: convexity (upward bowing) of the corpus callosum, loss of an identifiable isthmus, generalised thinning and a more posterior insertion of the fornix. These have been shown to be associated predominantly with pathologies that have volume gain, such as tumours. Unexplained thinning of the splenium was found in children with tuberous sclerosis and this should prompt further research.

Importantly morphology of the corpus callosum in younger children (< 5 years) may mimic that of pathologic MRI scans with a more tubular and convex CC appearance that has not yet undergone the pruning which results in an identifiable isthmus. We have shown, however, that in this age group a normal corpus callosum can be differentiated from pathologic morphology by the more anterior insertion of the fornix and through demonstration of normal thickness for age.

Future research is planned using customised software for determining the radial thickness of normal CC's and representing the normal morphological range in a graphic form.

Figures

Fig. 1 Midline T1 sagittal MRI scan demonstrates agenesis of the corpus callosum in a 1-day-old neonate

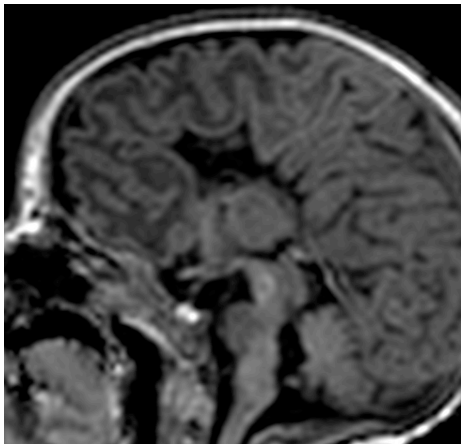


Fig. 2 Midline T1 sagittal MRI scan demonstrates an easily identifiable isthmus (arrow) in an 11-year-old boy. Note the absent posterior pituitary bight spot

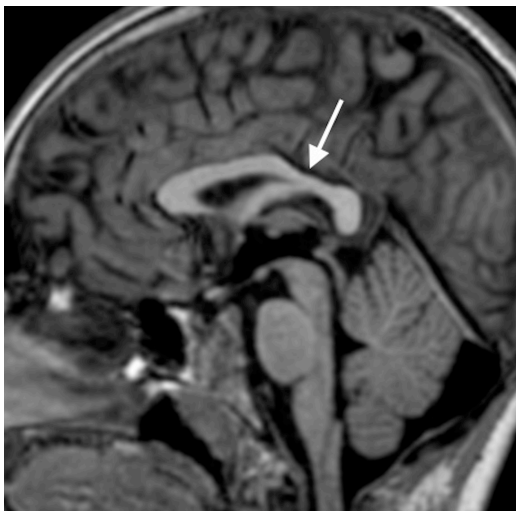


Fig. 3 Midline T1 sagittal MRI scan shows no identifiable isthmus (arrow) in a 3-year-old girl who was being followed up for previous tuberculosis meningitis

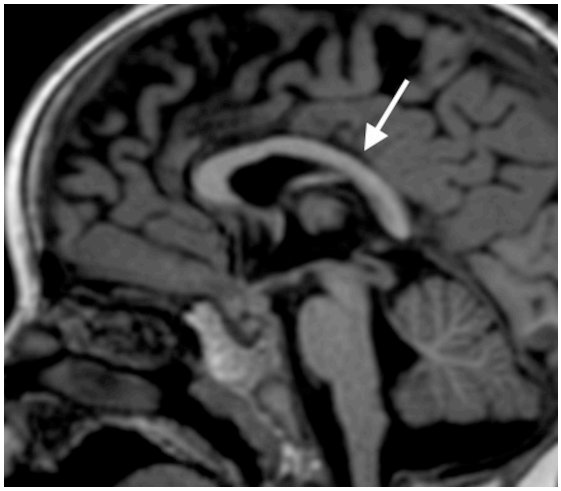


Fig. 4 Midline T1 sagittal MRI scan shows the fornix is seen inserting into zone 3 (arrow) in a 4-year-old girl presenting with seizures and a normal MRI scan

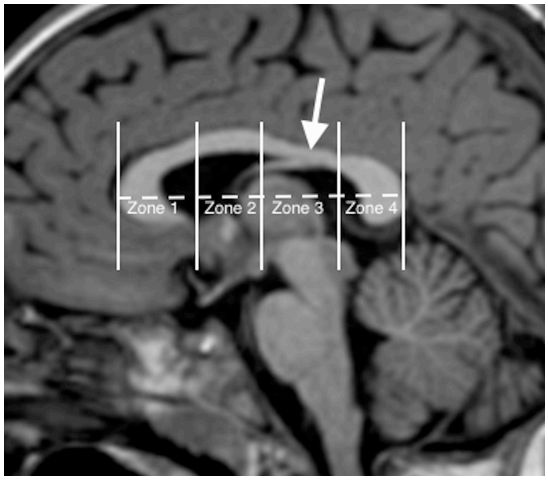


Fig. 5 Midline T1 sagittal MRI scan in a 5-year-old girl with transverse myelitis the fornix can be seen inserting into zone 4 (arrow)

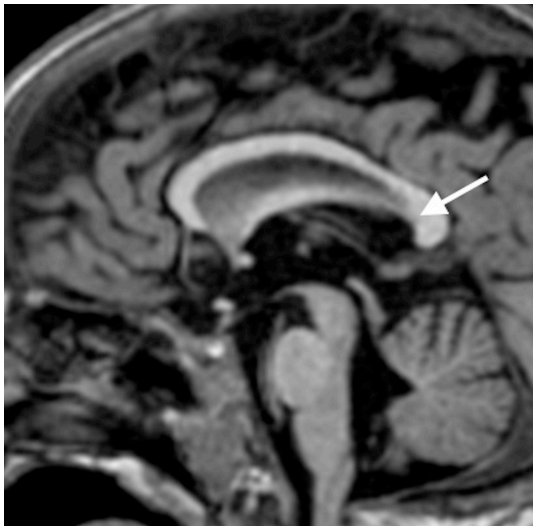


Fig. 6 Midline T1 sagittal MRI scan demonstrates a long and thin splenium (arrow) in a 9-month-old boy with tuberous sclerosis. Note also that the whole corpus callosum, including the splenium is thin

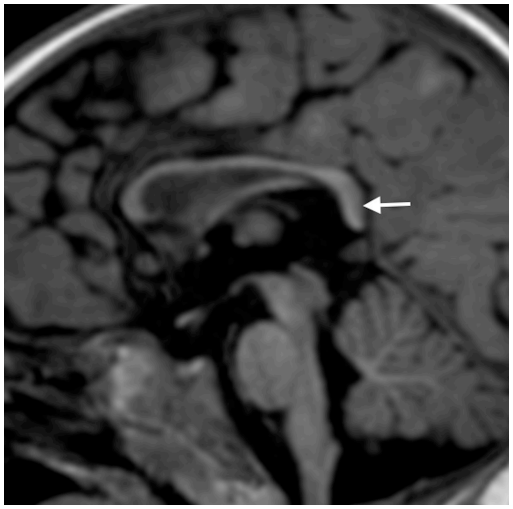


Fig. 7 Midline T1 sagittal MRI scan demonstrates a splenium that is circular and bulbous (arrow) in the 6-year-old boy known with transverse myelitis

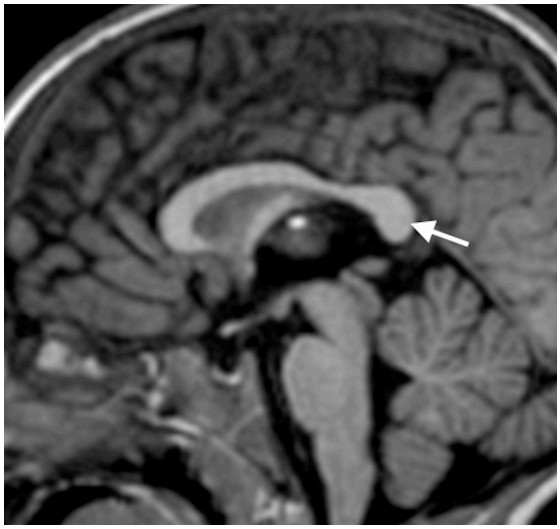


Fig. 8 Midline T1 sagittal MRI scan demonstrates a diffusely thick corpus callosum in a 10-year-old male with ADHD and seizures

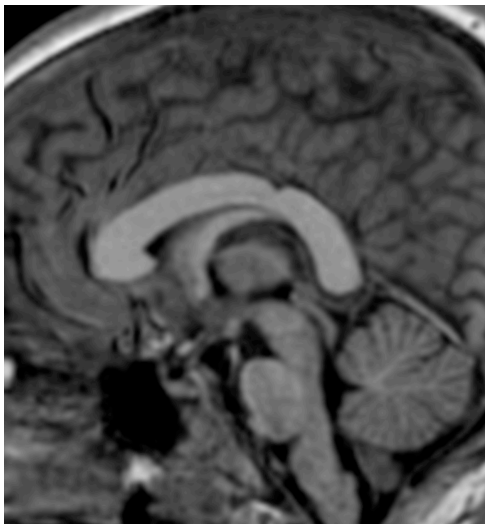


Fig. 9 Midline T1 sagittal MRI scan demonstrates a diffusely thin corpus callosum in a 13-month-old with Barth syndrome

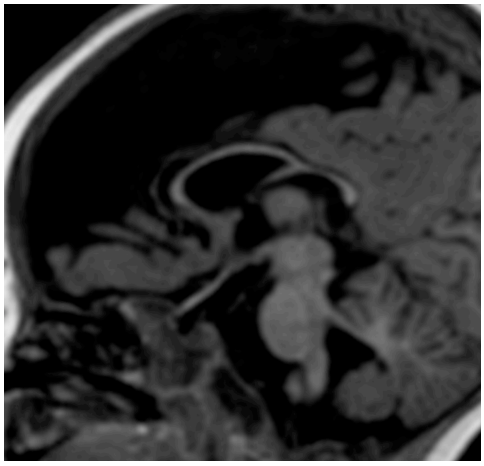


Fig. 10 Midline T1 sagittal MRI scan demonstrates upward bowing (convexity) of the corpus callosum in a 6-year-old with hypoxic ischaemic encephalopathy (HIE)

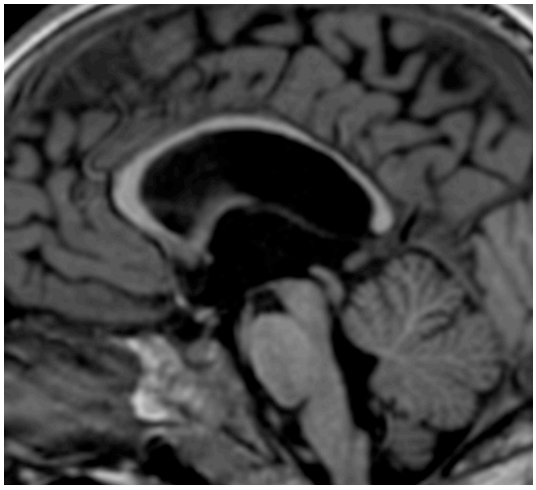


Fig. 11 Histogram of the Age distribution

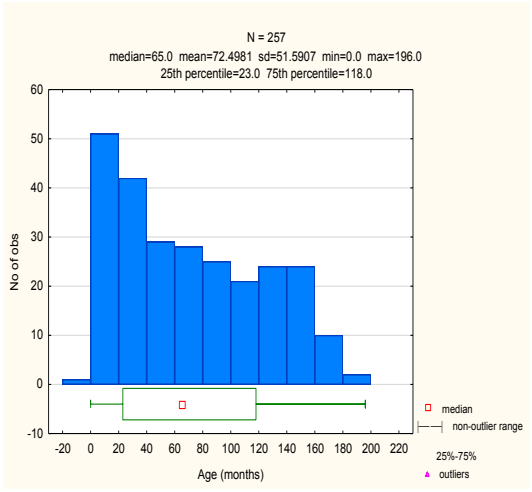


Fig. 12 Histogram of the Frequency and Proportions of the Indication for MRI

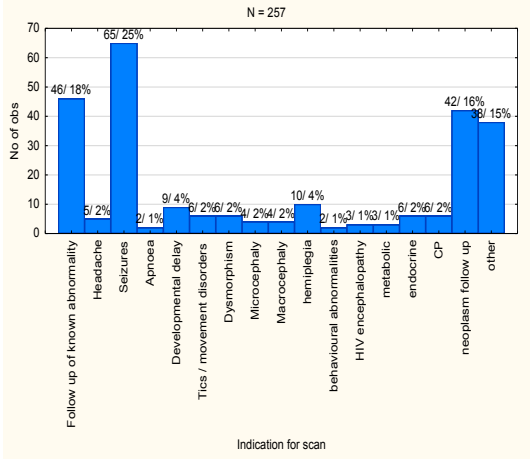


Fig. 13 Histogram Summarising the Frequency and Proportion of MRI Diagnoses

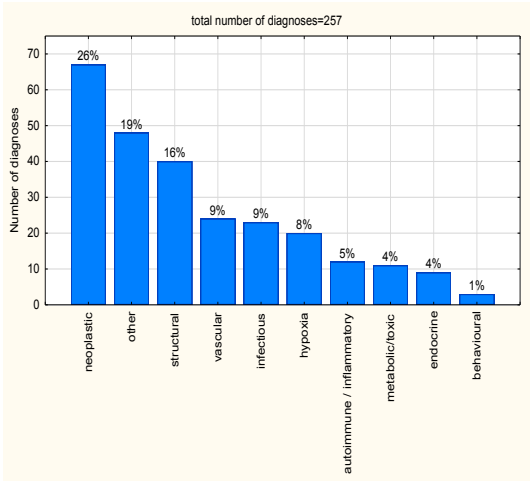


Fig. 14 Flow diagram summarising the categorisation of corpus callosum morphology as determined on MRI

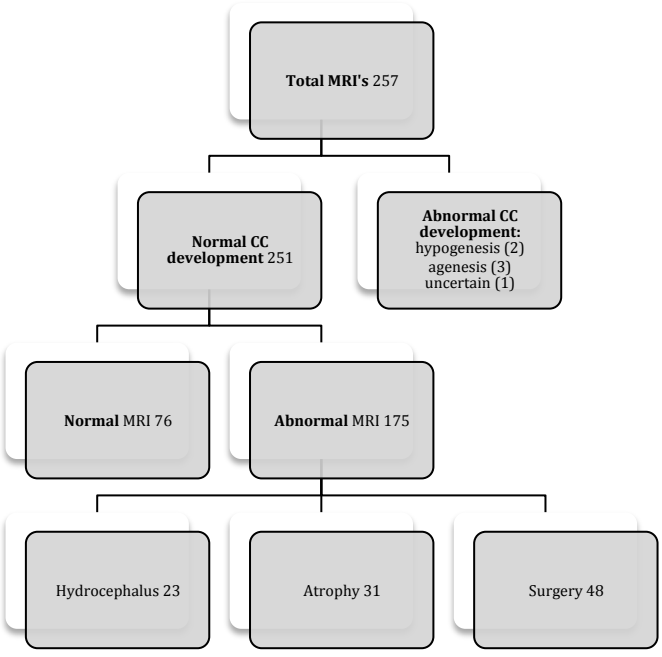


Fig. 15 Line drawing depicting pathologic corpus callosum morphology including convex shape, no identifiable isthmus, fornix insertion in zone 4 (broken arrow) and thin genu, body and splenium

Fig. 16 Age range of identifiable vs. no identifiable isthmus expressed in LS means

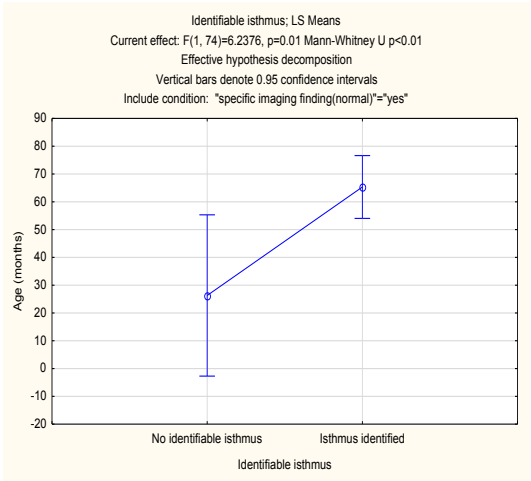


Fig. 17 Age range of fornix insertion levels expressed in LS means

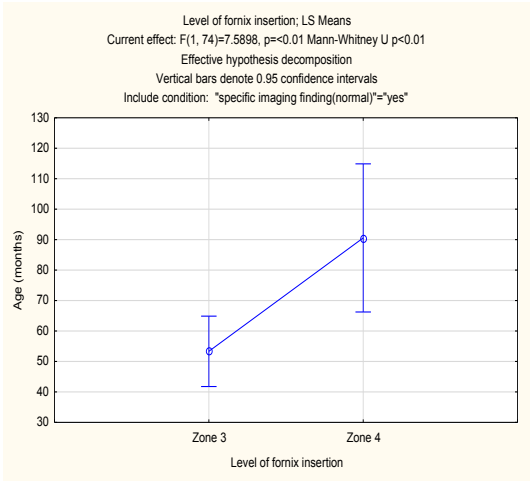


Fig. 18 Age range of overall shape of corpus callosum expressed in LS means

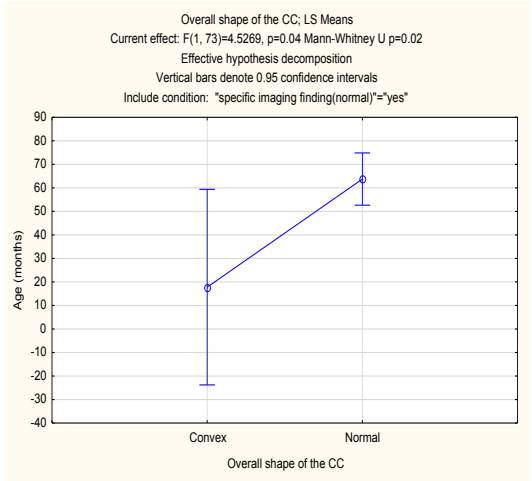


Fig. 19 Age range of splenium shape expressed in LS means

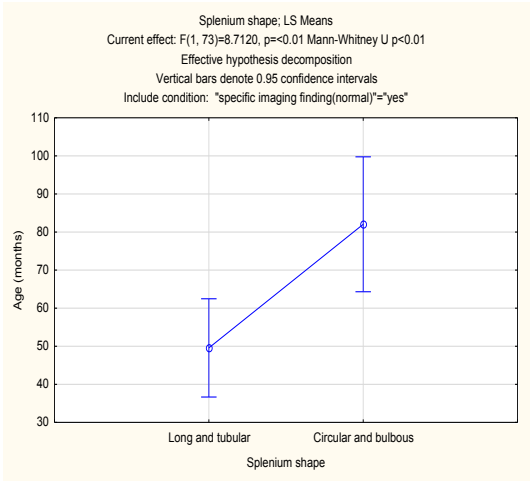
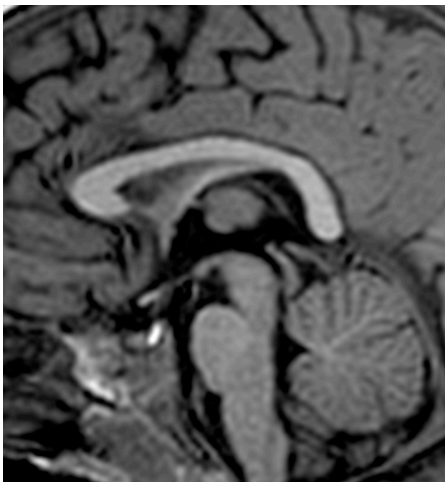


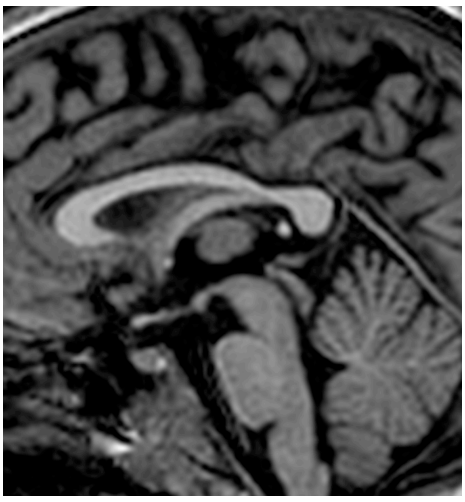
Fig. 20 a-d Comparison of normal morphology of the corpus callosum in children under 5 years of age with those 5 years of age and older using line diagrams and midline sagittal T1 MRI scans:

a) Line drawing illustrating normal corpus callosum shape for children < 5 years (no identifiable isthmus, long and tubular splenium, convex shape and fornix insertion in zone 3 demonstrated by broken arrow).



b) T1 sagittal MRI in a 3-year-old girl who was being followed up for previous tuberculosis meningitis with typical corpus callosum morphology for children < 5 years.

c) Line drawing depicting normal corpus callosum shape for children ≥ 5 years of age (identifiable isthmus, circular / bulbous splenium, normal shape and fornix insertion in zone demonstrated by broken arrow).



d) T1 sagittal MRI in an 9-year-old boy demonstrated normal corpus callosum shape for children > 5 years

References

1. Andronikou S, Pillay T, Gabuza L, Mahomed N, Naidoo J, Hlabangana LT, du Plessis V, Prabhu SP (2014) Corpus callosum thickness in children: an MR pattern-recognition approach on the midsagittal image. Pediatr Radiol. doi:10.1007/s00247-014-2998-9

2. Garel C, Cont I, Alberti C, Josserand E, Moutard ML, Ducou le Pointe H (2011) Biometry of the corpus callosum in children: MR imaging reference data. AJNR Am J Neuroradiol 32 (8):1436-1443. doi:10.3174/ajnr.A2542

3. Park HJ, Kim JJ, Lee SK, Seok JH, Chun J, Kim DI, Lee JD (2008) Corpus callosal connection mapping using cortical gray matter parcellation and DT-MRI. Hum Brain Mapp 29 (5):503-516. doi:10.1002/hbm.20314

4. Erdogan N, Ulger H, Tuna I, A. O (2005) A novel index to estimate the corpus callosum morphometry in adults: callosal/supratentorial-supracallosal area ratio. Diagn Interv Radiol 11 (4):179-181

5. Luders E, Di Paola M, Tomaiuolo F, Thompson PM, Toga AW, Vicari S, Petrides M, Caltagirone C (2007) Callosal morphology in Williams syndrome: a new evaluation of shape and thickness. Neuroreport 18 (3):203-207. doi:10.1097/WNR.0b013e3280115942

6. Ng WH, Chan YL, Au KS, Yeung KW, Kwan TF, To CY (2005) Morphometry of the corpus callosum in Chinese children: relationship with gender and academic performance. Pediatr Radiol 35 (6):565-571. doi:10.1007/s00247-004-1336-z

7. Wittelson SF (1985) The brain connection: the corpus callosum is larger in left-handers. Science 229 (665-668)

8. Byne W, Nleier R, Houston L (1988) Variations in Human Corpus Callosum Do Not Predict Gender: A Study Using Magnetic Resonance Imaging. Behvaioural Neuroscience 102 (2):222-227

9. Rice SA, Bigler ED, Cleavinger HB, Tate DF, Sayer JM, W., Ozonoff S, Lu J, Lainhart JE (2006) Macrocephaly, Corpus Callosum Morphology, and Autism. Journal of Child Neurology 20 (1):34-42

10. Walterfang M, Malhi GS, Wood AG, Reutens DC, Chen J, Barton S, Yucel M, Velakoulis D, Pantelis C (2009) Corpus callosum size and shape in established bipolar affective disorder. Australian and New Zealand Journal of Psychiatry 43:838-845

11. Witelson SF (1989) Hand and sex differences in the isthmus and genu of the human corpus callosum: a postmortem morphological study. Brain 112:799-835

12. Huang H, Zhang J, Jiang H, Wakana S, Poetscher L, Miller MI, van Zijl PC, Hillis AE, Wytik R, Mori S (2005) DTI tractography based parcellation of white matter: application to the mid-sagittal morphology of corpus callosum. Neuroimage 26 (1):195-205. doi:10.1016/j.neuroimage.2005.01.019

13. Hutchinson AD, Mathias JL, Banich MT (2008) Corpus callosum morphology in children and adolescents with attention deficit hyperactivity disorder: a meta-analytic review. Neuropsychology 22 (3):341-349. doi:10.1037/0894-4105.22.3.341

14. Narr KL, Cannon TD, Woods RP, Thompson PM, Kim S, Asunction D, van Erp TGM, Poutanen M, Lonnqvist J, Standerksjold-Nordenstam C, Kapro J, Mazziotta JC, Toga AW (2002) Genetic Contributions to Altered Callosal Morphology in Schizophrenia. The Journal of Neuroscience 22 (9):3720-3729

15. DeLacoste-Utamsing C, Holloway R (1982) Sexual dimorphism in the human corpus callosum. Science 216 (4553):1431-1432. doi:10.1126/science.7089533

16. Luders E, Thompson PM, Toga AW (2010) The development of the corpus callosum in the healthy human brain. The Journal of neuroscience : the official journal of the Society for Neuroscience 30 (33):10985-10990. doi:10.1523/JNEUROSCI.5122-09.2010

Appendices

Appendix A: Descriptions and definitions to aid the reader in MRI interpretation and recording of data

Category 1: Completeness in development

- Normal development: the CC shows normal antenatal development with a visible rostrum, genu, body and splenium (Figure 1).
- Hypogenesis: defined as absence of the posterior body, splenium and/or rostrum (Figure 2).
- Agenesis: no visible corpus callosum

Figure 1

Figure 2

Category 2: Identifiable isthmus

- Isthmus identified: Area of focal thinning easily identified between the posterior body and splenium of the CC (Figure 3).
- No identifiable isthmus: (Figure 4).

Figure 3

Figure 4

Category 3: Level of fornix insertion

A line representing the length of the CC should be drawn by connecting the most anterior to the most posterior points of the CC. This line is then divided into quarters. The fornix attachment is then recorded according to its position at one of 4 zones. The normal fornix usually attaches at the splenium i.e. zone 3 or 4 (Figure 5).

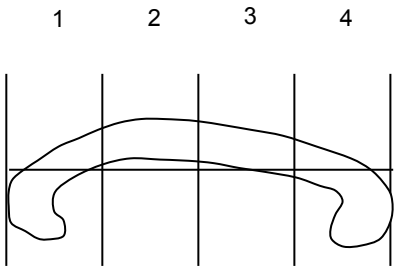


Figure 5

Category 4: Splenium shape

- Long and tubular (Figure 6).
- Circular and bulbous (Figure 7).

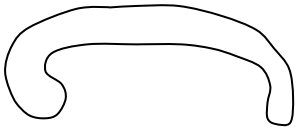


Figure 6

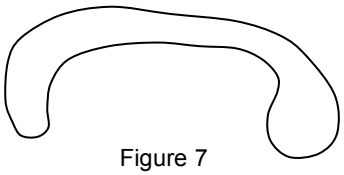


Figure 7

Category 5: Corpus callosum mid-sagittal thickness based on measurements of the thickest part of the genu, body and splenium (Table 2)

- Genu:
- Body:
- Splenium:

Table 2: Garel 2011

	Age (yrs)																
	0	0.5	1	1.5	2	2.5	3	4	5	6	7	8	9	10	11	12	13
Genu thickness																	
3rd percentile	2.5	3.7	4.6	5.2	5.7	6	6.3	6.7	6.9	7	7.1	7.2	7.3	7.3	7.4	7.5	7.5
97th percentile	8.3	8.9	9.7	10.4	11	11.4	11.8	12.3	12.6	12.9	13	13.1	13.2	13.3	13.4	13.5	13.5
Body thickness																	
3rd percentile	1.3	1.8	2.2	2.6	2.9	3.1	3.3	3.5	3.7	3.8	3.9	3.9	4	4	4	4	3.9
97th percentile	5	5.3	5.7	6.1	6.5	6.8	7	7.4	7.6	7.7	7.8	7.9	7.9	8	8	8	8.1
Splenium thickness																	
3rd percentile	1.9	3.4	4.4	5.1	5.6	6	6.2	6.7	6.9	7.2	7.4	7.5	7.6	7.7	7.7	7.7	7.5
97th percentile	9	9.2	9.9	10.5	10.9	11.3	11.5	11.9	12.2	12.5	12.7	12.8	13	13.1	13.3	13.5	13.7

Thin < 3rd centile
Normal 3rd – 97th centile
Thick > 97th centile

Category 6: Overall shape of the CC

- Convex - upwardly bowed (Figure 8).
- Flattened (Figure 9).
- Normal (Figure 10).

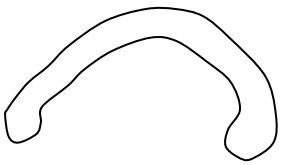


Figure 8

Figure 9

Figure 10

Appendix B: RCWMCH approval

Western Cape Government
Health

Dr AS Booysen
Manager: Medical Services
Email: Tony.Booysen@Westerncape.gov.za
Tel: +27 21 658 5788 fax: +27 21 658 5166

Dr L Raubenheimer
Red Cross War Memorial Children's Hospital

Dear Dr L Raubenheimer

APPROVAL OF RESEARCH

PROJECT TITLE: CORPUS CALLOSUM MORPHOLOGY IN CHILDREN ON MID-SAGITTAL MR IMAGING

It is a pleasure to inform you that approval is hereby granted to conduct the above-mentioned study at Red Cross War Memorial Children's Hospital.

Yours sincerely

Dr AS Booysen
Manager: Medical Services
Date: 27.06.16

Appendix C: Ethics approval

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
Email: shuretta.thomas@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

14 April 2016

HREC REF: 202/2016

Prof S Andronikou
Division of Radiology
C16, NGSH

Dear Prof Andronikou

PROJECT TITLE: CORPUS CALLOSUM MORPHOLOGY IN CHILDREN ON MID-SAGITTAL MR-IMAGING (MMED CANDIDATE - DR L RAUBENHEIMER)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30th April 2017.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval before the research may occur.

The HREC acknowledge that Dr Lauren Raubenheimer will also be involved in this study.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH

Appendix D: Instructions for authors, Journal of Paediatric Radiology

It is the Corresponding Author's responsibility to ensure that he/she has the correct authors' names, affiliations, addresses and author sequence when the final corrected proofs are submitted. Please keep in mind that corrections are no longer possible after online first publication. All additional corrections need the approval of the Managing Editors and would result in the publication of an erratum that will be hyperlinked to the article.

Important Information Regarding Radiation Dosimetry

In order to adhere to the ALARA concept, authors should not submit manuscripts that describe techniques that have used inappropriately high radiation exposures for children. Furthermore, when CT has been used, the text should include the CTDI (as a single value when there is one exam or as a range in multiple exams) in manuscript submissions. This will provide significant information for appropriate dosimetry.

TYPES OF PAPERS

Original article

This is the most important type of article because it provides new information based on original research. An original report is new because of the imaging findings in a disease or syndrome; it is new because of unique interventional processes; it is new because it expresses new manifestations or complications or follow-up of a disease or disorder. Original reports can be prospective or retrospective. They can be clinical or basic research. This type of article must not exceed 18 double–spaced typed pages excluding tables and pictures.

Format:

Structured Abstract which should be divided into the following sections:

1) Background – reason for study

2) Objective – give hypothesis being tested

3) Materials and methods – brief but specific to number of subjects, how collected, and what was done

4) Results – the findings of the study with statistical significance

5) Conclusion

Body of paper:

Introduction: Briefly describe the objective of the investigation and explain why it is important.

Materials and methods: Describe the research plan, the materials (or subjects), and the methods used, in that order.

Explain in detail how disease was confirmed and how subjectivity in observations was controlled.

Results: Present results in a clear, logical sequence. If tables are used, do not duplicate tabular data in text, but do describe important trends and points.

Discussion:

Describe the limitations of the research plan, materials (or subjects), and methods, considering both the objective and the outcome of the study. When results differ from those of previous investigators, explain the discrepancy.

Conclusion:

In one or two sentences, present the message to be remembered when all else is forgotten. Describe the conclusion of the study, based solely on the data provided in the body of the report. Conclusions must relate directly to the objective of the paper as defined in the title and first paragraph of the report. Do not use abbreviations. Do not use reference citations.

Editorial

Brief article (6 or fewer double spaced typed pages) stating the author's personal opinion on a contentious or timely topic. Minimum illustrations. Author will review articles to align his/her viewpoint.

Format:

> No abstract
> Sections divided by topic headings

Technical innovation

A short explanation of a certain method or procedure, alteration of a method, or new equipment of interest to radiologists. Limited to 6 double-spaced typed pages. References limited to 8.

Format:

> Abstract in paragraph form of less than 125 words
> A brief, one–paragraph introduction giving the general background
> Body of report: Introduction with general background. Description of new technical innovation. Discussion.

Case report

Short discussion of a single case with unique features not previously described. A case report must be unique by imaging findings, a unique manifestation of a disease or disorder or by making unique use of imaging to reveal a disease or disorder. Images of a second case may supplement either the discussion or the illustration of findings, but a single case must remain the concentration. Limited to 6 double-spaced typed pages. References limited to 8. Authors limited to 5 who are affiliated with the institution that managed the case.

Format:

> Abstract in paragraph form (<125 words) and includes: 1) Reason to report 2) What was unique 3) Ramification of this report
> Body of report: Introduction – is a brief paragraph giving general background and specific interest of the case. Case report – Stress should be on the radiologic aspects; clinical information must be limited to that which provides a background for the radiologist. Discussion – Concise and focuses on the specific message and significance of radiologic methods. A review of the literature is not appropriate.

Since we receive many case reports, we will attempt to publish those accepted as rapidly as possible. However, priority in getting to publication will be given to original articles and review articles.

Review

Scholarly examination of recent developments on a certain topic as reported in the literature. No new information is described but personal experiences may be expressed.

Reviews are not encyclopedic like a chapter in a textbook; rather, they include only the highlights. Limited to 20 double–space typed pages.

Format:

Abstract in paragraph form introducing scope of paper.
Body of report: Introduction – background and scope Headings – used to organize text

Pictorial essay

This is a teaching exercise with the message in the figures and their legends. Text is no more than 9 double–spaced typed pages, and there may be as many as 30 figure parts; however, no new information is included. The value of the paper turns on the quality of the illustrations as well as the timeliness and utility of the message.

Format:

Abstract in paragraph form defining the goals of the essay.
Body: Introduction Headings – used to organize text

Clinical image

Clinical images are no longer accepted

Letter to the Editor and Reply

Letters to the editor and replies should offer objective analysis of published articles. Letters may also discuss matters of general interest to pediatric radiologists. Material being submitted or published elsewhere should not be repeated in letters.

Format:

Double-spaced on non-letterhead paper, with a salutation of ‘‘Dear Editor’’. The title included on the letter should be short and relevant. The title for a reply is simply ‘‘Reply.’’ Do not use abbreviations in the title, letter, or reply.

Summary of Format for Articles

Types of articles	Maximum pages* (words)	Abstract
Original article	18 (4,500)	Structure
Editorial (Opinion/Commentary)	6 (1,500)	None
Technical innovation	6 (1,500)	Paragrap
Case report	6 (1,500)	Paragrap
Review	20 (5,000)	Paragrap
Pictorial essay	9 (2,250)	Paragrap
Letters to the Editor	2 (500)	None

*Each page double–spaced is approximately 250 words. Total pages include references but not pictures.

EDITORIAL PROCEDURE

Double-blind peer review

This journal follows a double-blind reviewing procedure. Authors are therefore requested to submit:

A blinded manuscript without any author names and affiliations in the text or on the title page. Self-identifying citations and references in the article text should be avoided.
A separate title page, containing title, all author names, affiliations, and the contact information of the corresponding author. Any acknowledgements, disclosures, or funding information should also be included on this page.

MANUSCRIPT SUBMISSION

Manuscript Submission

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been

approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

Permissions

Authors wishing to include figures, tables, or text passages that have already been published elsewhere are required to obtain permission from the copyright owner(s) for both the print and online format and to include evidence that such permission has been granted when submitting their papers. Any material received without such evidence will be assumed to originate from the authors.

Online Submission

Please follow the hyperlink “Submit online” on the right and upload all of your manuscript files following the instructions given on the screen.

TITLE PAGE

Title Page

The title page should include:

- The name(s) of the author(s)
- A concise and informative title
- The affiliation(s) and address(es) of the author(s)
- The e-mail address, and telephone number(s) of the corresponding author
- If available, the 16-digit ORCID of the author(s)

Abstract

Please provide an abstract of 150 to 250 words. The abstract should not contain any undefined abbreviations or unspecified references.

Keywords

Please provide 4 to 6 keywords which can be used for indexing purposes.

TEXT

Text Formatting

Manuscripts should be submitted in Word.

- Use a normal, plain font (e.g., 10-point Times Roman) for text.
- Use italics for emphasis.
- Use the automatic page numbering function to number the pages.
- Do not use field functions.
- Use tab stops or other commands for indents, not the space bar.
- Use the table function, not spreadsheets, to make tables.
- Use the equation editor or MathType for equations.
- Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Manuscripts with mathematical content can also be submitted in LaTeX.

- LaTeX macro package (zip, 181 kB)

Headings

Please use no more than three levels of displayed headings.

Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.

Always use footnotes instead of endnotes.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full.

SCIENTIFIC STYLE

Please always use internationally accepted signs and symbols for units (SI units).

REFERENCES

Citation

Reference citations in the text should be identified by numbers in square brackets. Some examples:

1. Negotiation research spans many disciplines [3].

2. This result was later contradicted by Becker and Seligman [5].

3. This effect has been widely studied [1-3, 7].

Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text. Do not use footnotes or endnotes as a substitute for a reference list.

The entries in the list should be numbered consecutively.

> Journal article Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. https://doi.org/10.1007/s00421-008-0955-8 Ideally, the names of all authors should be provided, but the usage of “et al” in long author lists will also be accepted: Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329
> Article by DOI Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. https://doi.org/10.1007/s001090000086
> Book South J, Blass B (2001) The future of modern genomics. Blackwell, London
> Book chapter Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257
> Online document Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007
> Dissertation Trent JW (1975) Experimental acute renal failure. Dissertation, University of California

Always use the standard abbreviation of a journal’s name according to the ISSN List of Title Word Abbreviations, see

> ISSN.org LTWA

If you are unsure, please use the full journal title.

For authors using EndNote, Springer provides an output style that supports the formatting of in-text citations and reference list.

> EndNote style (zip, 2 kB)

Authors preparing their manuscript in LaTeX can use the bibtex file spbasic.bst which is included in Springer's LaTeX macro package.

SPECIFIC REMARKS

References with correct punctuation can be found in EndNoteX1 (Windows 2000 SP3, XP [SP2] and Vista) (Mac OS X).

TABLES

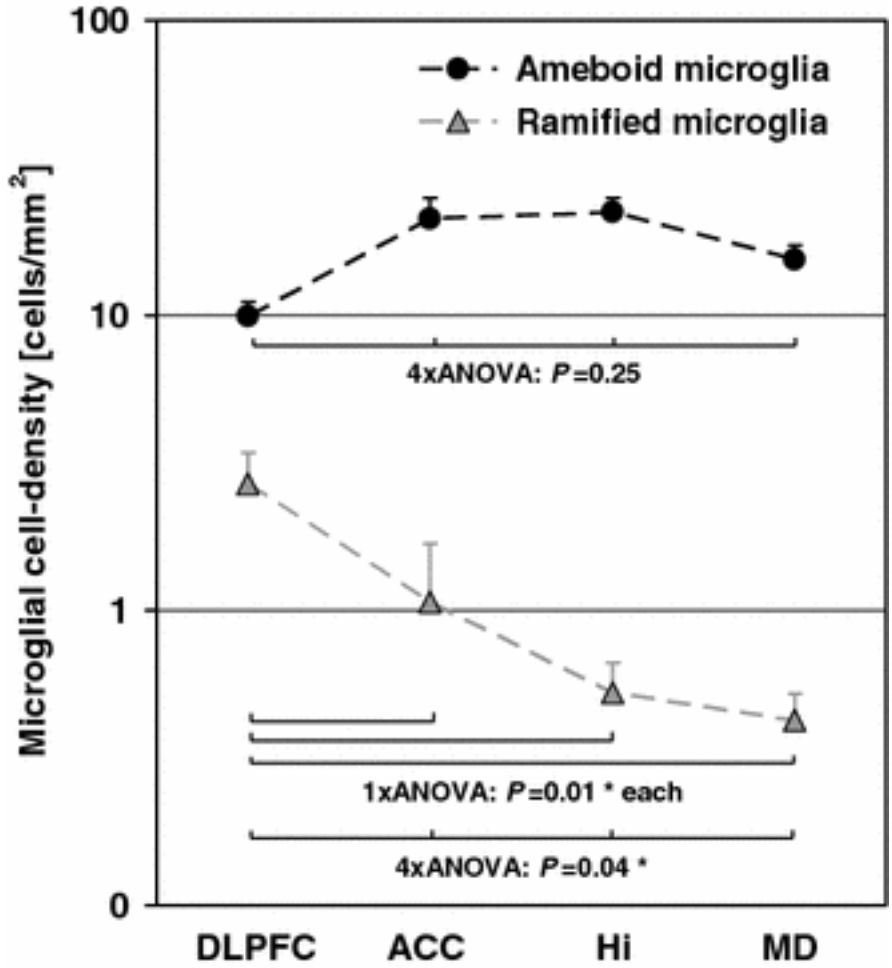
All tables are to be numbered using Arabic numerals.
Tables should always be cited in text in consecutive numerical order.
For each table, please supply a table caption (title) explaining the components of the table.
Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.
Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

ARTWORK AND ILLUSTRATIONS GUIDELINES

Electronic Figure Submission

Supply all figures electronically.
Indicate what graphics program was used to create the artwork.
For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MSOffice files are also acceptable.
Vector graphics containing fonts must have the fonts embedded in the files.
Name your figure files with "Fig" and the figure number, e.g., Fig1.eps.

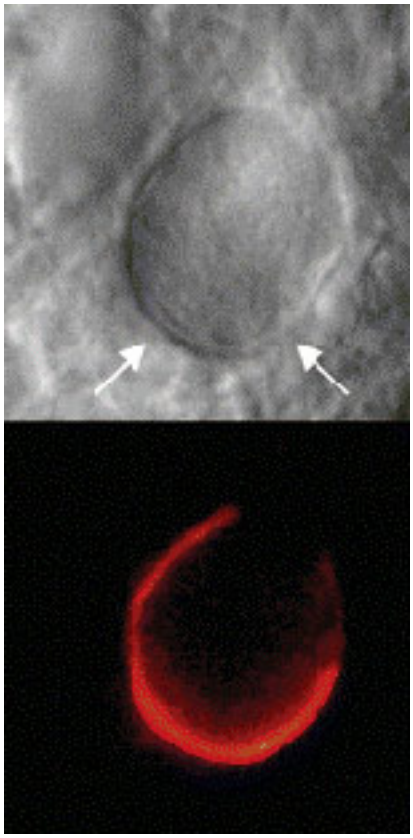
Line Art



Definition: Black and white graphic with no shading.
Do not use faint lines and/or lettering and check that all lines and lettering within the

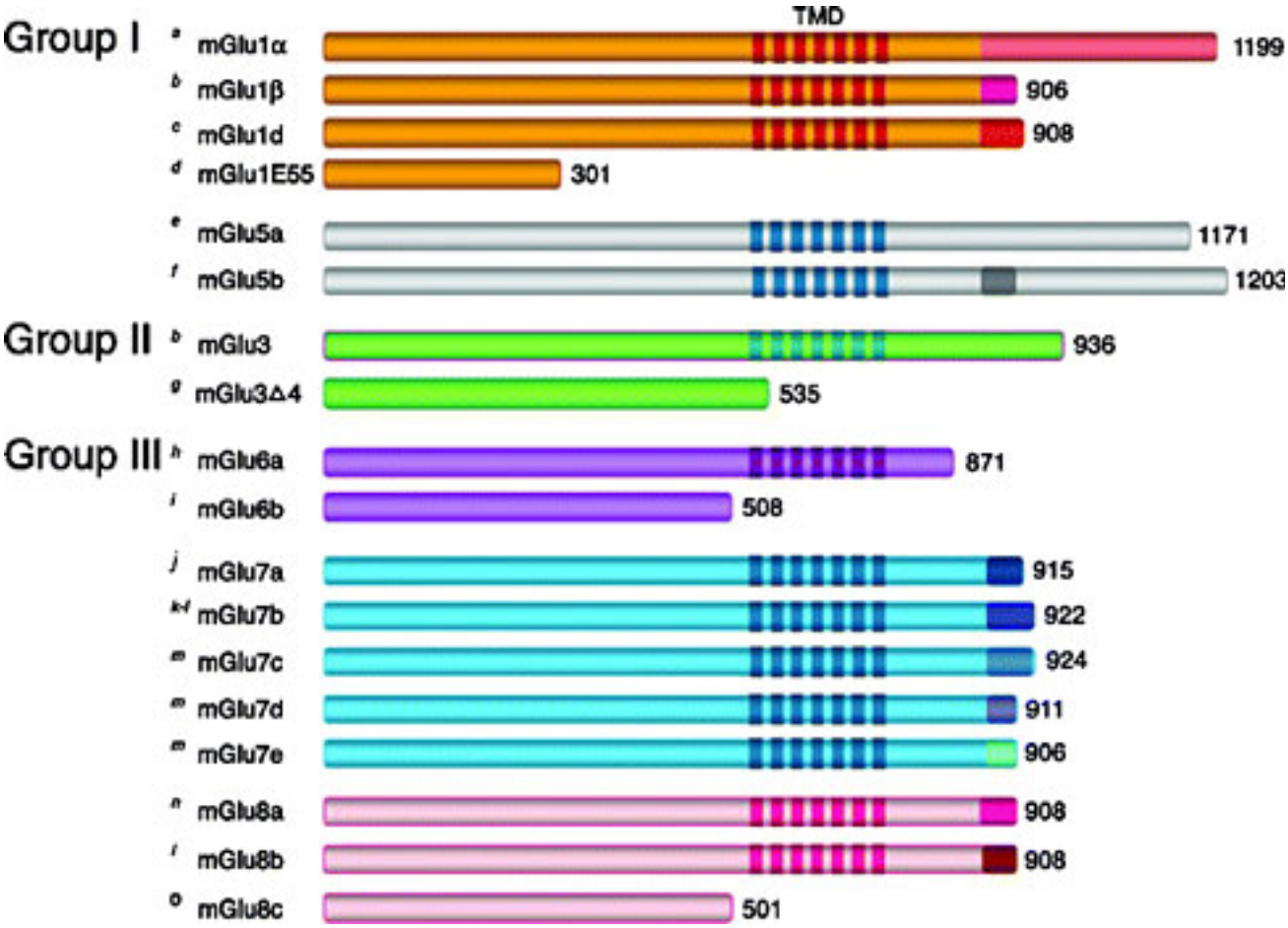
figures are legible at final size.
All lines should be at least 0.1 mm (0.3 pt) wide.
Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.
Vector graphics containing fonts must have the fonts embedded in the files.

Halftone Art



Definition: Photographs, drawings, or paintings with fine shading, etc.
If any magnification is used in the photographs, indicate this by using scale bars within the figures themselves.
Halftones should have a minimum resolution of 300 dpi.

Combination Art



Definition: a combination of halftone and line art, e.g., halftones containing line drawing, extensive lettering, color diagrams, etc.
Combination artwork should have a minimum resolution of 600 dpi.

Color Art

Color art is free of charge for online publication.
If black and white will be shown in the print version, make sure that the main information will still be visible. Many colors are not distinguishable from one another when converted to black and white. A simple way to check this is to make a xerographic copy to see if the necessary distinctions between the different colors are still apparent.
If the figures will be printed in black and white, do not refer to color in the captions.
Color illustrations should be submitted as RGB (8 bits per channel).

Figure Lettering

To add lettering, it is best to use Helvetica or Arial (sans serif fonts).
Keep lettering consistently sized throughout your final-sized artwork, usually about 2–3 mm (8–12 pt).
Variance of type size within an illustration should be minimal, e.g., do not use 8-pt type on an axis and 20-pt type for the axis label.
Avoid effects such as shading, outline letters, etc.
Do not include titles or captions within your illustrations.

Figure Numbering

All figures are to be numbered using Arabic numerals.
Figures should always be cited in text in consecutive numerical order.
Figure parts should be denoted by lowercase letters (a, b, c, etc.).
If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures, "A1, A2, A3, etc." Figures in online appendices (Electronic Supplementary

Material) should, however, be numbered separately.

Figure Captions

Each figure should have a concise caption describing accurately what the figure depicts. Include the captions in the text file of the manuscript, not in the figure file.
Figure captions begin with the term Fig. in bold type, followed by the figure number, also in bold type.
No punctuation is to be included after the number, nor is any punctuation to be placed at the end of the caption.
Identify all elements found in the figure in the figure caption; and use boxes, circles, etc., as coordinate points in graphs.
Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption.

Figure Placement and Size

Figures should be submitted separately from the text, if possible.
When preparing your figures, size figures to fit in the column width.
For most journals the figures should be 39 mm, 84 mm, 129 mm, or 174 mm wide and not higher than 234 mm.
For books and book-sized journals, the figures should be 80 mm or 122 mm wide and not higher than 198 mm.

Permissions

If you include figures that have already been published elsewhere, you must obtain permission from the copyright owner(s) for both the print and online format. Please be aware that some publishers do not grant electronic rights for free and that Springer will not be able to refund any costs that may have occurred to receive these permissions. In such cases, material from other sources should be used.

Accessibility

In order to give people of all abilities and disabilities access to the content of your figures, please make sure that

All figures have descriptive captions (blind users could then use a text-to-speech software or a text-to-Braille hardware)
Patterns are used instead of or in addition to colors for conveying information (colorblind users would then be able to distinguish the visual elements)
Any figure lettering has a contrast ratio of at least 4.5:1

ELECTRONIC SUPPLEMENTARY MATERIAL

Springer accepts electronic multimedia files (animations, movies, audio, etc.) and other supplementary files to be published online along with an article or a book chapter. This feature can add dimension to the author's article, as certain information cannot be printed or is more convenient in electronic form.

Before submitting research datasets as electronic supplementary material, authors should read the journal’s Research data policy. We encourage research data to be archived in data repositories wherever possible.

Submission

Supply all supplementary material in standard file formats.
Please include in each file the following information: article title, journal name, author names; affiliation and e-mail address of the corresponding author.
To accommodate user downloads, please keep in mind that larger-sized files may require very long download times and that some users may experience other problems during downloading.

Audio, Video, and Animations

Aspect ratio: 16:9 or 4:3
Maximum file size: 25 GB
Minimum video duration: 1 sec

Supported file formats: avi, wmv, mp4, mov, m2p, mp2, mpg, mpeg, flv, mxf, mts, m4v, 3gp

Text and Presentations

Submit your material in PDF format; .doc or .ppt files are not suitable for long-term viability.
A collection of figures may also be combined in a PDF file.

Spreadsheets

Spreadsheets should be submitted as .csv or .xlsx files (MS Excel).

Specialized Formats

Specialized format such as .pdb (chemical), .wrl (VRML), .nb (Mathematica notebook), and .tex can also be supplied.

Collecting Multiple Files

It is possible to collect multiple files in a .zip or .gz file.

Numbering

If supplying any supplementary material, the text must make specific mention of the material as a citation, similar to that of figures and tables.
Refer to the supplementary files as “Online Resource”, e.g., "... as shown in the animation (Online Resource 3)", “... additional data are given in Online Resource 4”.
Name the files consecutively, e.g. “ESM_3.mpg”, “ESM_4.pdf”.

Captions

For each supplementary material, please supply a concise caption describing the content of the file.

Processing of supplementary files

Electronic supplementary material will be published as received from the author without any conversion, editing, or reformatting.

Accessibility

In order to give people of all abilities and disabilities access to the content of your supplementary files, please make sure that

The manuscript contains a descriptive caption for each supplementary material
Video files do not contain anything that flashes more than three times per second (so that users prone to seizures caused by such effects are not put at risk)

ETHICAL RESPONSIBILITIES OF AUTHORS

This journal is committed to upholding the integrity of the scientific record. As a member of the Committee on Publication Ethics (COPE) the journal will follow the COPE guidelines on how to deal with potential acts of misconduct.

Authors should refrain from misrepresenting research results which could damage the trust in the journal, the professionalism of scientific authorship, and ultimately the entire scientific endeavour. Maintaining integrity of the research and its presentation can be achieved by following the rules of good scientific practice, which include:

The manuscript has not been submitted to more than one journal for simultaneous consideration.
The manuscript has not been published previously (partly or in full), unless the new work concerns an expansion of previous work (please provide transparency on the re-use of material to avoid the hint of text-recycling (“self-plagiarism”)).
A single study is not split up into several parts to increase the quantity of submissions and submitted to various journals or to one journal over time (e.g. “salami-publishing”).
No data have been fabricated or manipulated (including images) to support your conclusions
No data, text, or theories by others are presented as if they were the author’s own (“plagiarism”). Proper acknowledgements to other works must be given (this includes

material that is closely copied (near verbatim), summarized and/or paraphrased), quotation marks are used for verbatim copying of material, and permissions are secured for material that is copyrighted. **Important note:** the journal may use software to screen for plagiarism.
Consent to submit has been received explicitly from all co-authors, as well as from the responsible authorities - tacitly or explicitly - at the institute/organization where the work has been carried out, **before** the work is submitted.
Authors whose names appear on the submission have contributed sufficiently to the scientific work and therefore share collective responsibility and accountability for the results.
Authors are strongly advised to ensure the correct author group, corresponding author, and order of authors at submission. Changes of authorship or in the order of authors are **not** accepted **after** acceptance of a manuscript.
Adding and/or deleting authors and/or changing the order of authors **at revision stage** may be justifiably warranted. A letter must accompany the revised manuscript to explain the reason for the change(s) and the contribution role(s) of the added and/or deleted author(s). Further documentation may be required to support your request.
Requests for addition or removal of authors as a result of authorship disputes after acceptance are honored after formal notification by the institute or independent body and/or when there is agreement between all authors.
Upon request authors should be prepared to send relevant documentation or data in order to verify the validity of the results. This could be in the form of raw data, samples, records, etc. Sensitive information in the form of confidential proprietary data is excluded.

If there is a suspicion of misconduct, the journal will carry out an investigation following the COPE guidelines. If, after investigation, the allegation seems to raise valid concerns, the accused author will be contacted and given an opportunity to address the issue. If misconduct has been established beyond reasonable doubt, this may result in the Editor-in-Chief's implementation of the following measures, including, but not limited to:

If the article is still under consideration, it may be rejected and returned to the author.
If the article has already been published online, depending on the nature and severity of the infraction, either an erratum will be placed with the article or in severe cases complete retraction of the article will occur. The reason must be given in the published erratum or retraction note. Please note that retraction means that the paper is **maintained on the platform**, watermarked "retracted" and explanation for the retraction is provided in a note linked to the watermarked article.
The author's institution may be informed.

COMPLIANCE WITH ETHICAL STANDARDS

To ensure objectivity and transparency in research and to ensure that accepted principles of ethical and professional conduct have been followed, authors should include information regarding sources of funding, potential conflicts of interest (financial or non-financial), informed consent if the research involved human participants, and a statement on welfare of animals if the research involved animals.

Authors should include the following statements (if applicable) in a separate section entitled "Compliance with Ethical Standards" when submitting a paper:

Disclosure of potential conflicts of interest
Research involving Human Participants and/or Animals
Informed consent

Please note that standards could vary slightly per journal dependent on their peer review policies (i.e. single or double blind peer review) as well as per journal subject discipline. Before submitting your article check the instructions following this section carefully.

The corresponding author should be prepared to collect documentation of compliance with

ethical standards and send if requested during peer review or after publication.

The Editors reserve the right to reject manuscripts that do not comply with the above-mentioned guidelines. The author will be held responsible for false statements or failure to fulfill the above-mentioned guidelines.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST
Authors must disclose all relationships or interests that could influence or bias the work. Although an author may not feel there are conflicts, disclosure of relationships and interests affords a more transparent process, leading to an accurate and objective assessment of the work. Awareness of real or perceived conflicts of interests is a perspective to which the readers are entitled and is not meant to imply that a financial relationship with an organization that sponsored the research or compensation for consultancy work is inappropriate. Examples of potential conflicts of interests **that are directly or indirectly related to the research** may include but are not limited to the following:

Research grants from funding agencies (please give the research funder and the grant number)
Honoraria for speaking at symposia
Financial support for attending symposia
Financial support for educational programs
Employment or consultation
Support from a project sponsor
Position on advisory board or board of directors or other type of management relationships
Multiple affiliations
Financial relationships, for example equity ownership or investment interest
Intellectual property rights (e.g. patents, copyrights and royalties from such rights)
Holdings of spouse and/or children that may have financial interest in the work

In addition, interests that go beyond financial interests and compensation (non-financial interests) that may be important to readers should be disclosed. These may include but are not limited to personal relationships or competing interests directly or indirectly tied to this research, or professional interests or personal beliefs that may influence your research.

The corresponding author collects the conflict of interest disclosure forms from all authors. In author collaborations where formal agreements for representation allow it, it is sufficient for the corresponding author to sign the disclosure form on behalf of all authors. Examples of forms can be found
here:

The corresponding author will include a summary statement **on the title page that is separate from their manuscript,** that reflects what is recorded in the potential conflict of interest disclosure form(s).

Please make sure to submit all Conflict of Interest disclosure forms together with the manuscript.

See below examples of disclosures:

Funding: This study was funded by X (grant number X).

Conflict of Interest: Author A has received research grants from Company A. Author B has received a speaker honorarium from Company X and owns stock in Company Y. Author C is

a member of committee Z.

If no conflict exists, the authors should state:

Conflict of Interest: The authors declare that they have no conflict of interest.

RESEARCH INVOLVING HUMAN PARTICIPANTS AND/OR ANIMALS

1) Statement of human rights

When reporting studies that involve human participants, authors should include a statement that the studies have been approved by the appropriate institutional and/or national research ethics committee and have been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

If doubt exists whether the research was conducted in accordance with the 1964 Helsinki Declaration or comparable standards, the authors must explain the reasons for their approach, and demonstrate that the independent ethics committee or institutional review board explicitly approved the doubtful aspects of the study.

The following statements should be included in the text before the References section:

Ethical approval: "All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards."

For retrospective studies, please add the following sentence:

"For this type of study formal consent is not required."

2) Statement on the welfare of animals

The welfare of animals used for research must be respected. When reporting experiments on animals, authors should indicate whether the international, national, and/or institutional guidelines for the care and use of animals have been followed, and that the studies have been approved by a research ethics committee at the institution or practice at which the studies were conducted (where such a committee exists).

For studies with animals, the following statement should be included in the text before the References section:

Ethical approval: "All applicable international, national, and/or institutional guidelines for the care and use of animals were followed."

If applicable (where such a committee exists): "All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted."

If articles do not contain studies with human participants or animals by any of the authors, please select one of the following statements:

"This article does not contain any studies with human participants performed by any of the authors."

"This article does not contain any studies with animals performed by any of the authors."

“This article does not contain any studies with human participants or animals performed by any of the authors.”

INFORMED CONSENT
All individuals have individual rights that are not to be infringed. Individual participants in studies have, for example, the right to decide what happens to the (identifiable) personal data gathered, to what they have said during a study or an interview, as well as to any photograph that was taken. Hence it is important that all participants gave their informed consent in writing prior to inclusion in the study. Identifying details (names, dates of birth, identity numbers and other information) of the participants that were studied should not be published in written descriptions, photographs, and genetic profiles unless the information is essential for scientific purposes and the participant (or parent or guardian if the participant is incapable) gave written informed consent for publication. Complete anonymity is difficult to achieve in some cases, and informed consent should be obtained if there is any doubt. For example, masking the eye region in photographs of participants is inadequate protection of anonymity. If identifying characteristics are altered to protect anonymity, such as in genetic profiles, authors should provide assurance that alterations do not distort scientific meaning.

The following statement should be included:

Informed consent: “Informed consent was obtained from all individual participants included in the study.”

If identifying information about participants is available in the article, the following statement should be included:

“Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.”

AFTER ACCEPTANCE
Upon acceptance of your article you will receive a link to the special Author Query Application at Springer’s web page where you can sign the Copyright Transfer Statement online and indicate whether you wish to order OpenChoice and offprints.

Once the Author Query Application has been completed, your article will be processed and you will receive the proofs.

Copyright transfer
Authors will be asked to transfer copyright of the article to the Publisher (or grant the Publisher exclusive publication and dissemination rights). This will ensure the widest possible protection and dissemination of information under copyright laws.

Offprints
Offprints can be ordered by the corresponding author.
Color illustrations
Publication of color illustrations is free of charge.
Proof reading
The purpose of the proof is to check for typesetting or conversion errors and the completeness and accuracy of the text, tables and figures. Substantial changes in content, e.g., new results, corrected values, title and authorship, are not allowed without the approval of the Editor.

After online publication, further changes can only be made in the form of an Erratum, which will be hyperlinked to the article.
Online First

The article will be published online after receipt of the corrected proofs. This is the official first publication citable with the DOI. After release of the printed version, the paper can also be cited by issue and page numbers.

OPEN CHOICE

In addition to the normal publication process (whereby an article is submitted to the journal and access to that article is granted to customers who have purchased a subscription), Springer provides an alternative publishing option: Springer Open Choice. A Springer Open Choice article receives all the benefits of a regular subscription-based article, but in addition is made available publicly through Springer's online platform SpringerLink.

Open Choice

ENGLISH LANGUAGE EDITING

For editors and reviewers to accurately assess the work presented in your manuscript you need to ensure the English language is of sufficient quality to be understood. If you need help with writing in English you should consider:

Asking a colleague who is a native English speaker to review your manuscript for clarity.
Visiting the English language tutorial which covers the common mistakes when writing in English.
Using a professional language editing service where editors will improve the English to ensure that your meaning is clear and identify problems that require your review. Two such services are provided by our affiliates Nature Research Editing Service and American Journal Experts. Springer authors are entitled to a 10% discount on their first submission to either of these services, simply follow the links below.
English language tutorial
Nature Research Editing Service
American Journal Experts

Please note that the use of a language editing service is not a requirement for publication in this journal and does not imply or guarantee that the article will be selected for peer review or accepted.

If your manuscript is accepted it will be checked by our copyeditors for spelling and formal style before publication.

.

□便于□□和□□□家准确□估您稿件中□述的研究工作，您需要确保您的英□□言□量足以令人理解。如果您需要英文写作方面的帮助，您可以考□：

● □一位以英□□母□的同事□核您的稿件是否表意清晰。

● □看一些有关英□写作中常□□言□□的教程。

● 使用□□□言□□服□，□□人□会□英□□行□色，以确保您的意思表达清晰，并□□需要您复核的□□。我□的附属机构 Nature Research Editing Service 和合作伙伴 American Journal Experts 即可提供此□服□。

教程
Nature Research Editing Service
American Journal Experts

□注意，使用□言□□服□并非在期刊上□表文章的必要条件，同□也并不意味或保□文章将被□中□行同行□□或被接受。

如果您的稿件被接受，在□表之前，我□的文字□□会□□您的文稿拼写是否□范以及文体是否正式。

.
エディターと査読者があなたの論文を正しく評価するには、使用されている英語の質が十分に高いことが必要とされます。英語での論文執筆に際してサポートが必要な場合には、次のオプションがあります：

・英語を母国語とする同僚に、原稿で使用されている英語が明確であるかをチェックしてもらう。

・英語で執筆する際のよくある間違いに関する英語のチュートリアルを参照する。

・プロの英文校正サービスを利用する。校正者が原稿の意味を明確にしたり、問題点を指摘し、英語の質を向上させます。Nature Research Editing Service とAmerican Journal Experts の2つは弊社と提携しているサービスです。Springer の著者は、いずれのサービスも初めて利用する際には10%の割引を受けることができます。以下のリンクを参照ください。
英語のチュートリアル
Nature Research Editing Service
American Journal Experts

英文校正サービスの利用は、投稿先のジャーナルに掲載されるための条件ではないこと、また論文審査や受理を保証するものではないことに留意してください。

原稿が受理されると、出版前に弊社のコピーエディターがスペルと体裁のチェックを行います。

.
□□ 원고의 경우, 에디터 및 □□□□□ 귀하□ □□□ 실린 결과물을 정확□□ 평가할 수 있도록, 그□□ 충분히 □□□ □ □□ 만한 □□□□ 작성되□□ 합니다. □□ □□□□ 관련□□ □□□ 받기를 □□□□□ □□□ 사항□□ □□□□ 주십시오:

• □□□ □□□ 표현□ 명□□ □□ □□ □□□ 동료□ 찾아서 □□□ □□□□□.

• □□ □□□□ 페□□□ 방□□□ □□□ 글□ 쓸 때 자□□□ □□□□ □□□□□.

• □□□ 대비□□, □□□ □□□ □□□□ □□□ □□□□ 요구□□ □□□□□ 식별□□ □□ □□□ 향상□□□□ 전□ □□ 교□ □□□□ □□□□□. Nature Research Editing Service와 American Journal Experts□□ 저희□ 협□□ 통□ □□□□ □□□□ □□□□. Springer □□□□ 본 □□ □□□□ 첫 논□ 투□□ 위□ □□□□□ □□ 10%□ □□□ 적□□□, □□□ 링크□ □□□ □□□ □□□□□.

영어 튜토리얼 페이지
Nature Research Editing Service
American Journal Experts

□□ □□ □□□□ □□□ □□ □□□□□ □□□, □□ □□□□ □□□ 피□ □□□ □□□ 선택□□□ □□□ □□□□ 것□ □□□□□ 보장□□ 않□□□.

□□□ □□□ □□, 출판 □ □□□ 편집□□ □□ □□□ 철□ □ □□□ 검□□□ □□□

□□□ 됩□□.