Automatic magnetic resonance brain volume measurements to assess the effect of minimised blood sample extraction on brain development in extremely preterm infants

Automatiserade radiologiska hjärnvolymetriberäkningar för att bedöma effekten av minimerad blodprovstagning hos extremt prematurfödda barn

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Abbreviations

BPD- Bronchopulmonary dysplasia

DICOM- Digital Imaging and Communications in Medicine

DRAW-EM- Developing brain Region Annotation With Expectation-Maximization

EOP- Encephalopathy of prematurity

EPT- Extremely preterm

FSL- FMRIB Software Library

IVH- Intraventricular hemorrhage

LIM- Less is more, a study

M-CRIB- Melbourne Children's Regional Infant Brain

NICU- Neonatal intensive care unit

NIfTI- Neuroimaging Informatics Technology Initiative

PVL- Periventricular leukomalacia

TEA- Term equivalent age

Abstract

Aim: To evaluate a pipeline for automatic MRI brain segmentation and volumetric tissue structure quantification in a cohort of extremely preterm infants.

Methods: 13 extremely preterm neonates underwent MRI-scanning at a term equivalent age. Seven of these subjects had suitable MRI sequences and were analyzed in the pipeline. Segmentation of MRI brain images into brain tissue types and parcellation of the brain into its different anatomical structures was done using Developing Brain Region Annotation With Expectation-Maximization (DRAW-EM), a Medical Image Registration Toolkit package. Volumetric quantification of the regions of interest were done on the segmented images utilizing the FMRIB Software Library (FSL), a software library containing statistical tools for image analysis of MRI brain data. Thalami, hippocampi, white matter and cortical gray matter were analyzed.

Result: Six of the analyzed subjects had little or no imaging artefacts and one had severe imaging artefacts due to motion. One of the subjects with little or no artefacts had a prominent pathology in the left lateral ventricle. Segmentation in the patients with little or no imaging artefacts was overall satisfactory, with possible room for improvement in cortical gray matter segmentation based on one subject. Deep white matter was not demarcated by the program. The pipeline had difficulty segmenting the patient with ventricular pathology, but did surprisingly well with the patient with severe motion artefacts.

Conclusion: The pipeline is able to analyze images without severe pathology and little to no imaging artefacts. Even with severe artefact and no motion correction code, the segmentation in itself was satisfactory. Prominent ventricular pathologies were challenging for the program to handle and may require manual intervention. Segmentation of deep white matter needs to

be implemented. Further improvements are needed to address issues regarding accurate segmentation of cortical gray matter around sulci.

Populärvetenskaplig sammanfattning

I Sverige föds omkring 110 000 barn varje år, varav 5 % föds prematurt, det vill säga innan graviditetsvecka 37. Man räknas dessutom som extremt prematur (EPT) om man föds före graviditetsvecka 28, vilket cirka 0,3 % av alla barn i Sverige gör. EPT-barn har historiskt haft dystra chanser att överleva. Detta har varit av en rad olika anledningar, till exempel omogna lungor, dödliga infektioner och blödningar samt medfödda sjukdomar. Enorma framsteg har gjorts de senaste decennierna i neonatalvården (vård för nyfödda barn) och omkring 80% av EPT-barn i Sverige överlever. Dessa framsteg innefattar bl.a. administrering av kortison för att påskynda lungmognaden, modern teknisk utrustning, bättre kunskaper om neonatala sjukdomar m.m. Globalt är överlevnaden av EPT-barn lägre i länder med sämre ekonomiska förutsättningar och därför är vården ojämlik även här.

En del EPT-barn möter svårigheter senare i livet. Under deras uppväxt och som vuxna drabbas flera i högre utsträckning av olika hälsoproblem, såsom besvär med syn, minne och intelligens, än motsvarande individer födda vid fullgången tid. En del får allvarliga symtom såsom motoriska dysfunktion i form av cerebral pares (CP). Förutom ett stort personligt lidande för individen och familjen är de samhällsekonomiska kostnaderna inte obetydande för insatserna senare i livet. Detta understryker behovet av tidiga insatser för att förhindra hjärnskador och främja hjärnutvecklingen hos dessa barn.

Under vistelsen inom neonatala intensivvården provtas EPT-barn väldigt ofta. Man beräknar att de förlorar motsvarande 50 % av blodvolymen under de första 2 veckorna, och motar motsvarande 100% av blodvolymen under samma period i form av blodtransfusioner. Blodet i dessa transfusioner kommer från vuxna donatorer, och kallas då för adult blod. Fetalt blod,

det vill säga blodet hos fostret och hos nyfödda, innehåller höga halter av olika ämnen som främjar tillväxt, s.k. tillväxtfaktorer. Utöver tillväxtfaktorer innehåller fetalt blod stamceller och ämnen viktiga för syresättningen hos det nyfödda barnet. Man kan därför ställa sig frågan om ersättning av fetalt blod med vuxet blod p.g.a. de frekventa provtagningar och transfusioner kan vara skadlig för den normala utvecklingen av dessa barn. Less is More (LIM) är en studie som vill undersöka just detta.

LIM-studien genomförs på universitetssjukhusen i Lund, Göteborg och Stockholm, där 65 % av EPT-barn i Sverige vårdas. Barn som föds innan vecka 27 och som inte har betydliga missbildningar delas slumpmässigt in i två grupper: den ena gruppen provtas precis som vanligt och kallas för kontrollgruppen, den andra provtas med s.k. mikrometoder och kallas för interventionsgruppen. Genom att använda sig av mikrometoder för vissa vanligt beställda blodprov kan man reducera den totala blodvolymen som dras med mer än 50 %. LIM vill undersöka om interventionen, d.v.s. mikrometoder för provtagning, resulterar i bättre utfall för barnen. Man vill studera om det skiljer sig åt mellan de två grupperna gällande utveckling av lungsjukdomar, hjärnutveckling, generell överlevnad m.m. Hjärnutveckling bedöms genom psykologiska tester som görs senare i livet, men även genom avbildning av hjärnan med magnetkamera när barnens ålder motsvarar cirka fullgången tid (kring 40 veckor). I den studien som genomförts vill vi undersöka om våra automatiserade steg för att analysera hjärnan hos EPT-barn fungerar. Stegen där bilderna bearbetas kallas tillsammans för en pipeline och flera olika program har använts för framtagning av denna. Totalt har vi tittat på bearbetningen av bilderna hos 7 EPT-barn.

Pipelinen utför flera saker, bl.a. markerar den hjärnans olika strukturer på röntgenbilder, vilket kallas för segmentering. Efter segmentering har vissa hjärnstrukturer valts ut för att beräkna volymen på bl.a. hippocampus som är viktig för minnet. Det viktiga i detta arbete är att bedöma hur väl pipelinen genomför dessa steg, inte om LIM har har gett några effekter. Dels finns det för få inkluderade barn i LIM hittills för att dra slutsatser, dels måste man kvalitetssäkra pipelinen först. Vi har satt vår studie i relation till andra studier för jämförelse.

Pipelinen kunde segmentera de flesta strukturerna med god precision och även beräkna volymerna. Vissa strukturer som hjärnbarken hade den ibland svårare med. I ett barn med tydlig skador i hjärnans hålrum hade programmet svårare att segmentera korrekt, vilket innebär att man kommer behöva korrigera för detta framöver.

Arbetet är ett led i att kvalitetssäkra pipelinen för att den ska kunna användas på bilderna av EPT-barn i LIM framöver. I det stora hela är detta viktigt för att kunna utvärdera effekten av mikrometoder på hjärnutvecklingen. Om LIM kan påvisa positiva effekter när den avslutas kan det vara en viktig pusselbit i att främja god hälsa och höja livskvaliteten hos EPT-barn. Den enskilde individen, och även samhället i stort, gynnas av att barnen får optimala förutsättningar för att utvecklas till friska vuxna.

Introduction

Knowledge of early brain development is critical for understanding cerebral pathologies of the preterm neonate and their subsequent implications. Around 110 000 infants are born in Sweden annually, with preterm infants (born before 37 weeks of gestation) accounting for 5% of all live births (1). Of these live births, 0.3% are termed extremely preterm (EPT), which is defined as being born prior to 28 weeks of gestation (2). Being born prematurely can be accompanied by a myriad of short-term and long-term health complications (3). In the short term, many EPT infants face health issues in early life, including various cardiopulmonary anomalies, cerebral intraventricular hemorrhage, lethal infections and necrosis of the bowels (3,4). However, many survive in spite of this. In the long term, some go on to develop visual, cognitive and motor impairments, with cerebral palsy and deficits regarding intelligence, working memory and impulse control being major challenges faced by this patient group (3–5). Many of these disabilities are recognized as effects of injuries or disturbances to the developing immature brain (6).

The neuronal embryonic development is an intricate sequence of events which play a pivotal role in later cognitive and motor development. Up to around midgestation, neurogenesis and neural migration are the main events that occur in the developing fetal brain (7). This process is characterized by the formation and differentiation of neuronal stem cells in the ventricular zone and their migration to the cerebral cortex, a radial development. Around mid-gestation, focus shifts from structural crafting to establishing cerebral connections, in which each cortical layer establishes connectivities that define their future functions. Also, there exists a temporal developmental gradient of the six cortical layers where the deeper layers are laid first, i.e. layer six followed by layer five and so on. Likewise, the vascularization process of the brain follows a predictable temporal pattern with concurrent inside-out series of events. Gyration-sulcation is a result of the establishing connectivity between synaptic fibers in the

superficial layers of the brain, which leads to tangential growth necessitating cortical folding. Prior to gestational week 26, the surface of the developing human brain is mostly flat, lacking the characteristic sulci and gyri of a fully developed brain (8). It is only after this time that the brain's gyration-sulcation begins and follows characteristic temporal patterns, from primary to secondary to tertiary cortical areas, and not until term is the adult gyrification/sulcation pattern established. Embryological structures which are precursors to the white matter are flanked between the ventricular zone and forming cortical layers (7).

Typical brain lesions in EPT infants include intraventricular hemorrhage (IVH) and periventricular leukomalacia (PVL), both displaying correlates on MRI-imaging (6). PVL in combination with diffuse neuronal lesions affecting various brain structures is collectively known as encephalopathy of prematurity (EOP), predominantly affecting the white matter and, therefore, also called white-matter damage of immaturity (WMDI) (6). Imaging studies indicate that 50% or more of infants with very low birth weight (<1500 g) show findings consistent with EOP, whereas severe IVH (grade 4) and subsequent associated post-hemorrhagic infarcts are seen in around 5% of cases, but rises to 20-30% in infants born weighing less than 750 g (6). However, not all lesions are detectable on conventional MRI or may present rather diffusely. Therefore, recent research has focused on using more advanced MRI for analyzing specific features of the developing brain. Brain volumetric quantification is such a domain. Research focusing on brain assessment of EPT infants has largely focused on analyzing the volumes of cerebral structures on MRI and relating them to respective subject's future imaging and developmental assessments as children and adults (5). Previous studies have demonstrated that the volumes of cerebral structures differ between preterm neonates and their term-born, age-controlled counterparts, such as the hippocampi, deep gray nuclei and cortical gray matter (9). These differences, specifically of the hippocampus, were

shown to persist into childhood and early adulthood, and even become more pronounced (5,9,10). Different methods of testing have unequivocally proved that EPT infants perform worse than their term-born peers in different memory and intelligence tests later on in life (5,10). One can speculate whether there exists a causative correlation between the sizes of brain structures in EPT infants, e.g. that of the hippocampus, and the aforementioned cognitive domains. However, whether this link exists is not entirely clear, with some studies drawing significant results affirming this relationship, while others are not entirely conclusive (5,10).

For many of the health issues that EPT infants face, there exist treatments that have become indispensable in the modern neonatal intensive care unit (NICU). For example, administration of cortisone and surfactants is standard therapy to promote early pulmonary function and to manage infant respiratory distress syndrome. In infants with dangerously high levels of bilirubin in their blood, so called neonatal jaundice, light therapy is the established treatment option for the breakdown of these potentially toxic metabolites. A recent promising study conducted in Gothenburg demonstrated that the risk for severe retinopathy of prematurity was halved when the newborns were given a new supplement combining two fatty acids (11). Medical breakthroughs, such as the ones mentioned, constitute but a small part of the ever expanding arsenal of treatments and therapies that modern neonatal care has at its disposal. Today, modern medicine has been propelled to new heights when it comes to the care of EPT infants, and their chances of survival, once abysmal and still lagging in much of the developing world, have increased substantially (12). Cognitive impairments, however, persist for many of these infants later in life (4). This casts the light on the need for early interventions that aid early brain development.

During the first postnatal two weeks at the NICU, it is estimated that the median blood equivalent of 50% of total blood is drawn from EPT infants, and a 100% blood equivalent is given in various transfusions. All of these transfusions are given using adult donor blood. It has been shown that concentrations of growth factors after birth are positively associated with brain volumes at a term equivalent age (TEA) in very preterm infants (13). One can hypothesize that fetal blood components, such as fetal hemoglobin, circulating growth factors and stem cells, play a pivotal role in healthy neonatal development. These are mostly unique to the developing infant whose body undergoes rapid growth and adapts to sudden physiologic changes. By using micro-methods for blood extraction, instead of the current methods designed for adults, blood taken for clinical diagnostics can be decreased and the subsequent need for transfusions can be limited. Whether this intervention would promote brain development, measured in the form of volumes of different brain structures, is yet to be researched to our knowledge. Therefore, an analysis pipeline that performs volumetric quantification and tissue demarcation of neonatal brains is required. Ultimately, if these micro-methods for blood extraction show promising results, it can set precedent for how to optimally hospitalize EPT infants in the future.

Research question

Will the automatic brain volumetric quantification pipeline be able to perform analysis of brain structures in the two groups of extremely preterm infants: one subject to micromethods of blood extraction and the other solely to conventional methods?

Material and Methods

"Less is more" (LIM) is an ongoing multicentre study of EPT infants born before gestational week 27 in Lund, Stockholm and Gothenburg. EPT infants are randomized into two group: The intervention group, where minimally invasive blood sample extraction methods were used in order to reduce blood volume drawn by over 50%, and a control group of EPT infants which was subject to conventional blood extraction methods. The micro-method for blood collection provides enough blood volume for the analyses of blood gas and C-reactive protein, the two blood tests accounting for most blood extraction in EPT infants in the LIM study. The intervention group is, therefore, also subject to conventional blood sampling methods. The primary endpoint of the LIM study is to investigate if methods that minimize infant blood volume extracted and the subsequent decreased need for blood transfusions lead to prevention of broncho-pulmonary dysplasia (BPD) of these infants. However, this paper is interested in brain development of the infants assessed using MRI, one of LIM's secondary endpoints, and as such will be its scope.

EPT infants in the LIM study undergo an MRI scan at TEA. Up to the date of this study, 13 infants have been scanned in total in Lund. Patient information including name, date of MRI scan and archive numbers were logged by the MRI technicians. Also, an excel sheet containing anonymized study ID (LIM-ID), date of MRI scan, gestational age at birth, clinical parameters, and study randomization was provided to us by the neonatal ward. After MRI scanning, images were anonymized and exported to a computer server within the hospital firewall. On the computer server, each subject's dataset was associated with its corresponding LIM-ID for identification within the study. The 7 LIM-IDs are substituted with the letters a to g.

The MRI-protocol included a number of sequences, which were adjusted multiple times due to the late involvement of a responsible radiologist for the study. Scanning was done on 3 3-Tesla Siemens Prisma at VO Bild och Funktion, Skåne University Hospital Lund. MRI imaging included conventional MR images, used for clinical radiological assessment, and a set of additional research sequences. Subjects were fed and given sedation in the form benzodiazepine beforehand. T1-weighted MRI images normally show high signals in fatty structures and are useful in the adult neuroradiology diagnostics. However, in infants before the age of two years, T1-weighted MRI images do not show sufficient contrast between white and gray matter due to an incomplete myelination of nerve fibers. T2-weighted images prove much more useful in this regard with a better gray-white differentiation (figure 1) and is the sequence of choice for neonatal brain tissue segmentation. In the 13 EPT infants scanned, T1-weighted images were obtained in all of them. The more useful T2-weighted images, however, were not acquired in the first 3 patients scanned, which left us to assess 10 patients.

All computational work was done from an Ubuntu terminal in a Linux system workstation accessed remotely in the Lund University Hospital. The powerful work station was used to conduct the analyses. Longer software codes are available on GitHub (https://github.com/finnlennartsson/LIM), an online community where software code can be written and edited. A laptop as a working tool was provided by the Department of Radiology from the same hospital. The programming language utilized is not a language per se, but is called Bash (Bourne Again Shell) scripting and utilized in Linux-operating systems. In short, this is a type of interpreter that processes commands. This takes commands in plain format and calls Operating System services to execute that command, such as “mv” for moving a file.

Digital Imaging and Communications in Medicine (DICOM) is the standard format of

medical imaging information and data. DICOM is most commonly used for the transmission and storage of medical images. However, the Neuroimaging Informatics Technology Initiative (NIfTI) is the format of choice in the research neuroimaging community. All images were originally acquired in DICOM, but were subsequently converted into NIfTI using a conversion software code. Following this conversion, we assessed the quality of all the available T2-weighted images and scored them 0, 1 or 2; where 0 indicates little to no imaging artefacts, 1 indicates some imaging artefacts and 2 indicates severe imaging artefacts. The scoring is presented (table 1). We included all patients, regardless of artefacts, to observe how well the pipeline was able to analyze the images.

In the 10 infants with T2-weighted images, 3 had images acquired using the Edinburgh protocol sequence and 7 had images acquired using the Melbourne Children's Regional Infant Brain (M-CRIB) protocol sequence. These T2-weighted images can be used in the parcellation programmes, which segment the images and assign them with corresponding structures. M-CRIB is the protocol optimized for the Developing brain Region Annotation With Expectation-Maximization (DRAW-EM, https://github.com/MIRTK/DrawEM) parcellation toolkit, which was the one utilized in this study (14). Therefore, 7 of the EPT infants were chosen for further analysis in the pipeline.

Before the MRI images were parcellated and analyzed, they underwent pre-processing (figure 2). In this step, a software code was used to optimize the images prior to later segmentation. The images initially underwent upsampling from 1mm^3 isometric voxel resolution to 0.68 mm^3. Furthermore, a contour of the brain was extracted, a so-called “brain mask”. The brain extraction was done using FMRIB Software Library (FSL), a software library containing image analysis and statistical tools for MRI brain imaging data. The upsampling and brain mask are required for later segmentation using DRAW-EM.

Once the pre-processing was done, a software code that is based on the DRAW-EM segmentation algorithm was used. This step requires the previously pre-processed material. Additionally, a label-based encephalic region-of-interest template (ALBERT atlas) was used as the default atlas in the DRAW-EM parcellation. An atlas in this context is a reference MRI-image which the segmentation is based on. This reference changes with the change of the equivalent gestational age of the infants in tandem with neonatal brain development. Therefore, we input the corresponding gestational ages of the EPT infants at the time of the scan, rounded to the nearest whole week. This was calculated in the same excel sheet containing the previous scoring. Gestational age at birth and equivalent gestational age at scan are presented (table 1).

DRAW-EM segmentation took around 2 hours to conduct in each patient. The program output was 10 images in the NIfTI format with different segmentations. In this study, we chose to study the hippocampi, thalami, white matter and cortical gray matter. Selection of these structures was partly based on already existing data, partly on these structures' importance in different cognitive domains. The output images deemed most suitable for this purpose were the "Tissues template", for white matter and cortical gray matter, and the "Labels template", for the hippocampi and thalami.

Volumetric quantification of selected brain structures was done using FSL. Two software codes were used: one which extracted the structures we are studying into 4 different corresponding NIfTI files and one which calculated the volumes of each of these structures in cubic centimeters (cc) (Appendix- bash codes).

Ethical considerations

The LIM study has acquired ethical approval from the Swedish Ethical Review Authority (dnr: 2019-01786). Consent has been collected from the guardians of the included subjects in the LIM study. In the current project, all relevant patient data has been extracted and anonymized. There will be no need to check the subjects' medical records (i.e. no need for KVB approval).

The LIM intervention is minimally invasive and aims to drastically cut the amount of blood taken for routine blood testing in the NICU. Therefore, conventional blood-taking using syringes and peripheral venous catheters, which often prove challenging in EPT infants and can cause discomfort to the child, will be decreased in frequency. By extension, the children's guardians will also benefit emotionally.

Another aspect to take into consideration is gender. It is generally known that prematurely born female infants generally thrive better than their male counterparts (15). Randomization occurs with no differentiation between female and male infants. Whether this will prove problematic in later results will require a more comprehensive evaluation of the survival/disability outcomes, effects which are still too early to be seen in LIM.

Results

The T2-weighted images acquired with M-CRIB protocol of all 7 subjects were analyzed using the pipeline. 6 of the subjects were deemed to have little or no motion artefact (score 0 or 1), and 1 had a lot of motion artefact (score 2) (table 1). The pre-processing step and subsequent segmentations are exemplified on subject c, with no artefact (score 0) (Figures 2-4). In figure 2, the pre-processing step is shown. The brain mask is extracted in white. The brain mask is required for accurate brain segmentation in DRAW-EM. In addition to brain mask extraction, this step includes upsampling of the voxels. A voxel in computer graphics represents a value on a regular grid in 3D space, analogous to a pixel in 2D space. The voxel dimensions can be measured along the x, y and z axes in mm^3. In an image with isovolumetric voxel resolution, these values are equal. In upsampling, these dimensions are altered to convey greater detail, in our case by decreasing it to 0.68 mm^3 from an original isovolumetric voxel resolution of 1 mm^3. This is required by the DRAW-EM program to achieve optimal segmentation. It is in this step, the pre-processing step, where an ambition is to implement a motion-correction script that would partially compensate for possible images artefacts caused by movements of the infants, as well as other possible interferences.

In figure 3, we can see the segmentation done using the "tissues template". For example, the cortical gray matter (purple) in the second image is accurately demarcated in respect to winding architecture of the gyri and sulci. The white matter, which is shown in white in the last step, enjoys a sharp gray-white matter differentiation making its segmentation faithful to the true structure. The volume of the white matter is displayed in the bottom of the figure as calculated in FSL. In figure 4, the "labels template" contains 50 delineated structures in the same subject, where the names of these can be accessed in the link found below the figure. The segmentation of the hippocampi appears in the last image in cyan. The hippocampi have

a curving architecture which can be especially appreciated in the axial view. An accurate volumetric brain quantification necessitates an accurate brain segmentation, which is why the pipeline requires the latter to be especially scrutinized. As a segmentation toolkit, DRAW-EM has been validated previously by processing the images collected from 465 subjects ranging from 28 to 45 weeks postmenstrual age (14). Comparison to manual demarcation showed that significant errors were found in only 2% of cases, largely corresponding to the subjects with severe motion artefact (14)**.**

Subjects a, b, e and f, with no to some motion artefact (score 0 or 1), are also segmented (Figures 5-8) by undergoing similar steps to subject c. Errors in cortical gray matter segmentation are highlighted in subject f (figure 8). Subject d (score 0) and subject g (score 2) are notable, with the former having pronounced dilation of the ventricular system and the latter having a lot of motion artefact (figures 9, 10). Subject d's pathology is the result of an high-grade IVH, evidenced by the presence of residual blood breakdown products in the wall of the left ventricle, post-hydrocephalic ventricular dilation and periventricular white matter reduction (figure 9). Subject g was constantly waking up during the MRI scan, which caused motion artefacts in most sequences and the examination had to be terminated prematurely (figure 10).

In spite of glaring pathology and motion artefact, subjects d and g were processed in order to evaluate how well the pipeline could cope with deviation from normal anatomy (figures 11, 12).

Volumes of the thalami, hippocampi, white matter and cortical gray matter for each subject are presented (table 2). 6 of the 7 subjects were randomized into the intervention arm of LIM. These volumes are in absolute terms, and direct comparison between the subjects cannot be done. This is due to not correcting for intracranial volumes in each subject. An ambition is to

implement this correction in future improvements of the pipeline. When looking at the numbers, subject a, a control, seems to have the biggest absolute volumes in all structures. This is insignificant given that the volumes are not relative and sparse to begin with. When it comes to volumes of the remaining subjects in the intervention group, the volumes seem to be similar. The subjects have hippocampal volumes around 1.5 cc, thalamic volumes around 9 cc and cortical gray matter volumes and white matter volumes around 140 cc for both.

Discussion

The study has yielded an interesting set of data, both in terms of quantitative volumetric measurements as well as illustrative imaging results. However, it is important to acknowledge its shortcomings.

Even though 13 patients were scanned in the Lund LIM cohort at the time of writing this paper, only 7 could be analyzed in the end. This is unfortunate in the sense that more EPT infants could have been processed in the analysis pipeline and included had they undergone T2-weighted MRI acquisition according to the M-CRIB protocol. A late assignment of a responsible radiologist to the study was the reason behind this, which led to a suboptimal planning of the MRI protocols that were required for later use. Since radiologic assessment of brain development in LIM is a secondary endpoint, the importance of this might have been overlooked initially in favor of the primary endpoint of evaluating the development of BPD in the EPT infants. Nonetheless, relevant MRI images for radiologic assessment were acquired even in infants scanned prior to the implementation of the M-CRIB protocol.

There was an initial ambition to include a motion correction component in the preprocessing software code. This was not implemented at the time of this study and as such could not be assessed. Motion correction would adjust for some artefacts bias in the infants, but would most likely not be able to correct for it entirely. The code could have corrected for the severe artefacts seen in subject g, perhaps downscaling the score from 2 to a 1. There is an intention to implement motion correction, with a rerun of the performed analyses as well as incorporating it into the future pipeline used on EPT infants in LIM.

The pipeline was able to calculate the volumes of the selected structures, but with what precision it did this will be discussed further on. The quantification of the volumes it presents are in absolute numbers. It did not take into account the intracranial volumes and head sizes

of the EPT infants and make adjustments accordingly. Had it done this, the volumes calculated would be relative and comparable. Other studies which have done similar analyses have corrected for this fact by computing intracranial volumes and using it as a normalising factor (5,9,10). This correction is an ambition for the pipeline, possibly incorporating it into a future software code. Nevertheless, given the small sample size of the included subjects, a comparative statistical analysis would still not have been possible to conduct, even with the inclusion of all the MRI scanned infants thus far. Hence, based on the results, we cannot draw conclusions regarding the efficacy of the LIM intervention in blood extraction.

When it comes to the tissue segmentation of our 5 patients with little or no artefact (score of 0 and 1), the performance of the pipeline appears to be satisfactory, but with room for further improvement.

The software was able to capture the hippocampal contours with good precision. This is best exemplified in images of subject e, where the eponymous seahorse-like appearance can be appreciated. Compared to other studies calculating volumes of the hippocampus, our acquired numbers have significant differences. Thompson et al showed in 2014 that TEA preterm infants had an average total hippocampal volume of around 2.26 cc (10). Our 7 subjects show significantly lower volumes than this, with an average of around half that number. This could boil down to different reasons. Firstly, the methodology of the studies are vastly different. The quoted study had enough participants to draw significant conclusions, in addition to correcting for artefacts and head size adjustments (10). This stands in stark contrast to our own. Secondly, it included preterm infants born prior to gestational week 30, whereas LIM had an inclusion criterion of being born before gestational week 27 (10). Both, however, conducted MRI scans at TEA. Thirdly, and arguably most importantly, is the methods of segmentations used. While our process was entirely automated using DRAW-EM as the segmentation program and ALBERT as the parcellation reference, Thompson et al employed

instead the 3Dslicer 2.5 software (http://slicer.org/) and used two anatomical atlases from 1988 and 1997, in addition to further human delineation later (10,16). Therefore, the disparity in results could ultimately come down to an issue over definition: What constitutes the hippocampus on imaging and what does not? When looking at our segmentation of the hippocampus and the example images of Thompson et al, we can clearly see a vast difference of what areas we denote as the hippocampus (10). A strength in our study is using up-to-date reference material with a solid basis in the neonatal brain, in addition to a state-of-the-art segmentation software code.

Thalamic segmentation in the labels template shows a clear demarcation from other surrounding deep gray nuclei. The tissue labels template represents all the deep gray matter, including the thalamus and basal ganglia, in one homogeneous segmentation. The volume of the deep gray matter was not calculated as a whole, but rather that of the thalamus alone through the labels template. DRAW-EM and the ALBERT-atlas do not allow segmentation of deep white matter, e.g. internal capsule. This occurs in both the tissue template and the labels template, the former of which we base our white matter segmentation on and the latter our thalamic segmentation. This ultimately underestimates white matter volumes. Rectifying this issue is important if the pipeline is to be accurate and expanded to include analysis of additional structures.

At a glance, the segmentation of the cortical gray matter might look erroneous at times with specks of cortical gray matter embedded deep within the white matter. This appearance seems to be in all subjects and in the sagittal, coronal and axial views. However, it is not sufficient to just look at one view and judge the segmentation based on it. In medical radiology, one view is no view at all. When looking at all views simultaneously, these specks appear to be gray matter located in deep sulci. These might be misinterpreted as isolated islands of tissues when viewing the image at the deepest grooves in the different views, depending on the

sulci's spatial position. Another aspect that appears prominent in the images is that the cortical gray matter appears to be coalescing into conglomerations of cortical gray matter, especially exemplified in subject f. The program seems to have difficulty in detecting thin sulci and consequently labels the surrounding cortex as one unified mass, which anatomically is incorrect in the selected patients. In an older paper published in Thompson et al in 2008, volumes of cortical gray matter better matched our measurements on our 7 patients than the hippocampal measurements (9,10). The differences in power and methodology between our study and that of Thompson et al have been discussed previously, but the greater similarities regarding cortical gray matter volumes echo an anatomical consensus that is lacking in hippocampal measurements. This showcases the importance of the method employed.

The white matter segmentations in the subjects performed well overall, however, with notable exceptions. In almost all subjects, the program was able to accurately demarcate the borders of white matter relative to the surrounding subject. However, as mentioned earlier, white matter within the deeper gray matter is not included, which casts doubt on whether the quantification is a true representation. Furthermore, white matter assignment in subject d with ventricular pathology seems to be flawed, which will be discussed in greater detail later on. However, a better than expected white matter parcellation was performed in subject g with severe motion artefact.

When it comes to the tissue segmentation of subject d, with post-hydrocephalic dilation of the ventricular system, the pipeline had mixed results. We wanted to investigate how well the segmentation program could cope with the enlarged ventricles in general and the pronounced ventricular anomaly in the left occipital horn. At a first glance, one would say that the pipeline fared quite well in its segmentation. The cortical gray matter, thalamus and cerebellum seem to be well demarcated. The right lateral ventricle also seems to be well delineated despite pathological enlargement. However, upon closer inspection of the occipital

horn of the left ventricle, the segmentation program performed quite poorly. In the images shown of subject d's segmentation, we can see an area of darker blue along the occipital walls of the left ventricle and protruding well into the ventricular space at different points, seen in figure 11. According to the segmentation program, it has recognized these areas as white matter, an obvious error. The residual blood break-down products of the previous IVH seems to have been challenging for the program to label and assigned it instead as white matter, which could have resulted in a spill-off effect to the adjacent voxels in the ventricular space. This could be explained by the fact that the program uses a whole range of information when labeling tissues, including taking into consideration the tissue types of neighboring voxels. Additionally, which is not entirely apparent in the attached images, there were light yellow specks, corresponding to cerebrospinal fluid, inside this portion of the ventricle. While not de facto wrong, it is a mislabeling given that the ventricular system is supposed to have a distinct color and code from the extra-ventricular cerebrospinal fluid surrounding the brain. It was surprising to us that the volume of the white matter in subject d was within the range of the volumes of white matter in the other subjects and not diverging. Given the pathology of subject d and the subsequent reduction of white matter, we would have assumed that the volume of white matter would be toward the lower end of the spectrum. This was probably compensated for by erroneously overestimating the true volume of white matter due to the previous mislabeling. Therefore, the program requires further input from patients with similar pathologies in order to accurately distinguish these biological differences. Severe IVH, such as in this EPT infant, is not uncommon in the patient population, and if the pipeline is to have high precision in future segmentations then it must be trained to better recognize similar ventricular pathologies, or be manually corrected for.

The pipeline seems to have coped well with segmenting the brain of subject g (score of 2). Even though the MRI images contain too much artefact to be reliably assessed, the program

was able to demarcate the studied brain structures in a realistic manner. This processing can be said to be adequate, and the volumes calculated do not seem to deviate in a remarkable way from the volumes of the other subjects. However, motion artefacts have probably led to the overestimation of some tissues at the expense of others. Since motion can mimic or mask pathologic processes, correcting for it through implementing a future software correction programme remains desirable. Not only would this provide more reliable results, but it would also prove useful for the interpreting clinical radiologist by increasing diagnostic accuracy.

One needs to look at care of EPT infants in a wider perspective, beyond the presented results. Previously, most of these infants did not survive the postnatal period. With the advent of modern medicine and adequate supportive care, almost all infants born before 32 weeks of gestation survive in high-income countries (12). This is in stark contrast to survival rates in the developing world, where half of infants born before 32 weeks gestational age do not survive, a statistic far worse for EPT infants (12). This disparity showcases one of many healthcare inequalities that the developing world suffers from. A major contributor to this discrepancy is ready access to high-tech supportive care which many developing countries lack due to economic constraints. The LIM intervention is a low-cost, minimally invasive blood extraction method which could prove interesting in this respect. If later data is able to show favorable survival and disability outcomes in the intervention group, the LIM intervention could prove to be an accessible method that countries can utilize regardless of income levels. This would pave the way to a more equitable world in healthcare.

Even long-term effects of the increasing survival rates of EPT infants pose ethical dilemmas. As previously stated, many EPT infants suffer from a range of disabilities as adults. Cognitive impairments are a particular cause of disability later on in life. With an increasing number of these infants making it into adulthood, the scientific community has a responsibility towards these children, a responsibility which does not end at ensuring their

survival in their most critical period of life. Ensuring that EPT infants enjoy an adequate quality of life as they age is of paramount importance, not only on an individual level, but on a societal level as a whole. This would lessen the emotional distress of parents and decrease the economic costs for the care of individuals with severe disabilities. By introducing methods that potentially could ameliorate cognitive deficits early on in their development, which the LIM intervention aspires to do, EPT infants are given a fairer start which sets them on a trajectory to lead healthy, fulfilling lives as adults.

Conclusion

The pipeline is able to conduct adequate analysis on images without severe pathology and little to no imaging artefacts. However, even with severe artefact and no motion correction code, the segmentation in itself was satisfactory. Prominent ventricular pathologies seemed to be challenging for the program to handle and may require manual intervention. Segmentation of deep white matter needs to be implemented. Further improvements are needed to address issues regarding accurate segmentation of gray matter around sulci.

Personal work effort

Matching the subjects in the LIM study to their corresponding images and collecting their respective data was done independently, in collaboration with the responsible research nurses. The author also performed the segmentations of all subjects on the workstation based on software code provided by the supervisor. Compilation, editing of images and volumetric quantification was performed by the author. The volumetric quantification software code in the attached appendix was also developed independently, with valuable input for code legibility from the supervisor.

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Figures and tables

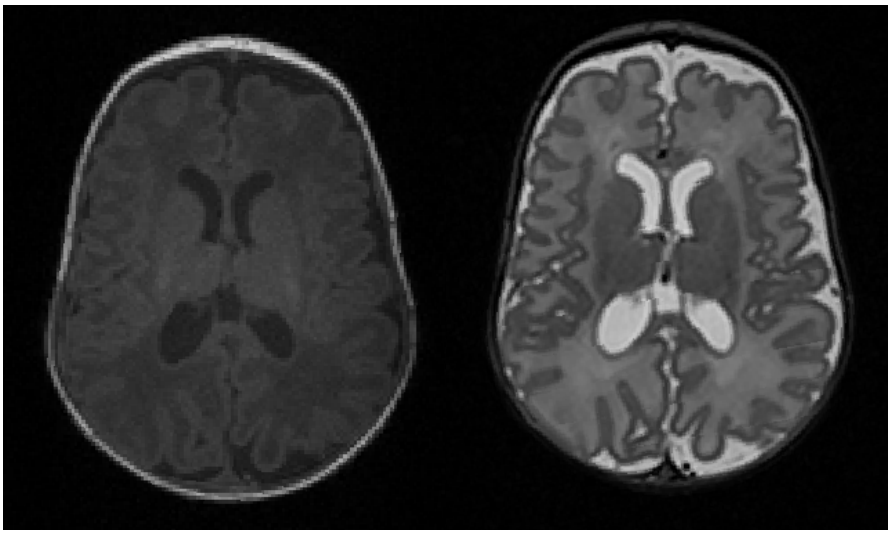

Figure 1 T1-weighted (left) and T2-weighted (right) MRI images in subject c, axial view. Note the superior contrast of deep and cortical gray matter to white matter in the T2-weighted image compared to the T1-weighted image.

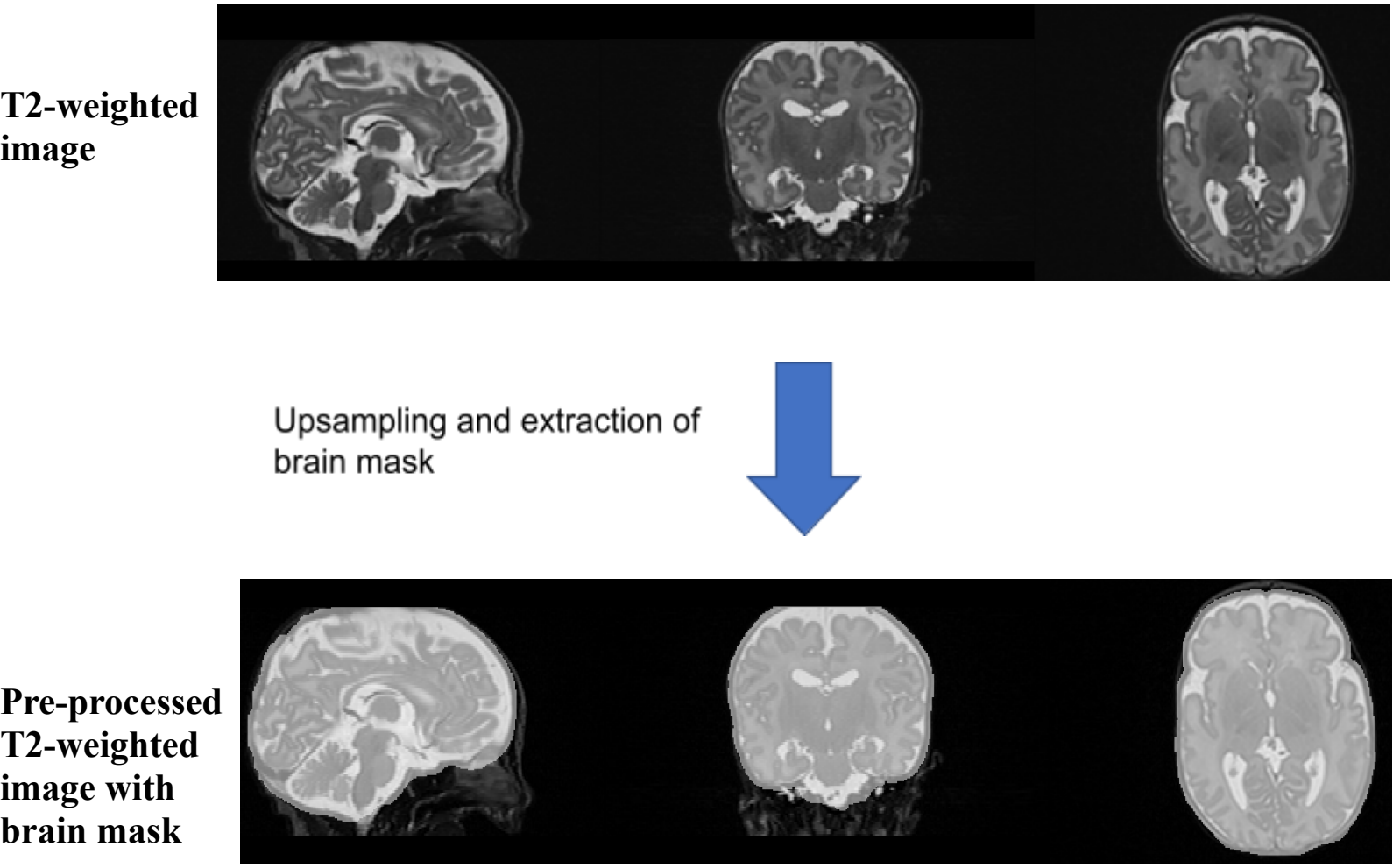

Figure 2 Pre-processing of T2-weighted MRI image acquired using M-CRIB protocol in subject c: brain mask extraction (white outline) and upsampling to isometric voxel resolution of 0.68 mm^3.

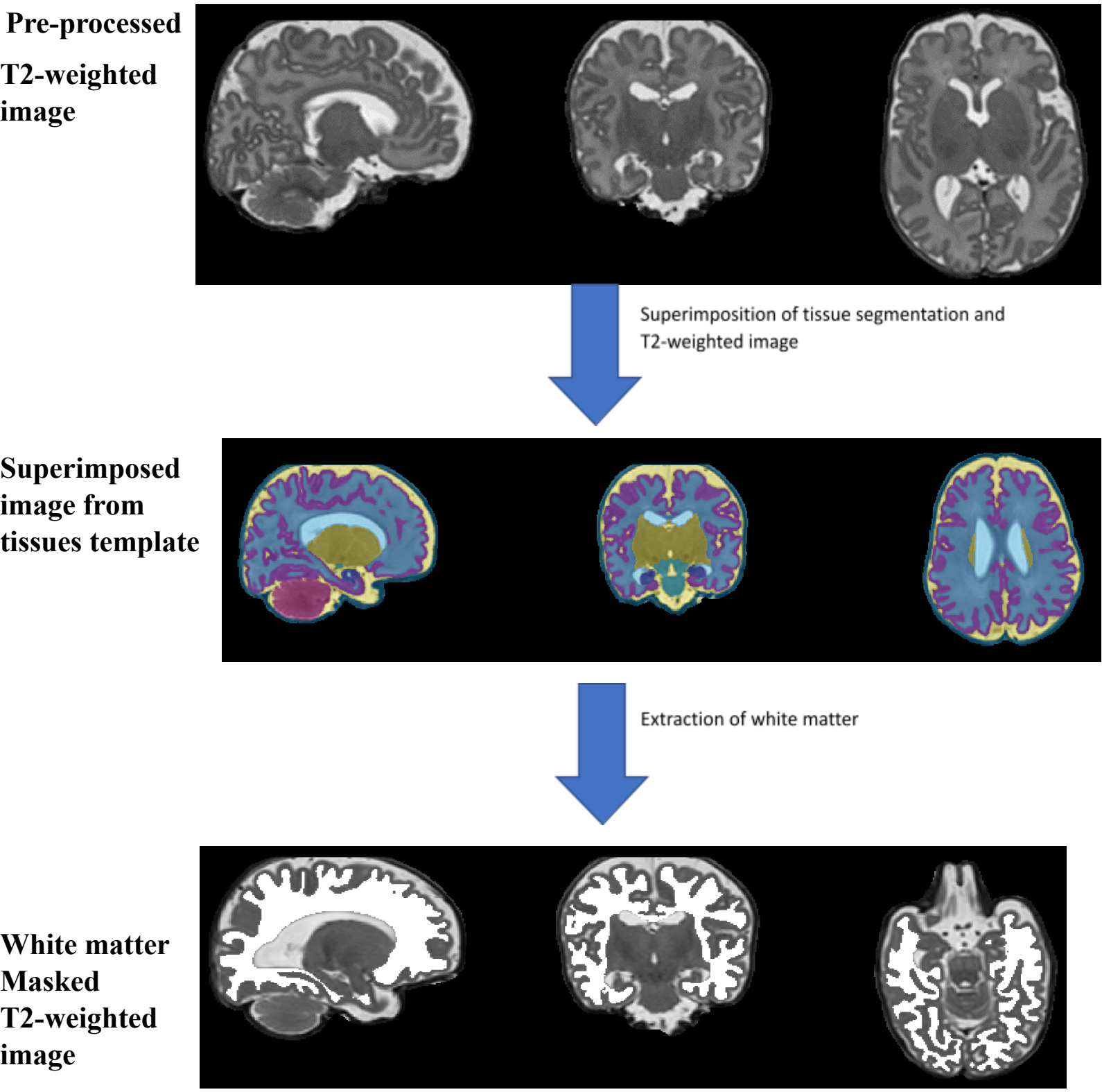

Figure 3 Flowchart illustrating the extraction of white matter from "Tissues Template" and its volumetric quantification in subject with LIM-ID c. Color scheme in superimposed image: Ventricles in light blue, white matter in blue, amygdala and hippocampus in dark blue, cortical gray matter in purple, deep gray matter in dark yellow, CSF in light yellow, cerebellum in pink (for delineated structures, see Appendix-tissue labels). In the T2-weighted image masked in white matter, the latter is denoted in white.

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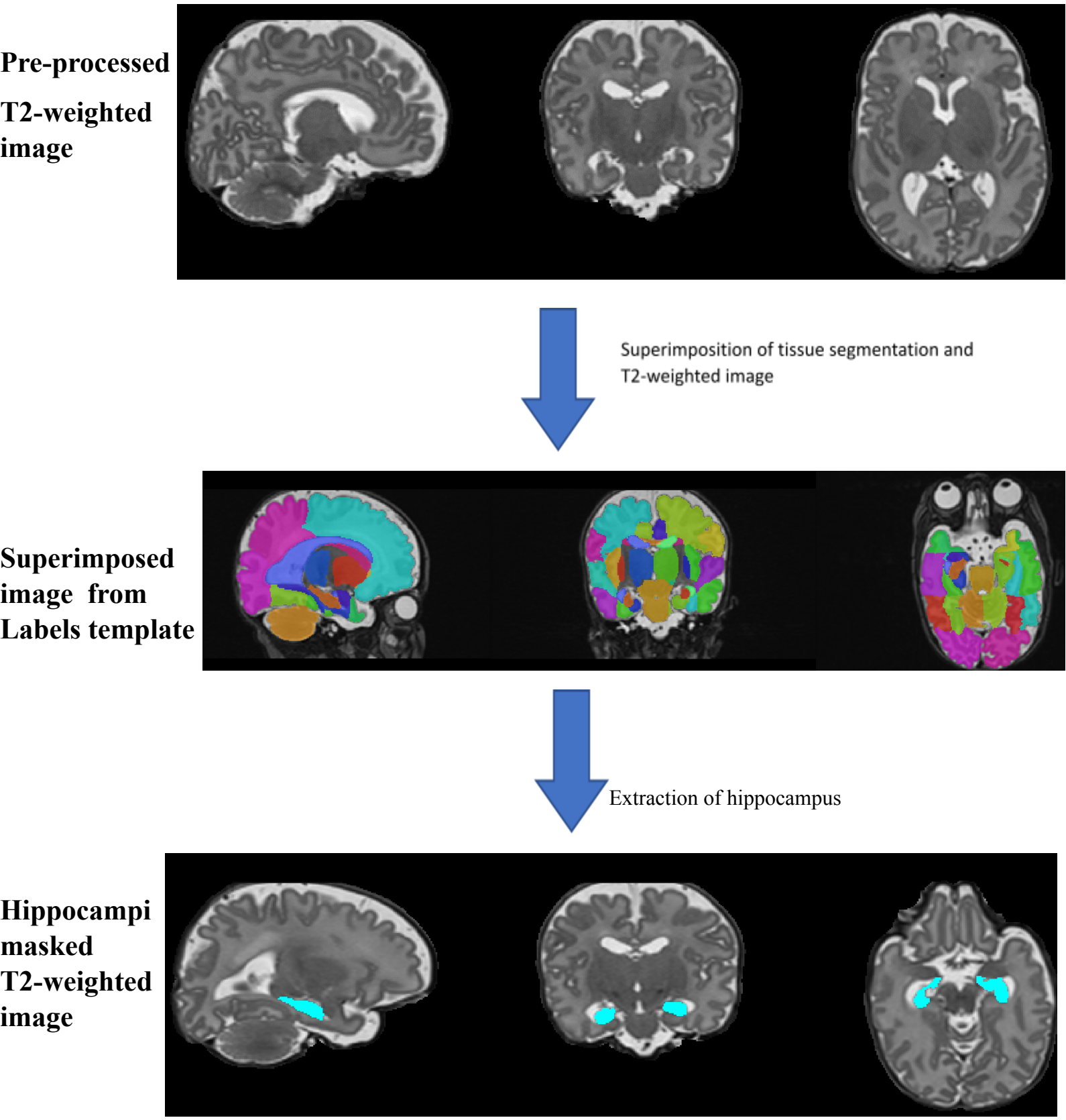

Volume of hippocampus using FSL: 1.418 cc

Figure 4 Subject c**.** Flowchart illustrating the extraction of hippocampus from "Labels Template" and its volumetric quantification. In the superimposed image, 50 structures are labeled (for the 50 structures, see https://github.com/finnlennartsson/zagreb_dhcp/blob/pmr/label_names/ALBERT/labels.txt). In the masked image, hippocampi are in cyan.

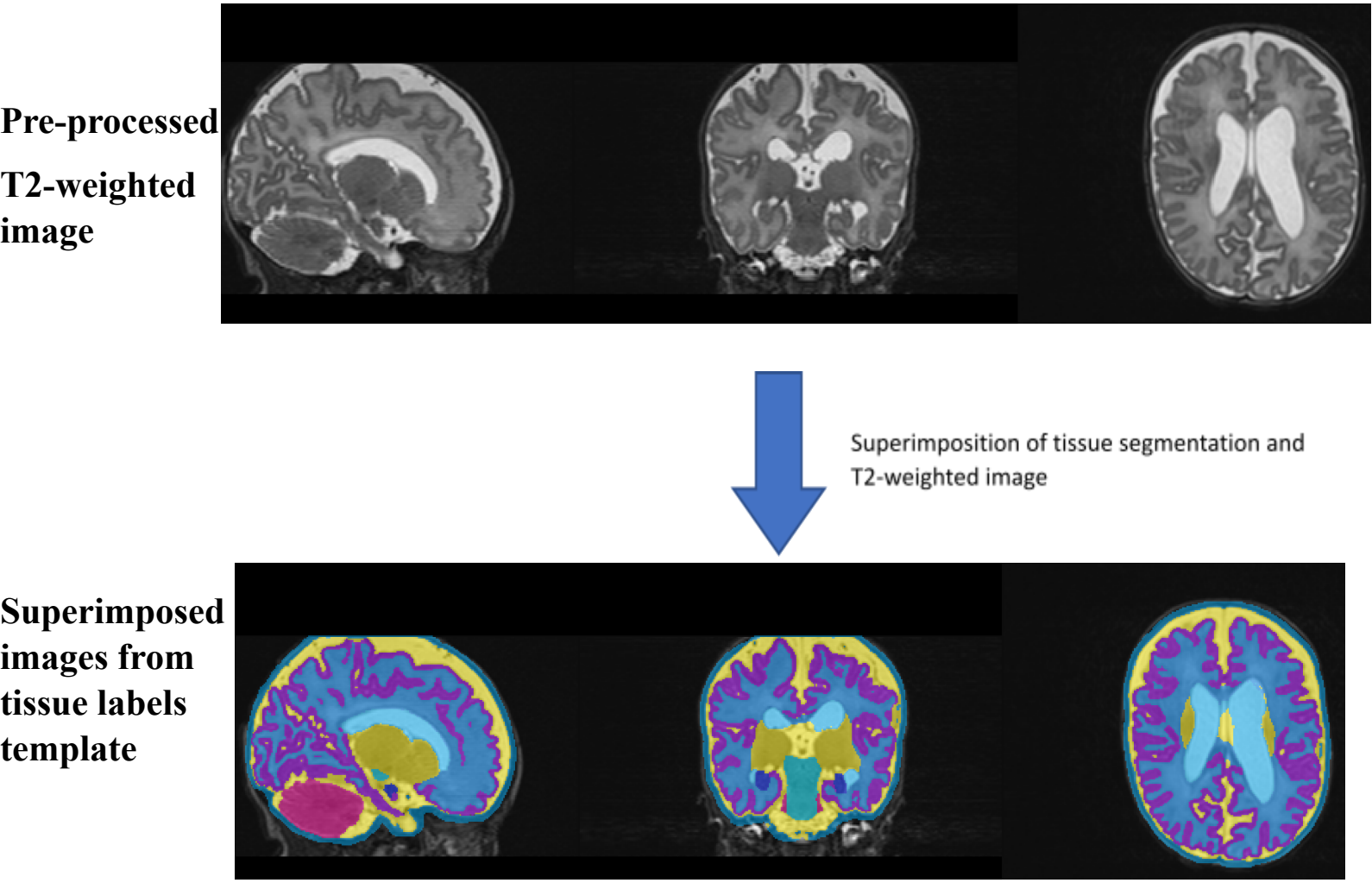

Figure 5 Subject a undergoing tissue segmentation according to tissue labels template. Color scheme in superimposed image: Ventricles in light blue, white matter in blue, amygdala and hippocampus in dark blue, cortical gray matter in purple, deep gray matter in dark yellow, CSF in light yellow, cerebellum in pink (for delineated structures, see Appendix- tissue labels).

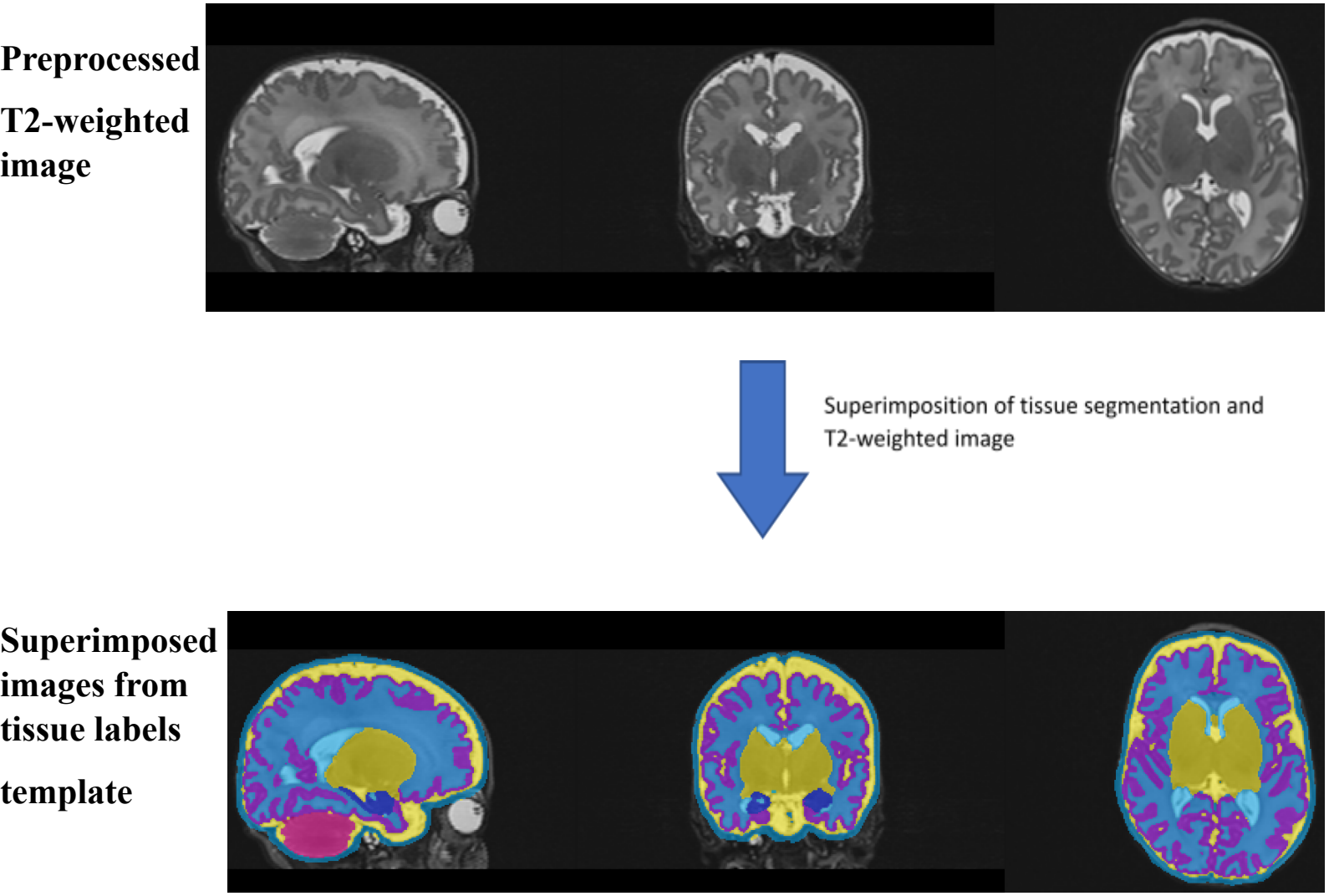

Figure 6 Subject b undergoing tissue segmentation according to tissue labels template. Color scheme in superimposed image: Ventricles in light blue, white matter in blue, amygdala and hippocampus in dark blue, cortical gray matter in purple, deep gray matter in dark yellow, CSF in light yellow, cerebellum in pink (for delineated structures, see Appendix- tissue labels).

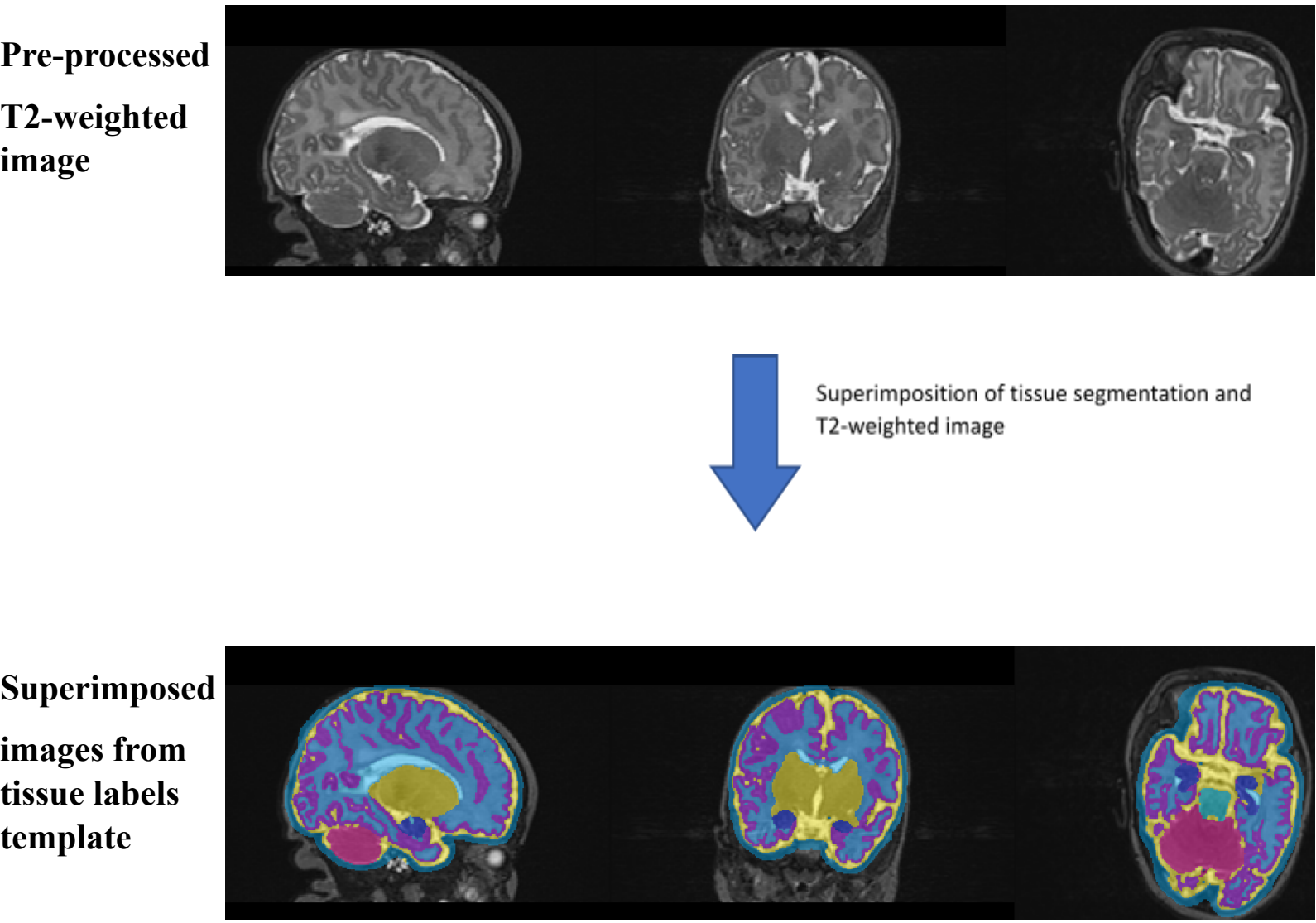

Figure 7 Subject e undergoing tissue segmentation according to tissue labels template. Color scheme in superimposed image: Ventricles in light blue, white matter in blue, amygdala and hippocampus in dark blue, cortical gray matter in purple, deep gray matter in dark yellow, CSF in light yellow, cerebellum in pink (for delineated structures, see Appendix- tissue labels).

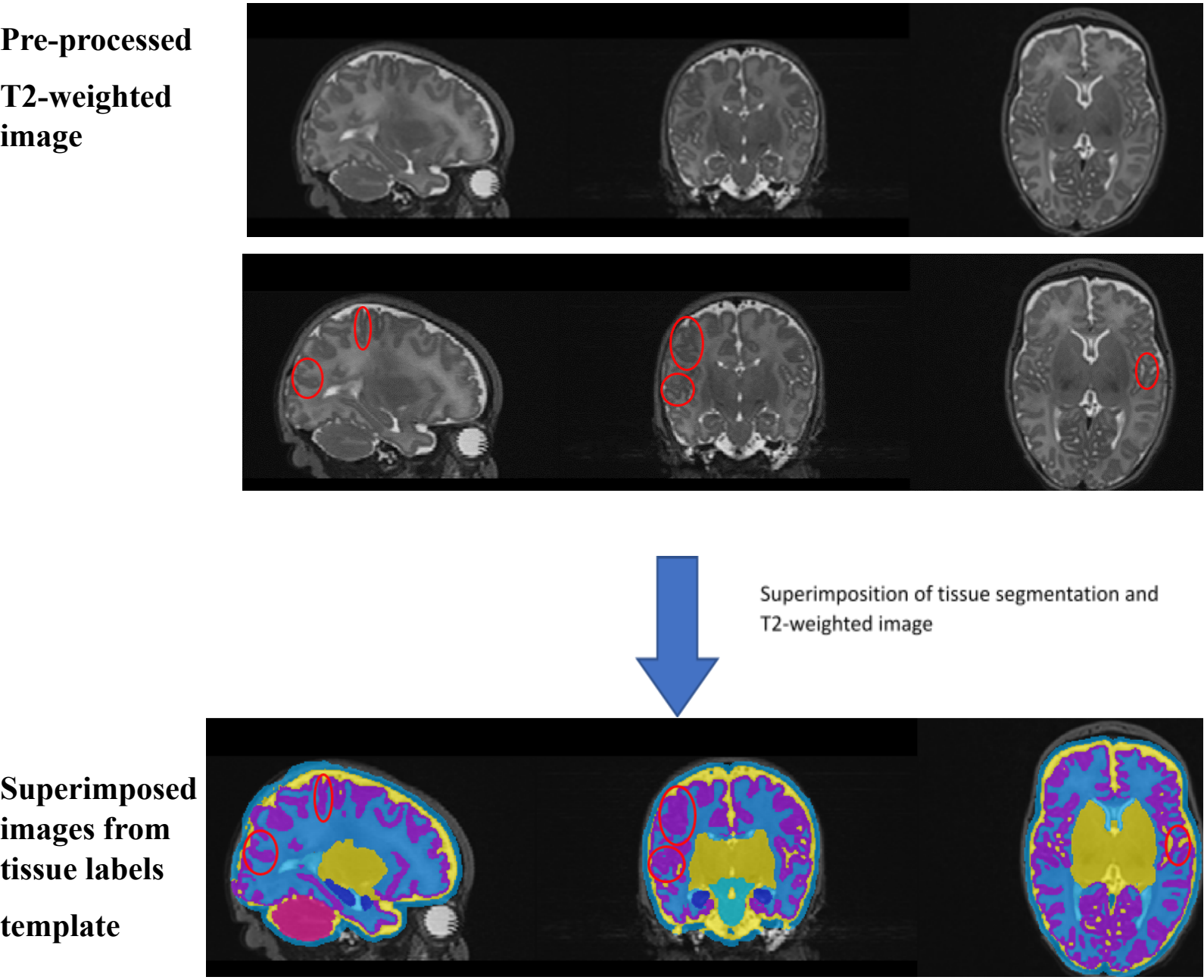

Figure 8 Subject f undergoing tissue segmentation according to tissue labels template. Red circles: Cortical gray matter around sulci which the program had difficulty segmenting correctly. Color scheme in superimposed image: Ventricles in light blue, white matter in blue, amygdala and hippocampus in dark blue, cortical gray matter in purple, deep gray matter in dark yellow, CSF in light yellow, cerebellum in pink (for delineated structures, see Appendix- tissue labels).

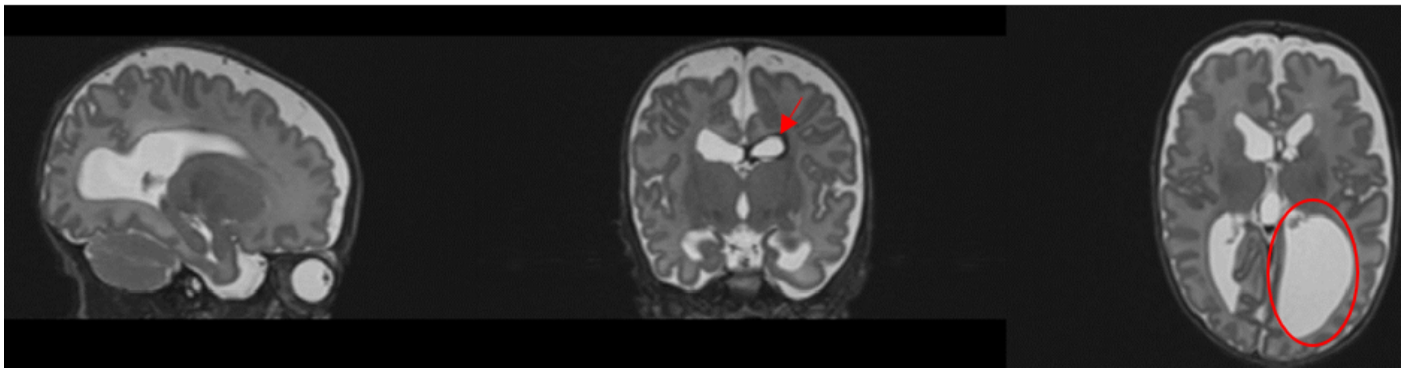

Figure 9 Subject d. Red arrow: low signal rim representing residual blood break-down products, indicating prior germinal matrix bleeding with intraventricular hemorrhage and post-hydrocephalic dilatation of the lateral ventricles. Red circle: pronounced left occipital horn dilatation with severe periventricular white matter reduction

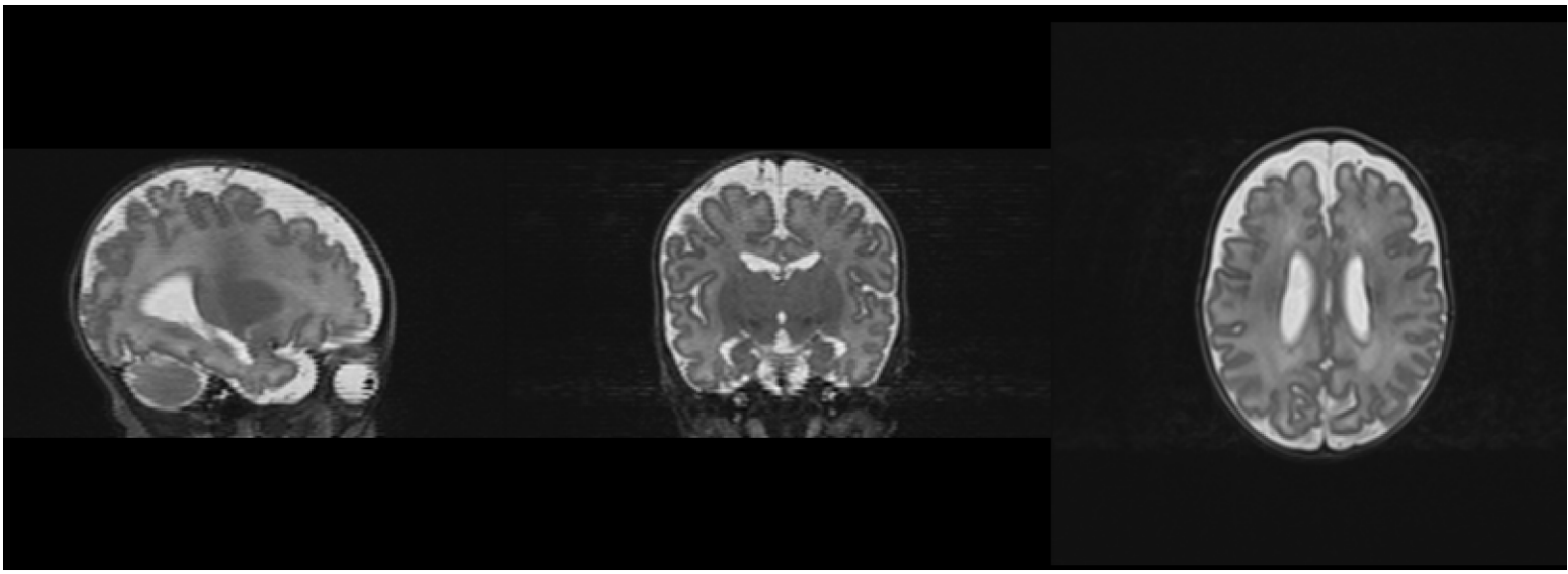

Figure 10 Subject g with considerable motion artefacts, seen here as a “fuzziness” of the images.

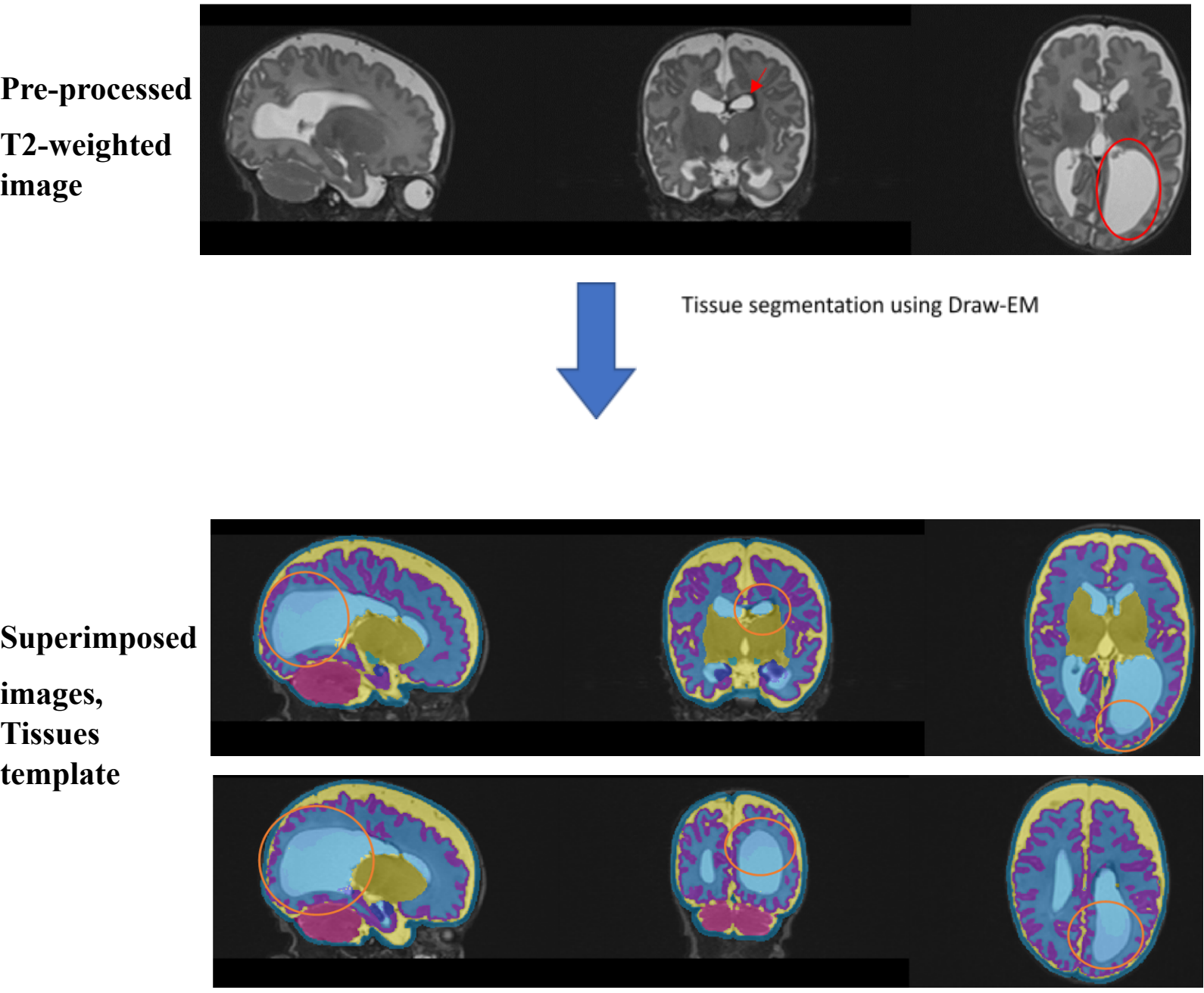

Figure 11 Subject d with prominent ventricular pathology undergoing tissue segmentation according to tissue labels template. Red arrow: low signal rim representing residual blood break-down products, indicating prior germinal matrix bleeding with intraventricular hemorrhage and post-hydrocephalic dilatation of the lateral ventricles. Red circle: pronounced left occipital horn dilatation with severe periventricular white matter reduction. Orange circles: Areas of ventricular space mislabeled as white matter. Color scheme in superimposed images: Ventricles in light blue, white matter in blue, amygdala and hippocampus in dark blue, cortical gray matter in purple, deep gray matter in dark yellow, CSF in light yellow, cerebellum in pink (for delineated structures, see Appendix- tissue labels).

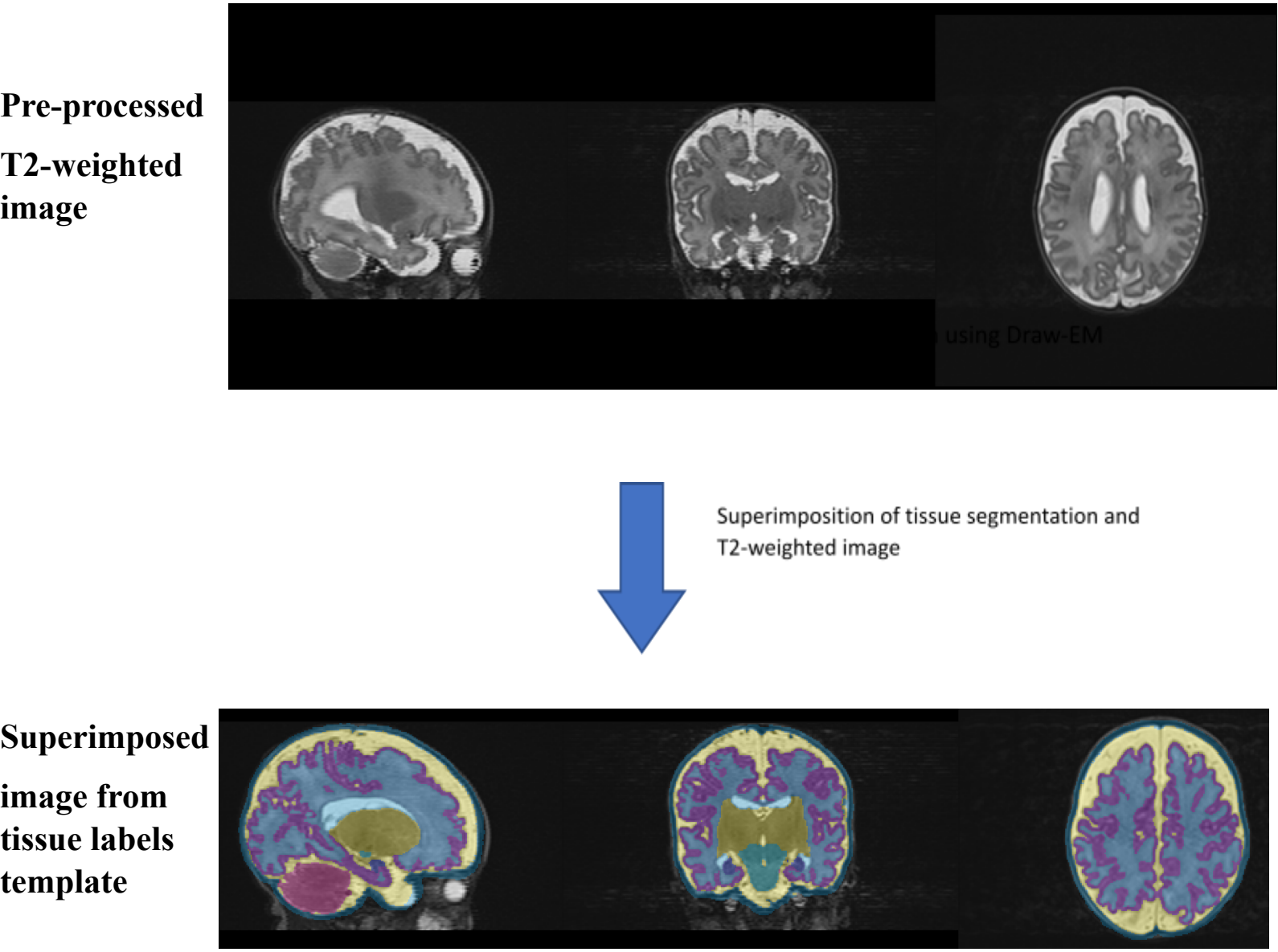

Figure 12 Subject g with a lot of motion artefact undergoing tissue segmentation according to tissue labels template. Color scheme: Color scheme in superimposed image: Ventricles in light blue, white matter in blue, amygdala and hippocampus in dark blue, cortical gray matter in purple, deep gray matter in dark yellow, CSF in light yellow, cerebellum in pink (Appendix- tissue labels).

LIM-ID	Gestational age at birth (weeks)	Equivalent gestational age at MRI scan (weeks)	Scoring of T2-weighted image	Conventional blood sampling or intervention
a	23	42	1	Conventional
b	25	41	0	Intervention
c	25	41	0	Intervention
d	25	41	0	Intervention
e	27	42	1	Intervention
f	27	42	1	Intervention
g	25	41	2	Intervention

Table 1 Table showing the 7 included subjects with respective LIM-ID (Less is More ID), equivalent gestational week at birth and at MRI scan (both rounded to the nearest week), scoring of T2-weighted image (0 no or little image artefact, 1 some artefact, 2 severe artefact) and randomization into each study arm.

LIM-ID	Volume hippocampi (cc)	Volume thalami (cc)	Volume cortical gray matter (cc)	Volume white matter (cc)	Conventional blood sampling or intervention
a	1.923	10.168	163.909	157.920	Conventional
b	1.443	8.999	132.742	150.589	Intervention
c	1.418	9.333	136.846	150.230	Intervention
d	1.597	9.357	131.192	147.069	Intervention
e	1.225	8.129	137.431	128.295	Intervention
f	1.426	9.054	155.413	140.693	Intervention
g	1.332	8.886	126.094	134.033	Intervention

Table 2 Table showing volumes of hippocampi, thalami, cortical gray matter and white matter in each subject, identified by LIM (Less is More) ID. Volumes obtained using FMRIB Software Library (FSL). Study randomization is also shown. cc=cubic centimeter

Appendix - bash scripts

Software code for segmentation

```
#!/bin/sh

# Script for extracting segmentation maps of thalami, hippocampi, cortical
gray matter and white matter from DRAW-EM segmentation map

# usage: bash Gray_White_Hippocampus_Thalamus_Extractor.sh sID

# Input: sID = Subject ID


#Defining variables

sID=$1

tissue_labels=sub-$sID/segmentations/sub-${sID}_desc-preproc_T2w_tissue_label
s.nii.gz

labels=sub-$sID/segmentations/sub-${sID}_desc-preproc_T2w_labels.nii.gz


#Commands for extracting cortical gray matter

echo Extracting cortical gray matter

fslmaths $tissue_labels -thr 1.9 -uthr 2.1  sub-${sID}_cortical_gray_matter


#Commands for extracting white matter

echo Extracting white matter

fslmaths $tissue_labels -thr 2.9 -uthr 3.1  sub-${sID}_white_matter


#Commands for extracting hippocampi

echo Extracting hippocampi

fslmaths $labels -thr 0 -uthr 2.1 sub-${sID}_hippocampus


#Commands for extracting thalami

echo Extracting thalami

fslmaths $labels -thr 41.9 -uthr 43.1  sub-${sID}_thalamus


#Moving generated files into respective patient's segmentation folder

echo Moving into segmentations folder

mv sub-${sID}_*.nii.gz sub-$sID/segmentations/


echo Finished
```

Software code for volume calculation

```
#!/bin/sh

# Script for calculating volumes of thalami, hippocampi, cortical gray matter
and white matter from DRAW-EM segmentation map

# Usage: bash Volume_calculator.sh sID

# Input: sID = Subject ID


# Setting variables

sID=$1


# Command for calculating voxels and volume (mm^3) of cortical gray matter
respectively


echo Voxels and volume mm^3 of cortical gray matter

fslstats sub-$sID/segmentations/sub-${sID}_cortical_gray_matter -V


# Command for calculating voxels and volume (mm^3) of white matter
respectively

echo Voxels and volume mm^3 of white matter

fslstats sub-$sID/segmentations/sub-${sID}_white_matter -V


# Command for calculating voxels and volume (mm^3) of hippocampus
respectively

echo Voxels and volume mm^3 of hippocampus

fslstats sub-$sID/segmentations/sub-${sID}_hippocampus -V


# Command for calculating voxels and volume (mm^3) of thalamus respectively

echo Voxels and volume mm^3 of thalamus

fslstats sub-$sID/segmentations/sub-${sID}_thalamus -V


echo Done
```