ΕΘΝΙΚΟ ΚΑΙ ΚΑΠΟΔΙΣΤΡΙΑΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ

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ΔΙΔΑΚΤΟΡΙΚΗ ΔΙΑΤΡΙΒΗ

"Διερεύνηση συσχέτισης LSV και cCMV λοίμωξης σε πρόωρα νεογνά"

ΑΙΚΑΤΕΡΙΝΗ ΚΥΡΙΑΚΟΠΟΥΛΟΥ

ΕΙΔΙΚΕΥΟΜΕΝΗ ΠΑΙΔΙΑΤΡΙΚΗΣ ΑΠΠΚ'

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Abstract

Congenital Cytomegalovirus (cCMV) infection has a global prevalence ranging from 0.2% to 2.5%. Despite its status as the most common congenital infection associated with various neurodevelopmental issues, the absence of screening programs leads to a significant number of undiagnosed neonates, missing timely intervention opportunities. This thesis aims to identify neuroimaging biomarkers of cCMV at birth to enable early diagnosis and identification of high-risk newborns who would benefit from antiviral therapy and intensive follow-up. Subsequently, the implementation of targeted neonatal screening programs in Greece will be discussed. A systematic review and meta-analysis were initially conducted to evaluate the predictive capability of prenatal imaging modalities (MRI and/or US) in determining clinical outcomes in cCMV. Despite challenges in interpreting the heterogeneous findings across studies, a significant observation was the high negative predictive value of normal fetal US and MRI for adverse outcomes in cCMV-infected fetuses. Fetal microcephaly exhibited a strong correlation with neurodevelopmental impairment. The comparison between neonatal and fetal imaging was then explored. Neonatal MRI is commonly recommended when abnormalities are detected on fetal or neonatal ultrasound to identify relevant cerebral anomalies and predict long-term neurological sequelae. However, limitations related to accessibility, cost, and sedation hinder its widespread use. Conversely, fetal MRI, a less invasive procedure performed during the prenatal stage, allows more time for decision-making. In a retrospective cohort study involving 10 asymptomatic neonates with congenital CMV infection who underwent both fetal and neonatal MRI scans, preliminary findings suggested that fetal MRI could provide comparable information to neonatal imaging. The lack of specificity of US abnormalities associated with cCMV presents a significant challenge as many of these findings are also observed in other pathologies. Therefore, identifying a cerebral abnormality specific to cCMV is crucial. Lenticulostriate vasculopathy (LSV), a recently discovered cerebral abnormality, has been investigated in relation to CMV. We conducted a prospective case-control study of 163 neonates (83 cases: 83 controls) to examine the role of LSV and prematurity as imaging biomarkers for cCMV infection. Overall, our results demonstrated that

LSV was not significantly associated to cCMV. Furthermore neonates with LSV had significantly larger z-head circumference, z-weight and more commonly depicted other concomitant cerebral abnormalities. Severe LSV, was further associated with LGA (large for gestational age) neonates and abnormal head circumference (above or below 2 SD from mean of z-score HC). Larger studies are needed to establish the relevance of these findings.

Περίληψη

Η συγγενής λοίμωξη από κυτταρομεγαλοϊό (cCMV) έχει παγκόσμιο επιπολασμό από 0,6% έως 2,5%[1]. Παρά το γεγονός ότι αποτελεί τη συνηθέστερη συγγενή λοίμωξη, αποτελεί το σημαντικότερο μη γενετικό αίτιο νευροαισθητήριας βαρηκοίας και προκαλεί πληθώρα νευροαναπτυξιακών προβλημάτων, η έλλειψη προγραμμάτων ανίχνευσης οδηγεί σε σημαντικό αριθμό μη διαγνωσμενων νεογνών, με αποτέλεσμα να χαθούν ευκαιρίες έγκαιρης παρέμβασης. Η παρούσα διατριβή έχει ως στόχο την μελέτη νευροαπεικονιστικών βιοδεικτών του cCMV, προκειμένου να διευκολυνθεί η έγκαιρη διάγνωση και ο εντοπισμός νεογνών με υψηλό κίνδυνο μακροπρόθεσμων διαταραχών.

Αρχικά, πραγματοποιήσαμε μια συστηματική ανασκόπηση και μετα-ανάλυση της βιβλιογραφίας, με θέμα την προγεννητική χρήση υπερήχου (US) και μαγνητικής τομογραφίας (MRI) στο έμβρυο με cCMV, ως μέσα πρόγνωσης της κλινικής έκβασης στην μεταγεννητική περίοδο[2]. Παρά τις δυσκολίες στην ερμηνεία των ευρημάτων λογω της υψηλής ετερογένειας μεταξύ των μελετών, υπογραμμίζουμε τον συμπληρωματικό ρόλο της MRI και του υπερήχου στην απεικονιση εγκεφαλικών ευρημάτων, καθώς και την υψηλή αρνητική προγνωστική αξία του φυσιολογικού εμβρυϊκού υπέρηχου & MRI για συμπτωματική cCMV λοίμωξη. Η μικροκεφαλία ήταν το μοναδικό εύρημα που εμφάνιζε στατιστικά σημαντική συσχέτιση με νευροαναπτυξιακές επιπλοκές. Μέσα απο αυτην την μελέτη, έγινε αισθητή η ανάγκη για δημιουργία διεθνών οδηγιών και πρωτοκόλλων για το cCMV σε κλινικό αλλα και ερευνητικό επίπεδο.

Στη συνέχεια, επικεντρωθήκαμε στην χρήστη της μαγνητικής τομογραφίας και μελετήσαμε την συσχέτιση μεταξύ νεογνικής και εμβρυϊκής MRI. Η νεογνική MRI συνήθως συνιστάται όταν παρουσιάζονται ανωμαλίες στον εμβρυϊκό ή νεογνικό υπέρηχο για τον εντοπισμό εγκεφαλικών ευρημάτων. Ωστόσο, περιορισμοί σχετικοί με την προσβασιμότητα, το κόστος και ηθικά ζητήματα που αφορούν την καταστολή των βρεφών, περιορίζουν την ευρεία χρήση της. Αντιθέτως, η εμβρυϊκή MRI, καθιστά μια λιγότερο επεμβατική εξέταση που επιτρέπει περισσότερο χρόνο για τη λήψη αποφάσεων σχετικά με την αντιμετώπιση και παρακολούθηση της λοίμωξης. Πραγματοποιήσαμε μια αναδρομική μελέτη 10 ασυμπτωματικών νεογνών με συγγενή CMV λοίμωξη που υποβλήθηκαν τόσο σε εμβρυϊκή όσο και σε νεογνική

MRI[3]. Παρατηρήσαμε ότι η εμβρυϊκή MRI μπορούσε να παρέχει συγκρίσιμες πληροφορίες με τη νεογνική απεικόνιση. Αν και μικρή μελέτη, τα ευρήματα της υποδεικνύουν ότι η εμβρυϊκή MRI αποτελεί μια εναλλακτική πρακτική.

Τέλος, η έλλειψη ειδικότητας των ευρημάτων στον υπέρηχο εγκεφάλου που σχετίζονται με cCMV αντιπροσωπεύει μια σημαντική πρόκληση, καθώς πολλά από αυτά τα ευρήματα παρατηρούνται και σε άλλες παθολογίες. Επομένως, ο εντοπισμός ενός ειδικού ευρήματος για το cCMV είναι μεγάλης σημασίας. Η επίταση των θαλαμοραβδωτων αγγειων (LSV) έχει πρόσφατα συζητηθεί αρκετά σε σχέση με τη cCMV. Πραγματοποιήσαμε μια προοπτική μελέτη ασθενών μαρτύρων με 166 νεογνά για να εξετάσουμε τον ρόλο του LSV ως νευροαπεικονιστικός βιοδεικτης για τη λοίμωξη από cCMV. Τα αποτελέσματα έδειξαν ότι η παρουσία του LSV δεν συσχετίζεται σημαντικά με την cCMV και πως η ανεύρεση του δεν επαρκεί για να δικαιολογήσει τον έλεγχο για cCMV. Επιπλέον, τα νεονά με LSV είχαν σημαντικά μεγαλύτερη περίμετρο κεφαλής, μεγαλύτερο βάρος και συχνότερα παρουσία άλλων συνυπάρχουσων εγκεφαλικών ανωμαλιών συγκριτικά με τους μάρτυρες της μελέτης. Μελετήθηκαν επιπλέον τα νεογνα με σοβαρή εικόνα LSV όπου βρέθηκε στατιστικά σημαντική συσχέτιση με τα μεγάλα για την ηλικία κύησης νεογνά (LGA) καθώς και την μη φυσιολογική περίμετρο κεφαλής (+ / - 2 SD από το μέσο z-score). Μεγαλύτερες μελέτες χρειάζονται για να επιβεβαιωθεί και να εκτιμηθεί η σημασία αυτών των ευρημάτων.

Βιογραφικό σημείωμα

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Ιατρικό Elective (2 rotation) ως κομμάτι του τελευταίου έτους Ιατρικής σχολής 2014
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Εθελοντική εργασία στο «Περιβολάκι» 2012 -13
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Ερευνητικό Internship, Πανεπιστημίου της Πενσυλβανίας (UPENN) 2008
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Δημοσιεύσεις/Εργασίες

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PLoS Biol. 2015 Oct 13; 13(10)

Πτυχιακή εργασία κατά την διάρκεια του intercalated BSc Biomedical Sciences

"Ανασκόπηση βιβλιογραφίας για την παθοφυσιολογία της Νόσου Alzheimer" 2012

Πιστοποιήσεις

Royal College of Paediatricians and Child Health (RCPCH)
- MRCPCH Part 1 **(FOP and TAS)** 2019
- MRCPCH Part 1 **(AKP)** 2021

European Resuscitation Council (ERC)
- Neonatal Life Support **(NLS)** 2018
- Advanced Life Support **(ALS)** 2016
- Immediate Life Support **(ILS)** 2015

Συνέδρια/ Παρουσιάσεις/ Posters

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European Society of Paediatric Indectious Diseases (ESPID) 2022
Παρουσίαση Poster " Fetal brain imaging provides valuable information in cCMV infected infants"

The 7th Congress of the European Academy of Paediatric Societies (EAPS) 2018

European Congenital Cytomegalovirus Initiative (ECCI) 2018
- Παρουσίαση Poster " Antenatal imaging and clinical outcome in congenital CMV infection: A field-wide systematic review and meta-analysis"

Εξωσχολικές/ Δραστηριότητες- Ιατρικές/ Εθελοντισμός

Διοργάνωση 1ης Ημερίδας Ειδικευομένων της ΑΠΠΚ του Παίδων « Η Αγία Σοφία» 2023

Επιτροπή ειδικευομένων της ΑΠΠΚ' Παίδων «Η Αγία Σοφία. 2021-2023
Ομάδα ειδικευομένων με στόχο την βελτίωση θεμάτων εκπαίδευσης ειδικευομένων

Παράδοση μαθημάτων πρώτων βοηθειών 2009-2013
Παράδοση μαθημάτων πρώτων βοηθειών σε μαθητές δημοτικών σχολείων στα πλαίσια του κοινωνικού project "**Teddy Bear Hospital**" του Πανεπιστημίου του Εδιμβούργου.

Peer learning program Edinburgh Medical School 2011-2013
Παράδοση μαθημάτων σε νεότερους φοιτητές στα πλαίσια διδασκαλίας του Πανεπιστημίου του Εδιμβούργου.

Οργάνωση και συμμετοχή Θεατρικής Παράστασης Musical της Ιατρικής σχολής του Πανεπιστημίου 2014
Ημι-επαγγελματική παράσταση που παίχτηκε για 10 ημέρες σε κεντρικό θέατρο του Εδιμβούργου

Εκλεγμένο μέλος του φοιτητικού συμβουλίου του Πανεπιστημίου του Εδιμβούργου 2012-2014
Εκπρόσωπος ευρωπαίων φοιτητών

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German - Καλή χρήση
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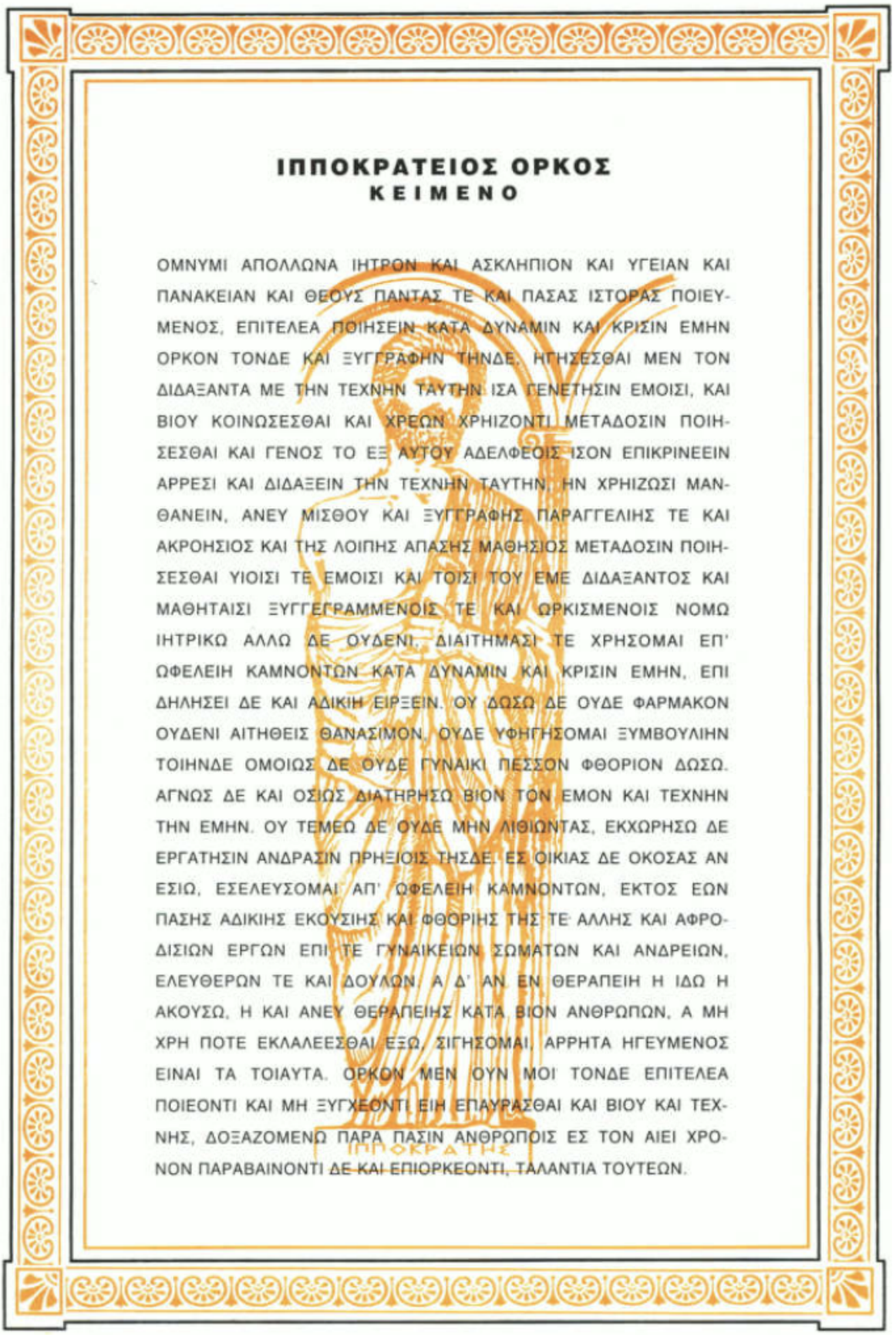
Όρκος του Ιπποκράτη

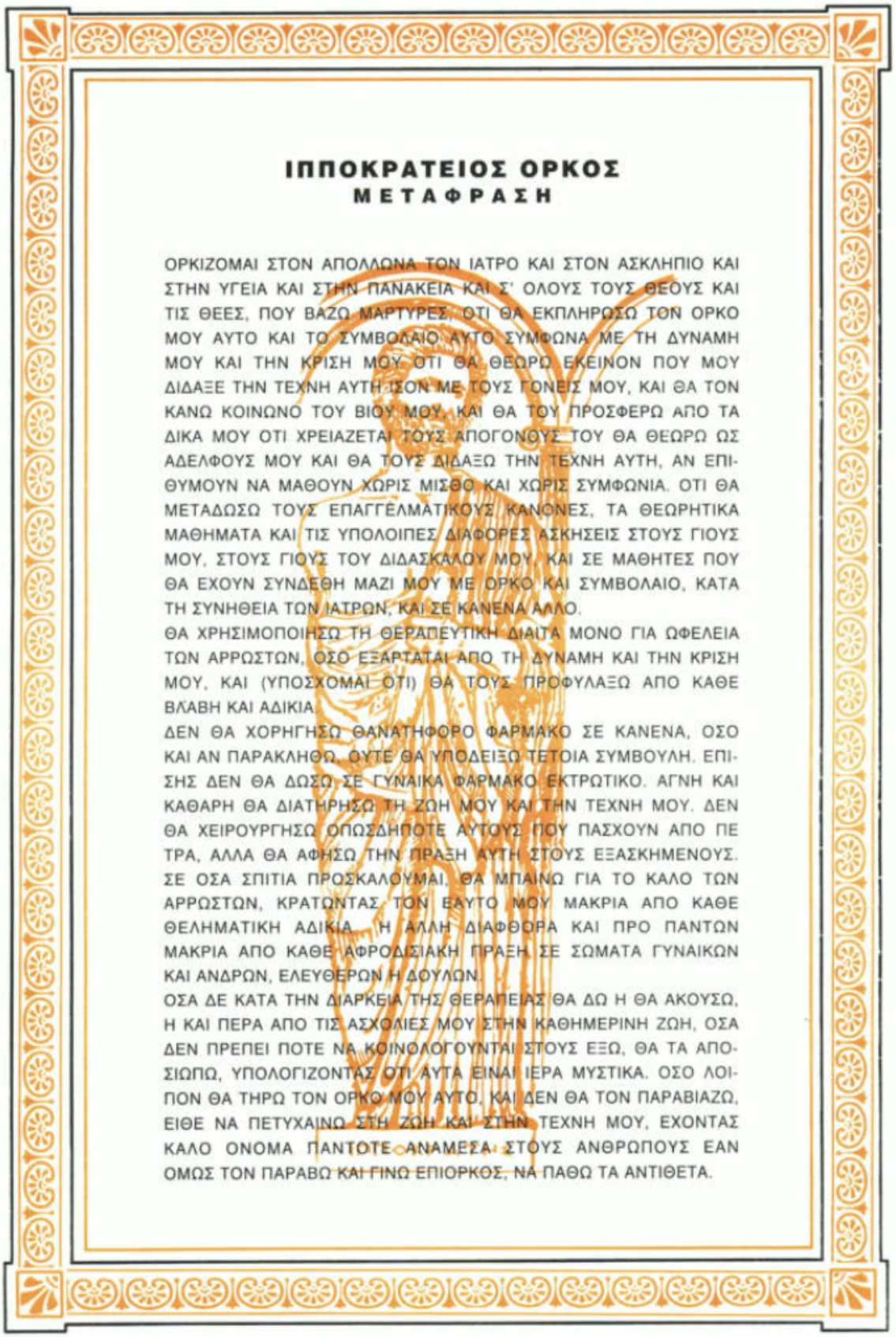


ΙΠΠΟΚΡΑΤΕΙΟΣ ΟΡΚΟΣ
ΚΕΙΜΕΝΟ

ΟΜΝΥΜΙ ΑΠΟΛΛΩΝΑ ΙΗΤΡΟΝ ΚΑΙ ΑΣΚΛΗΠΙΟΝ ΚΑΙ ΥΓΕΙΑΝ ΚΑΙ ΠΑΝΑΚΕΙΑΝ ΚΑΙ ΘΕΟΥΣ ΠΑΝΤΑΣ ΤΕ ΚΑΙ ΠΑΣΑΣ ΙΣΤΟΡΑΣ ΠΟΙΕΥΜΕΝΟΣ, ΕΠΙΤΕΛΕΑ ΠΟΙΗΣΕΙΝ ΚΑΤΑ ΔΥΝΑΜΙΝ ΚΑΙ ΚΡΙΣΙΝ ΕΜΗΝ ΟΡΚΟΝ ΤΟΝΔΕ ΚΑΙ ΞΥΓΓΡΑΦΗΝ ΤΗΝΔΕ. ΗΓΗΣΕΣΘΑΙ ΜΕΝ ΤΟΝ ΔΙΔΑΞΑΝΤΑ ΜΕ ΤΗΝ ΤΕΧΝΗΝ ΤΑΥΤΗΝ ΙΣΑ ΓΕΝΕΤΗΣΙΝ ΕΜΟΙΣΙ, ΚΑΙ ΒΙΟΥ ΚΟΙΝΩΣΕΣΘΑΙ ΚΑΙ ΧΡΕΩΝ ΧΡΗΙΖΟΝΤΙ ΜΕΤΑΔΟΣΙΝ ΠΟΙΗΣΕΣΘΑΙ ΚΑΙ ΓΕΝΟΣ ΤΟ ΕΞ ΑΥΤΟΥ ΑΔΕΛΦΕΟΙΣ ΙΣΟΝ ΕΠΙΚΡΙΝΕΕΙΝ ΑΡΡΕΣΙ ΚΑΙ ΔΙΔΑΞΕΙΝ ΤΗΝ ΤΕΧΝΗΝ ΤΑΥΤΗΝ, ΗΝ ΧΡΗΙΖΩΣΙ ΜΑΝΘΑΝΕΙΝ, ΑΝΕΥ ΜΙΣΘΟΥ ΚΑΙ ΞΥΓΓΡΑΦΗΣ ΠΑΡΑΓΓΕΛΙΗΣ ΤΕ ΚΑΙ ΑΚΡΟΗΣΙΟΣ ΚΑΙ ΤΗΣ ΛΟΙΠΗΣ ΑΠΑΣΗΣ ΜΑΘΗΣΙΟΣ ΜΕΤΑΔΟΣΙΝ ΠΟΙΗΣΕΣΘΑΙ ΥΙΟΙΣΙ ΤΕ ΕΜΟΙΣΙ ΚΑΙ ΤΟΙΣΙ ΤΟΥ ΕΜΕ ΔΙΔΑΞΑΝΤΟΣ ΚΑΙ ΜΑΘΗΤΑΙΣΙ ΞΥΓΓΕΓΡΑΜΜΕΝΟΙΣ ΤΕ ΚΑΙ ΩΡΚΙΣΜΕΝΟΙΣ ΝΟΜΩ ΙΗΤΡΙΚΩ ΑΛΛΩ ΔΕ ΟΥΔΕΝΙ. ΔΙΑΙΤΗΜΑΣΙ ΤΕ ΧΡΗΣΟΜΑΙ ΕΠ' ΩΦΕΛΕΙΗ ΚΑΜΝΟΝΤΩΝ ΚΑΤΑ ΔΥΝΑΜΙΝ ΚΑΙ ΚΡΙΣΙΝ ΕΜΗΝ, ΕΠΙ ΔΗΛΗΣΕΙ ΔΕ ΚΑΙ ΑΔΙΚΙΗ ΕΙΡΞΕΙΝ. ΟΥ ΔΩΣΩ ΔΕ ΟΥΔΕ ΦΑΡΜΑΚΟΝ ΟΥΔΕΝΙ ΑΙΤΗΘΕΙΣ ΘΑΝΑΣΙΜΟΝ, ΟΥΔΕ ΥΦΗΓΗΣΟΜΑΙ ΞΥΜΒΟΥΛΙΗΝ ΤΟΙΗΝΔΕ ΟΜΟΙΩΣ ΔΕ ΟΥΔΕ ΓΥΝΑΙΚΙ ΠΕΣΣΟΝ ΦΘΟΡΙΟΝ ΔΩΣΩ. ΑΓΝΩΣ ΔΕ ΚΑΙ ΟΣΙΩΣ ΔΙΑΤΗΡΗΣΩ ΒΙΟΝ ΤΟΝ ΕΜΟΝ ΚΑΙ ΤΕΧΝΗΝ ΤΗΝ ΕΜΗΝ. ΟΥ ΤΕΜΕΩ ΔΕ ΟΥΔΕ ΜΗΝ ΛΙΘΙΩΝΤΑΣ, ΕΚΧΩΡΗΣΩ ΔΕ ΕΡΓΑΤΗΣΙΝ ΑΝΔΡΑΣΙΝ ΠΡΗΞΙΟΙΣ ΤΗΣΔΕ. ΕΣ ΟΙΚΙΑΣ ΔΕ ΟΚΟΣΑΣ ΑΝ ΕΣΙΩ, ΕΣΕΛΕΥΣΟΜΑΙ ΑΠ' ΩΦΕΛΕΙΗ ΚΑΜΝΟΝΤΩΝ, ΕΚΤΟΣ ΕΩΝ ΠΑΣΗΣ ΑΔΙΚΙΗΣ ΕΚΟΥΣΙΗΣ ΚΑΙ ΦΘΟΡΙΗΣ ΤΗΣ ΤΕ ΑΛΛΗΣ ΚΑΙ ΑΦΡΟΔΙΣΙΩΝ ΕΡΓΩΝ ΕΠΙ ΤΕ ΓΥΝΑΙΚΕΙΩΝ ΣΩΜΑΤΩΝ ΚΑΙ ΑΝΔΡΕΙΩΝ, ΕΛΕΥΘΕΡΩΝ ΤΕ ΚΑΙ ΔΟΥΛΩΝ. Α Δ' ΑΝ ΕΝ ΘΕΡΑΠΕΙΗ Η ΙΔΩ Η ΑΚΟΥΣΩ, Η ΚΑΙ ΑΝΕΥ ΘΕΡΑΠΕΙΗΣ ΚΑΤΑ ΒΙΟΝ ΑΝΘΡΩΠΩΝ, Α ΜΗ ΧΡΗ ΠΟΤΕ ΕΚΛΑΛΕΕΣΘΑΙ ΕΞΩ, ΣΙΓΗΣΟΜΑΙ, ΑΡΡΗΤΑ ΗΓΕΥΜΕΝΟΣ ΕΙΝΑΙ ΤΑ ΤΟΙΑΥΤΑ. ΟΡΚΟΝ ΜΕΝ ΟΥΝ ΜΟΙ ΤΟΝΔΕ ΕΠΙΤΕΛΕΑ ΠΟΙΕΟΝΤΙ ΚΑΙ ΜΗ ΞΥΓΧΕΟΝΤΙ ΕΙΗ ΕΠΑΥΡΑΣΘΑΙ ΚΑΙ ΒΙΟΥ ΚΑΙ ΤΕΧΝΗΣ, ΔΟΞΑΖΟΜΕΝΩ ΠΑΡΑ ΠΑΣΙΝ ΑΝΘΡΩΠΟΙΣ ΕΣ ΤΟΝ ΑΙΕΙ ΧΡΟΝΟΝ ΠΑΡΑΒΑΙΝΟΝΤΙ ΔΕ ΚΑΙ ΕΠΙΟΡΚΕΟΝΤΙ, ΤΑΛΑΝΤΙΑ ΤΟΥΤΕΩΝ.

ΙΠΠΟΚΡΑΤΗΣ

ΙΠΠΟΚΡΑΤΕΙΟΣ ΟΡΚΟΣ
ΜΕΤΑΦΡΑΣΗ



ΟΡΚΙΖΟΜΑΙ ΣΤΟΝ ΑΠΟΛΛΩΝΑ ΤΟΝ ΙΑΤΡΟ ΚΑΙ ΣΤΟΝ ΑΣΚΛΗΠΙΟ ΚΑΙ ΣΤΗΝ ΥΓΕΙΑ ΚΑΙ ΣΤΗΝ ΠΑΝΑΚΕΙΑ ΚΑΙ Σ' ΟΛΟΥΣ ΤΟΥΣ ΘΕΟΥΣ ΚΑΙ ΤΙΣ ΘΕΕΣ, ΠΟΥ ΒΑΖΩ ΜΑΡΤΥΡΕΣ, ΟΤΙ ΘΑ ΕΚΠΛΗΡΩΣΩ ΤΟΝ ΟΡΚΟ ΜΟΥ ΑΥΤΟ ΚΑΙ ΤΟ ΣΥΜΒΟΛΑΙΟ ΑΥΤΟ ΣΥΜΦΩΝΑ ΜΕ ΤΗ ΔΥΝΑΜΗ ΜΟΥ ΚΑΙ ΤΗΝ ΚΡΙΣΗ ΜΟΥ. ΟΤΙ ΘΑ ΘΕΩΡΩ ΕΚΕΙΝΟΝ ΠΟΥ ΜΟΥ ΔΙΔΑΞΕ ΤΗΝ ΤΕΧΝΗ ΑΥΤΗ ΙΣΟΝ ΜΕ ΤΟΥΣ ΓΟΝΕΙΣ ΜΟΥ, ΚΑΙ ΘΑ ΤΟΝ ΚΑΝΩ ΚΟΙΝΩΝΟ ΤΟΥ ΒΙΟΥ ΜΟΥ, ΚΑΙ ΘΑ ΤΟΥ ΠΡΟΣΦΕΡΩ ΑΠΟ ΤΑ ΔΙΚΑ ΜΟΥ ΟΤΙ ΧΡΕΙΑΖΕΤΑΙ. ΤΟΥΣ ΑΠΟΓΟΝΟΥΣ ΤΟΥ ΘΑ ΘΕΩΡΩ ΩΣ ΑΔΕΛΦΟΥΣ ΜΟΥ ΚΑΙ ΘΑ ΤΟΥΣ ΔΙΔΑΞΩ ΤΗΝ ΤΕΧΝΗ ΑΥΤΗ, ΑΝ ΕΠΙΘΥΜΟΥΝ ΝΑ ΜΑΘΟΥΝ ΧΩΡΙΣ ΜΙΣΘΟ ΚΑΙ ΧΩΡΙΣ ΣΥΜΦΩΝΙΑ. ΟΤΙ ΘΑ ΜΕΤΑΔΩΣΩ ΤΟΥΣ ΕΠΑΓΓΕΛΜΑΤΙΚΟΥΣ ΚΑΝΟΝΕΣ, ΤΑ ΘΕΩΡΗΤΙΚΑ ΜΑΘΗΜΑΤΑ ΚΑΙ ΤΙΣ ΥΠΟΛΟΙΠΕΣ ΔΙΑΦΟΡΕΣ ΑΣΚΗΣΕΙΣ ΣΤΟΥΣ ΓΙΟΥΣ ΜΟΥ, ΣΤΟΥΣ ΓΙΟΥΣ ΤΟΥ ΔΙΔΑΣΚΑΛΟΥ ΜΟΥ, ΚΑΙ ΣΕ ΜΑΘΗΤΕΣ ΠΟΥ ΘΑ ΕΧΟΥΝ ΣΥΝΔΕΘΗ ΜΑΖΙ ΜΟΥ ΜΕ ΟΡΚΟ ΚΑΙ ΣΥΜΒΟΛΑΙΟ, ΚΑΤΑ ΤΗ ΣΥΝΗΘΕΙΑ ΤΩΝ ΙΑΤΡΩΝ, ΚΑΙ ΣΕ ΚΑΝΕΝΑ ΑΛΛΟ.

ΘΑ ΧΡΗΣΙΜΟΠΟΙΗΣΩ ΤΗ ΘΕΡΑΠΕΥΤΙΚΗ ΔΙΑΙΤΑ ΜΟΝΟ ΓΙΑ ΩΦΕΛΕΙΑ ΤΩΝ ΑΡΡΩΣΤΩΝ, ΟΣΟ ΕΞΑΡΤΑΤΑΙ ΑΠΟ ΤΗ ΔΥΝΑΜΗ ΚΑΙ ΤΗΝ ΚΡΙΣΗ ΜΟΥ, ΚΑΙ (ΥΠΟΣΧΟΜΑΙ ΟΤΙ) ΘΑ ΤΟΥΣ ΠΡΟΦΥΛΑΞΩ ΑΠΟ ΚΑΘΕ ΒΛΑΒΗ ΚΑΙ ΑΔΙΚΙΑ.

ΔΕΝ ΘΑ ΧΟΡΗΓΗΣΩ ΘΑΝΑΤΗΦΟΡΟ ΦΑΡΜΑΚΟ ΣΕ ΚΑΝΕΝΑ, ΟΣΟ ΚΑΙ ΑΝ ΠΑΡΑΚΛΗΘΩ. ΟΥΤΕ ΘΑ ΥΠΟΔΕΙΞΩ ΤΕΤΟΙΑ ΣΥΜΒΟΥΛΗ. ΕΠΙΣΗΣ ΔΕΝ ΘΑ ΔΩΣΩ ΣΕ ΓΥΝΑΙΚΑ ΦΑΡΜΑΚΟ ΕΚΤΡΩΤΙΚΟ. ΑΓΝΗ ΚΑΙ ΚΑΘΑΡΗ ΘΑ ΔΙΑΤΗΡΗΣΩ ΤΗ ΖΩΗ ΜΟΥ ΚΑΙ ΤΗΝ ΤΕΧΝΗ ΜΟΥ. ΔΕΝ ΘΑ ΧΕΙΡΟΥΡΓΗΣΩ ΟΠΩΣΔΗΠΟΤΕ ΑΥΤΟΥΣ ΠΟΥ ΠΑΣΧΟΥΝ ΑΠΟ ΠΕΤΡΑ, ΑΛΛΑ ΘΑ ΑΦΗΣΩ ΤΗΝ ΠΡΑΞΗ ΑΥΤΗ ΣΤΟΥΣ ΕΞΑΣΚΗΜΕΝΟΥΣ. ΣΕ ΟΣΑ ΣΠΙΤΙΑ ΠΡΟΣΚΑΛΟΥΜΑΙ, ΘΑ ΜΠΑΙΝΩ ΓΙΑ ΤΟ ΚΑΛΟ ΤΩΝ ΑΡΡΩΣΤΩΝ, ΚΡΑΤΩΝΤΑΣ ΤΟΝ ΕΑΥΤΟ ΜΟΥ ΜΑΚΡΙΑ ΑΠΟ ΚΑΘΕ ΘΕΛΗΜΑΤΙΚΗ ΑΔΙΚΙΑ, Η ΑΛΛΗ ΔΙΑΦΘΟΡΑ ΚΑΙ ΠΡΟ ΠΑΝΤΩΝ ΜΑΚΡΙΑ ΑΠΟ ΚΑΘΕ ΑΦΡΟΔΙΣΙΑΚΗ ΠΡΑΞΗ ΣΕ ΣΩΜΑΤΑ ΓΥΝΑΙΚΩΝ ΚΑΙ ΑΝΔΡΩΝ, ΕΛΕΥΘΕΡΩΝ Η ΔΟΥΛΩΝ.

ΟΣΑ ΔΕ ΚΑΤΑ ΤΗΝ ΔΙΑΡΚΕΙΑ ΤΗΣ ΘΕΡΑΠΕΙΑΣ ΘΑ ΔΩ Η ΘΑ ΑΚΟΥΣΩ, Η ΚΑΙ ΠΕΡΑ ΑΠΟ ΤΙΣ ΑΣΧΟΛΙΕΣ ΜΟΥ ΣΤΗΝ ΚΑΘΗΜΕΡΙΝΗ ΖΩΗ, ΟΣΑ ΔΕΝ ΠΡΕΠΕΙ ΠΟΤΕ ΝΑ ΚΟΙΝΟΛΟΓΟΥΝΤΑΙ ΣΤΟΥΣ ΕΞΩ, ΘΑ ΤΑ ΑΠΟΣΙΩΠΩ, ΥΠΟΛΟΓΙΖΟΝΤΑΣ ΟΤΙ ΑΥΤΑ ΕΙΝΑΙ ΙΕΡΑ ΜΥΣΤΙΚΑ. ΟΣΟ ΛΟΙΠΟΝ ΘΑ ΤΗΡΩ ΤΟΝ ΟΡΚΟ ΜΟΥ ΑΥΤΟ, ΚΑΙ ΔΕΝ ΘΑ ΤΟΝ ΠΑΡΑΒΙΑΖΩ, ΕΙΘΕ ΝΑ ΠΕΤΥΧΑΙΝΩ ΣΤΗ ΖΩΗ ΚΑΙ ΣΤΗΝ ΤΕΧΝΗ ΜΟΥ, ΕΧΟΝΤΑΣ ΚΑΛΟ ΟΝΟΜΑ ΠΑΝΤΟΤΕ ΑΝΑΜΕΣΑ ΣΤΟΥΣ ΑΝΘΡΩΠΟΥΣ ΕΑΝ ΟΜΩΣ ΤΟΝ ΠΑΡΑΒΩ ΚΑΙ ΓΙΝΩ ΕΠΙΟΡΚΟΣ, ΝΑ ΠΑΘΩ ΤΑ ΑΝΤΙΘΕΤΑ.

Ευχαριστίες

Με το πέρας της διδακτορικής μου διατριβής, θα ήθελα να εκφράσω την ευγνωμοσύνη μου στην Καθηγήτρια Παιδιατρικής Λοιμωξιολογίας της Ιατρικής Σχολής του ΕΚΠΑ, κα Βασιλική Παπαευαγγέλου. Η συνάντησή μου μαζί της ήταν μία από τις σημαντικότερες συγκυρίες της ζωής μου. Υπήρξε για μένα μέντορας και νιώθω ιδιαίτερα τυχερή που είχα την τύχη να την γνωρίσω και να δουλέψω δίπλα της. Ελπίζω να έχω την χαρά να συνεχίσουμε να συνεργαζόμαστε και στο μέλλον. Με την αφοσίωσή της στην επιστημονική έρευνα, την διεθνώς αναγνωρισμένη εμπειρία της στον τομέα των συγγενών λοιμώξεων, τον ενθουσιασμό της και την καθοδήγησή της κατάφερα να γνωρίσω αυτόν τον καινούριο για μένα ως τότε κόσμο της έρευνας. Της είμαι ευγνώμων, όχι μόνο για την μέχρι τώρα σταδιοδρομία μου, αλλά και για την συμμετοχή της στην διαμόρφωση της επιστημονικής μου γνώσης και της προσωπικότητάς μου, αναδεικνύοντας τα προτερήματά μου και ταυτόχρονα, βελτιώνοντας τα ελαττώματα μου. Της είμαι ευγνώμων για την εμπιστοσύνη που μου έδειξε, το ενδιαφέρον της για μένα, για την υποστήριξή της και για όλα αυτά που μου δίδαξε.

Στην συνέχεια, θα ήθελα να εκφράσω την βαθύτατη ευγνωμοσύνη μου στην Καθηγήτρια Θεραπευτικής, Επιδημιολογίας και Προληπτικής Ιατρικής του ΕΚΠΑ κα Θεοδώρα Ψαλτοπούλου χωρίς την οποία δεν θα είχα την ευκαιρία να έρθω σε επαφή με τον κόσμο της ιατρικής έρευνας. Η στήριξή της και η συμβολή της ήταν καθοριστικής σημασίας και υπήρξε ανεκτίμητη για την εκπόνηση της μελέτης και την συνακόλουθη συγγραφή.

Επίσης θα ήθελα να ευχαριστήσω την καθηγήτρια Παιδιατρικής και Επεμβατικής Ακτινολογίας της Ιατρικής Σχολής του Πανεπιστημίου Αθηνών, κα Ευθυμία Αλεξοπούλου, χωρίς την οποία δεν θα μπορούσα να έχω πραγματοποιήσει την έρευνα και τη συλλογή δεδομένων. Η κα Αλεξοπούλου καθ'όλη την πορεία της διατριβής μου βρίσκοταν δίπλα μου να με συμβουλέψει και να με καθοδηγήσει στον κόσμο της ακτινολογίας και των προκλήσεων που αφορούν την απεικόνιση του εγκεφάλου των νεογνων. Η εμπειρία της και η συμβολή της ήταν ανεκτίμητη όχι μόνο

στο πρακτικό κομμάτι αλλα και στο επιστημονικό. Ήταν πάντα διαθέσιμη και πρόθυμη να με βοηθήσει όπου το χρειαζόμουν και την ευχαριστώ από καρδιάς.

Επίσης, θα ήθελα να ευχαριστήσω τους Ιατρούς και Ακτινολόγους κο Κυριάκο Σαμικό και κα Κατερίνα Καναβάκη με τους οποίους είχα επίσης την τιμή να συνεργαστώ κυρίως στο πρακτικό κομμάτι της συλλογής δεδομένων. Ήταν πάντα πρόθυμοι να με βοηθήσουν και να με ακούσουν, να αφιερώνουν τον δικό τους προσωπικό χρόνο για να μπορέσω να βρω τα δεδομένα που χρειαζόμουν αλλα και να μου διδάξουν τα μυστικά του νεογνικού υπέρηχου. Θέλω να εκφράσω και στους δύο ένα μεγάλο ευχαριστώ γιατι δεν θα ήταν ίδια αυτή η εμπειρία χωρίς εκείνους. Ειναι και οι δυο εξαιρετικοί επιστήμονες αλλα και πανω απο ολα άνθρωποι και ήταν πραγματικά τιμή μου να τους γνωρίσω και να συνεργαστώ μαζί τους.

Θα ήταν μεγάλη παράλειψη αν δεν εξέφραζα την βαθύτατη ευγνωμοσύνη μου στον συμφοιτητή και φίλο μου Στέλιο Σεργίου, Ιατρό και Διδάκτωρ του Πανεπιστημίου του Stanford. Η αφοσίωση και το πάθος του για την βελτίωση της ποιότητας της ιατρικής έρευνας καθώς και η εξειδίκευση του στον τομέα της επιδημιολογίας και ανάλυσης ιατρικών δεδομένων τον καθιστούν ενα απο τα λαμπρότερα μυαλά με τα οποία είχα την τύχη να δουλέψω αλλα και να μάθω από εκείνον. Πέρα από τον πολύ σημαντικό του ρόλο που αφορά την ανάλυση των δεδομένων μου, τον ευχαριστώ από καρδιάς για την ιδιαίτερη βοήθεια του καθ'όλη την διάρκεια αυτού του ταξιδιού. Βρισκόταν πάντα δίπλα μου, να με εμψυχώνει και να με βοηθησει να αντιμετωπίσω την όποια δυσκολία παρουσιαζόταν.

Θα ήθελα να ευχαριστήσω θερμά την Καθηγήτρια Λοιμωξιολογίας κα. Αναστασία Αντωνιάδου, μέλος της τριμελούς επιτροπής, καθώς και τον Επίκουρο Καθηγητή Διαγνωστικής Ιολογίας κο Νικόλαο Σιαφάκα για την πραγματοποίηση των μοριακών εξετάσεων, την καθοδήγησή τους, τις συμβουλές, την προθυμία τους να με βοηθήσουν και φυσικά, τις συστάσεις τους. Οι ευχαριστίες μου δεν θα μπορούσαν να παραλείψουν το ιατρικό προσωπικό και κυρίως όλες τις μαιες των μονάδων νεογνών του Γ.Ν ΑΤΤΙΚΟΝ και του Μαιευτηρίου ΙΑΣΩ και ιδιαιτερα την κα. Ηλιανή Φίλιππα, οι οποίες επαιξαν καθοριστικό ρόλο στο να με βοηθήσουν με την συλλογή των δειγμάτων. Τους ειμαι ευγνώμον.

Τέλος, ευχαριστώ ιδιαίτερα τους υπόλοιπους συμμετέχοντες της 7μελούς επιτροπής, τους καθηγητές κα Χριστίνα Κανακά - Gantenbein, την κα Μαρία

Αργυροπούλου και τον κο Αργύρη Ντινόπουλο για την υποστήριξή τους και για την τιμή που μου έκαναν να είναι μέλη αυτής της επιτροπής. Αν μπόρεσε να φτάσει στο τέλος της αυτή η ερευνητική πορεία ήταν γιατί είχα την τύχη να περιβάλλομαι από ξεχωριστούς δασκάλους που με εμπιστεύτηκαν και μου έδωσαν τα πρώτα εφόδια ώστε να συνεχίσω αυτή την πορεία στο μέλλον. Τους είμαι πραγματικά ευγνώμων και τους ευχαριστώ θερμά.

General Part

Human Cytomegalovirus (HCMV) infection is a leading cause of both congenital infections and end organ diseases (EODs) especially in the immunocompromised. Even though it is the commonest congenital infection leading to a wide range of neurodevelopmental problems, there is still a large number of infected neonates who remain undiagnosed until later in childhood, where the window for intervention has been usually missed.[4] The lack of national screening programs for cCMV and the fact that not all clinically affected children are born symptomatic, both contribute to the burden of disease. More specifically 10-15% of initially asymptomatic neonates at birth will develop problems later in life, with sensorineural hearing loss (SNHL) being the commonest clinical manifestation diagnosed in 50% of symptomatic cases[5].

When suspicion of a congenital infection is present, detection of CMV-DNA in amniotic fluid is the most reliable method of prenatal diagnosis with a positive predictive value of 100%[6]. Nevertheless, even in cases where prenatal diagnosis is achieved, prediction of clinical outcome of newborns with cCMV still remains a challenge.

To date, despite the lack of screening programmes in place, there are no guidelines for treatment of asymptomatic cCMV infected newborns, those with 'minor' manifestations of disease and those with isolated SNHL. In order to achieve better prognosis of those infected congenitally we need to raise awareness for cCMV and improve the diagnostic process. Furthermore it is imperative to identify reliable biomarkers of disease severity in order to predict which infected neonates may develop sequelae later in life and therefore benefit from antiviral therapy and rigorous follow-up.

Chapter 1: CMV infection

1.0 CMV virology

1.1 Primary infection

The Human Cytomegalovirus (HCMV) belongs to the herpes viridae family of viruses, and is part of the beta herpes virinae subfamily - HHV5. It is a double-stranded DNA virus and it's genome has been estimated to approximately 230kb encoding 165 genes. Having approximately twice the size of the varicella zoster virus, it is the biggest human herpes virus described to date[7]. Despite enclosing the largest genome, it's capsid is similar to other herpes-viruses with an icosahedral nucleocaspid, enveloped by a protein matrix (the tegument) enclosed by a lipid bilayer composed by glycoproteins[8]. It has been suggested that the specific tegument protein pp150 owned by the virus creates a netlike dense layer which might be responsible for stabilizing the capsid and accelerating infectious virion formation[9] (figure 1). As a member of the herpesvirus group it has various typical characteristics such as its restricted host-range and the establishment of a persistent latent infection with the potential of reactivation in the future.

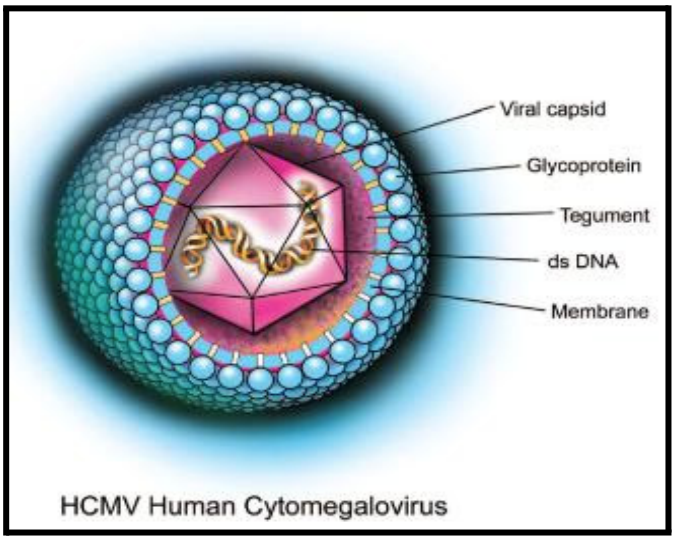


Figure 1. Schematic of the molecular structure of the Human Cytomegalovirus (HCMV). Adapted from Physiopedia.

During the primary infection, a broad immune response ensues, involving all the components of the adaptive immune system including neutralizing antibodies, specific to various viral proteins [10,11]. Furthermore, the primary infection leads to the production of CD4+ and CD8+ T cells which are also targeted against a number of viral proteins. The virus initially undergoes active cycles of replication in various cell tissues such as epithelial cells, smooth muscle cells, macrophages and especially differentiated human fibroblasts (HFs) showing the greatest vulnerability to viral infection. This wide variety of tissues involved, contribute to the systemic spread of the virus in the human body [12,13]

At the microscopic level, once the virus has entered the cell, it releases its tegument proteins as well as the capsid which is transferred inside the nucleus to ensure the delivery of the genome (figure 2). The tegument proteins control the efficiency of viral replication through triggering the synchronized expression of genes in three overlapping stages based on time of expression after infection[14]. The first to be expressed are viral I immediate early (IE) genes, followed by delayed early (DE) genes and finally late (L) genes. The major IE genes play a vital role in the viral gene expression and replication as they encode the key proteins for viral DNA replication [12,15,16], whereas the L genes encode structural proteins for the virion. Inhibiting the expression of the true late genes has to be achieved to prevent viral DNA synthesis. Despite the extensive research on the interaction of the CMV early and late gene expression, the exact mechanisms of late gene regulation are yet to be determined. After the immune's immediate response to the primary lytic phase of the viral infection, it fails to clear the HCMV, leading to a state of viral latency and periodic reactivation[17].

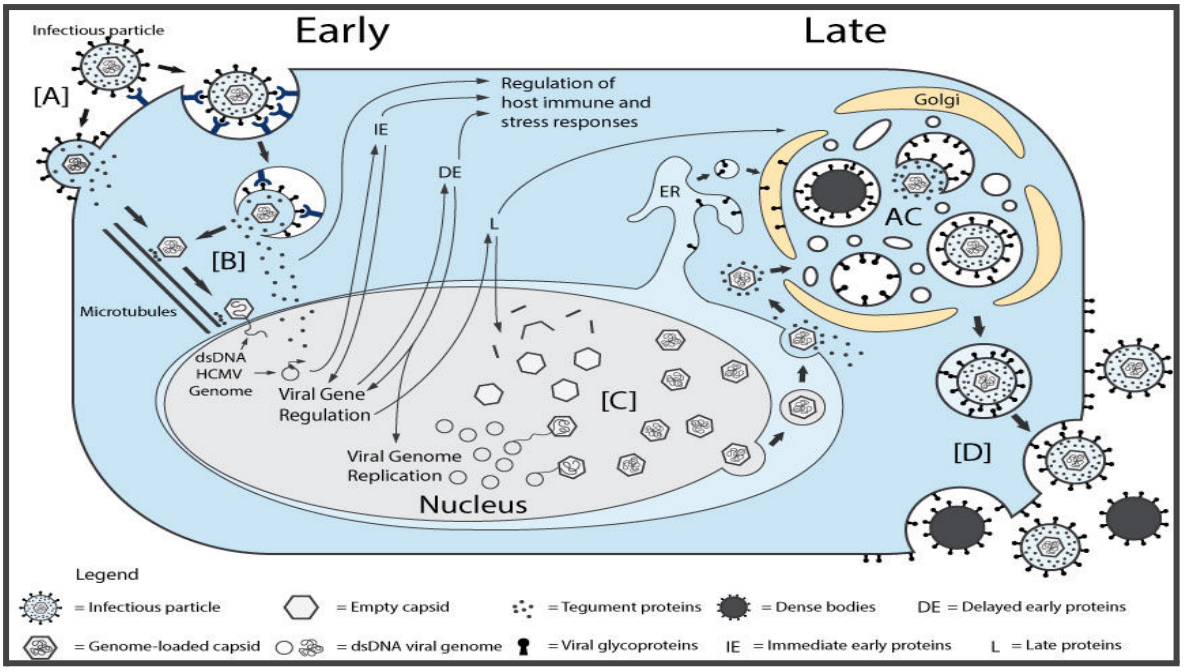


Figure 2. HCMV life cycle and host infection. Adapted from Beltran et al, 2015 [12]

1.2 Latency and Reactivation

The mechanisms by which the CMV virus achieves viral latency are still poorly understood. It is known however that the main site of latency seems to be in cells of the myeloid lineage, such as progenitors of granulocytes, macrophages and dendritic cells.[18] The presence of viral genome in those cells can either occur due to active viral replication or it could also be the result of phagocytosed virions[13]. Nevertheless, latency is a phase characterized by a very low or absent viral replication while the viral genome can be quiescently present in CD14 peripheral mononuclear cells (i.e monocytes) or even in CD33 and CD44 progenitor cells in the bone marrow of those infected (figure 3).[19]

An essential aspect of latency is that viral gene expression in progenitor cells is restricted to latency-associated transcripts, meaning that it is a state of transcriptional repression, which prevents the production of infectious virions.[20] This quiescent state is maintained until the pattern of gene expression is altered during progenitor differentiation into monocytes or dendrocytes. Conditions of inflammation,

infection, stress and immunocompromisation facilitate viral activation during this process.

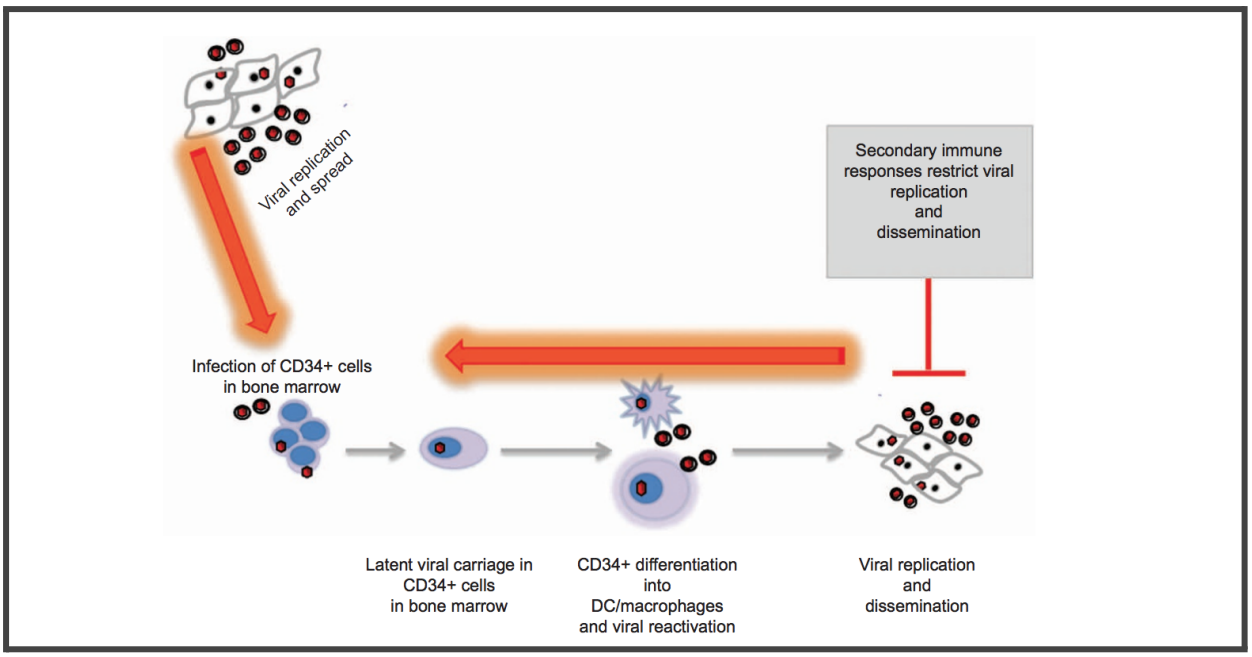


Figure 3. Establishment of HCMV latent infection through infection of myeloid progenitors. Adapted from MR Will et al, 2015[21]

It is important to stress that the tendency of a cell to allow for viral replication to take place is directly associated with its state of differentiation. In differentiated cells, active viral replication is more likely to be triggered than in undifferentiated cells. This does not mean however that the contribution of undifferentiated cells in the process of viral spread throughout the body is not essential. [10,22]

As it can be expected, the termination of latency is closely related to the expression of the major immediate early proteins (IE72 and IE86) which are vital for viral reactivation.[23] Research has shown that the tumor necrosis factor alpha (TNF) also plays a key role in this process as it attracts TNF receptors of latently infected cells which in turn activate protein kinases (C and NF-B) resulting in transcription of the CMV IE genes, initiating viral replication[24]. Other pathways resulting in CMV reactivation are those related to stress and inflammation. Both the release of catecholamines during stress as well as the production of prostaglandins during

inflammatory situations, increase cyclic AMP production, resulting in IE gene transcription [24,25].

Therefore, the establishment of lifelong latency and sporadic reactivation is a fine balance between the presence of viral genome in myeloid cells and transcriptional reactivation of the latent virus through the expression of early proteins in the absence of host-derived immune response[26]. Even though the key components of a primary CMV infection have been extensively studied, the precise elements controlling latency and reactivation are yet to be determined.[18]

1.3 Reinfection

A common misunderstanding regarding a CMV infection is that once someone has been infected with the virus, they cannot become reinfected. The CMV genome has a large sequence variability generating an extensive diversity of genotypes[27]. A symptomatic disease after the initial infection could reflect either a reactivation of the CMV virus from latency, or a new infection with a different exogenous strain. This is especially important when assessing women at a prenatal stage for CMV antibodies. It is important to stress that the presence of CMV IgG antibodies only provides partial protection against the fetus due to endogenous reactivation but also reinfection with a novel strain[28]. Gynecologists need to maintain high clinical suspicion in the presence of maternal flu-like symptoms as intrauterine transmission can occur and congenitally infected neonates can be born to previously seropositive women[29].

Furthermore, CMV reinfection has been shown to be involved in renal transplantation causing adverse outcomes such as allograft rejection[30]. CMV persists as a latent virus in the kidney and can easily become reactivated or even reinfect the immunocompromised transplant recipient. Recent research has shown poorer outcomes in cases where both the donor and the recipient are CMV-seropositive but with a mismatch in the glycoprotein H antibodies, suggesting that reinfection with a different CMV strain results in more serious complications.[31]

2.0 CMV Epidemiology

Human CMV infection affects 40 to 100 percent of the global adult population.[32] It is endemic and has no seasonal variation. Epidemiological studies have shown that age, sex, race, ethnicity, geographical location and socioeconomic status all affect the seroprevalence of the CMV virus.[33] A large study aiming to determine the CMV seroprevalence in the US is one of many to show that there is also an age-dependent rise of the CMV-specific antibody. Specifically the difference in seroprevalence between the pediatric and elderly population was significant, calculated at 36.3% in children from 6 to 11 years of age versus up to 90.8% in over 80 year olds.[34] Additionally higher seroprevalence is depicted within the female gender with a recent longitudinal study from germany reporting it as 62.3% in women vs 51% in males. [35] As mentioned in following sections, factors associated with the increased risk of congenital infection include low socioeconomic background, non-white race, living in low-income countries, prematurity and seropositive status in a previous pregnancy .[4,36] In developing countries, the burden of congenital CMV infection has been reported to be 3-fold greater than higher income countries [1]. Furthermore in smaller countries of sub-saharan africa the prevalence of cCMV has been reported to range from 0.5% up to 6%[37,38]. This translates into much higher level of maternal seroprevalence and therefore CMV infection during pregnancy in those areas.[39,] Higher prevalences in these countries in addition to maternal comorbidities such as HIV, facilitate vertical transmission of the virus.[40] Moreover, the deprived access to healthcare services poses a greater risk to the infected child and increases the chances of neurodevelopmental disabilities related to cCMV.

3.0 Transmission

One of the many reasons CMV is so prevalent in the population, is related to the numerous routes of transmission it has access to. Human cytomegalovirus can spread from one person to the other in various ways such as direct contact of bodily fluids (i.e. saliva, urine and breast milk), sexual intercourse, blood transfusions and transplanted organs, as well as from mother to child during pregnancy.

3.1 Sexual transmission

Evidence shows that in the developing world, CMV is acquired at a slow seroconversion rate of 1%-2% per year. Even with the rates of seroprevalence differing among countries, ethnicities and socioeconomic groups, it has been shown that the largest rise in seropositivity is found between the ages of 15 to 35. More specifically, studies show that when comparing adolescent levels of seroprevalence with those of people in their 30s, there is a large increase in seroprevalence of approximately 40%, making it the period with the highest rate of seroconversion.[41]

Looking into the reason behind this, it seems that the virus does not spread easily from pure close person-to-person contact. This fact is supported by studies that have taken place in crowded situations where there is close contact with a newly infected person shedding the virus, such as a military base or a hospital, where almost no seroconversions occurred.[42,43] On the other hand, it has been shown that transmission is greatly facilitated when there is contact with urine or an exchange of saliva and other bodily fluids through sexual contact. Proof for the role of sexual intercourse in the transmission of HCMV is also found in the high levels of seroprevalence among people who report to have had multiple sexual partners, a previous history of other sexually transmitted infections and recent onset of sexual activity. A study of women attending an STD clinic showed a 6.5 times higher rate of CMV positive results among women who have been diagnosed with other STDs in the past. This fact could indicate that recent sexual activity rather than the presence of concomitant sexually transmitted infection is a risk factor for CMV.[44] Furthermore the presence of high viral DNA load in the genital tract also supports the role of sexual intercourse in HCMV transmission.[45]

3.2 Other Body fluids

The most common mode of transmission of the HCMV is through bodily fluids such as saliva, urine and breastmilk. Even though epidemiological studies indicate that seroprevalence increases with age[46], we know that young children shed the virus at much higher levels, making them a very important vector of infection.[47] The high levels of viral load in the body fluids of infants have made viral culture of urine and

saliva samples the gold standard for diagnosis of both post-natal and congenital CMV infection.[48,49]

Furthermore, high levels of viral load are not only found among infants with congenital CMV, but also those who acquire the infection after the perinatal period mainly through exposure to saliva and urine.[50] A study investigating the presence of CMV on surfaces of numerous homes, found that of those testing positive for CMV, 90% lived in households with other young children also shedding the virus.[51] Importantly, they concluded that viral loads were much higher in younger children between the ages of 1-2 compared to older children, probably due to the increased likelihood of a recent primary infection at this age. Younger age was also associated with lower IgG avidity, lower IgG titres and prolonged shedding for up to a few months.[52]

This is particularly important as this group of young children is usually in close contact with their mothers or other women of childbearing age, and are therefore responsible for transmitting the virus to pregnant women[53]. This in turn gives rise to congenital infections and increases the risk of neurosensory hearing loss (SNHL) and neurodevelopmental handicap caused by the intrauterine transmission of the virus. Research on the most appropriate maternal preventative measures to avoid contracting the virus during pregnancy suggests taking measures to reduce exposure to bodily fluids such as hand washing after changing diapers, wiping a child's nose and avoiding contact with saliva through kissing, sharing food, drinks and towels.[54]

3.3 Blood and tissue products

Being a herpes virus, CMV has the ability to remain latent in white blood cells, allowing it to be transmitted through blood products.[15] One of the major issues related to this mode of transmission, is that people in need of blood or other blood products are usually immunocompromised due to cancer, chemotherapy, or a recent solid organ or haematopoietic stem cell transplant.[55] Contracting CMV through blood products can pose a serious risk to the immunocompromised by causing life-threatening disease, as well as organ rejection. In a large study of over 600 immunocompetent CMV-seronegative blood product recipients, CMV seroconversion

occurred in 0.9%.[56] However, when looking at immunocompromised patients after heart or liver transplant surgery the incidence of CMV has been calculated between 9 and 29%, and has been calculated to up to 32% after kidney transplantation.[57] Furthermore, 30% and 5% of patients who have gone through an allogeneic hematopoietic stem cell transplantation (HSCT) and an autologous HSCT respectively, become infected by CMV.[57,58] Due to the high incidence of CMV related mortality and morbidity, lot of effort has been dedicated in reducing the rates of transmission of CMV through the blood.

It is established that rates of seroconversion are higher when blood moves from a seropositive donor to a seronegative recipient.[55] Even though it would be ideal to solely use a CMV negative donor-population, the prevalence of CMV is so high among the adult population and the need for blood products and transplants is so large that it is merely impossible to exclude this valuable large group of donors.[59] Two techniques have been commonly used to reduce the risk of blood related CMV transmission. Those include only using CMV seronegative blood products or selecting blood which has undergone a process called leukocyte reduction.

The first has various limitations such as the possibility of falsely labeling the blood as being negative for CMV. The risk of a false negative result is small but exists depending on the sensitivity and specificity of the methodology used for testing the blood.[60] Furthermore, CMV infection can take up to a few weeks to appear in the blood, which means that for a period of time the virus can exist in disguise. Therefore it is vital for both the physician and the patient to keep in mind that when given a blood product which is labeled as being CMV-negative, the risk of CMV disease related complications still exist.[61]

The second and most commonly used technique is the one of leukocyte reduction.[62] Leukocytes contribute to a number of complications related to transfusion of blood products such as reperfusion injury, immunological effects (i.e transplant rejection, graft vs. host disease) and transmission of viral infections such as CMV and EBV.[59,62] Filters currently used in this process allow for the blood product to have less than 1×106 leukocytes per unit making it extremely unlikely to be infectious.[63,64] This process has been shown to greatly decrease the risk of transmission, nevertheless the choice between the two techniques is case specific. Finally, studies aiming to

assess the risk reduction when combining the two techniques have not shown any further benefit in doing so.[65,66]

4.0 Vertical transmission

Vertical or “mother-to-child” transmission of the CMV virus can occur both prenatally and postnatally. Prenatal intrauterine transmission poses the greatest risk to the fetus as it leads to congenital CMV infection. Clinical aspects and implications of congenital CMV is the main scope of this thesis and will be discussed in detail in the next chapter (Part B), however it is important to mention that cCMV is the commonest congenital infection and that it can lead to severe neurodevelopmental complications of which the most common is sensorineural hearing loss (SNHL).[67] Even though a primary CMV infection during pregnancy poses the greatest risk to the fetus, with a transmission rate from 30-70%, it is important to note that seroimmune women may also give birth to a cCMV infected child. This can happen whenever an immune pregnant woman has CMV viremia (non-primary infection;NPI) either from a reactivation or reinfection with a different CMV genotype. The risk of vertical transmission is significantly smaller (~1-3%) in women with NPI.[68] However, it has been well established that cCMV infected children born post non-primary maternal CMV infection have a similar risk to develop severe abnormalities as in the case of a primary infection during pregnancy. [69,70]

4.1 Perinatal transmission

When discussing postnatal or perinatal transmission, the two main routes implied are intrapartum (during labor) and through breast milk.74 Even though transuterine infection is the most dangerous to the growing fetus, postnatal transmission is the most important route in terms of global impact on the population prevalence of CMV. This is because neonates who conducted the infection during or soon after birth, start shedding the virus between the age of 3 and 6 weeks and continue to do so for several years.[71] This is critical, as these infants will shed the virus in saliva and urine while being in close contact to their caregivers and other children in pre-school settings. This in turn increases the prevalence of CMV in the population but more

importantly the seroprevalence of women of childbearing age who then transmit the virus to the fetus leading to congenital infection.

4.2 Intrapartum

As the newborn passes through the birth canal, the CMV virus can be transmitted to the newborn. This occurs because in seropositive women, CMV is present in the cervix. During labour, cervicovaginal shedding of the virus is enhanced, which is likely responsible for transmission of the virus during this stage.[71,72] An important study supporting this argument, showed that 50% of neonates born to mothers with a positive CMV vaginal culture, had conducted the infection.[71] Nevertheless, as the infection conducted at this stage is not congenital, the effects of CMV on the otherwise healthy newborn are usually minimal with no long-term abnormalities.[73]

However, more invasive disease phenotypes do exist, with evidence showing that they are related to the degree of viral excretion, which in turn represents viral replication. An example in which this is depicted, is in the case of a maternal concomitant HIV infection.[74] In cases where HIV infection coexists, viral replication is enhanced in the background of immunosuppression, which increases the risk of transmission.[74] Poor control of AIDS and low CD4 counts are contributing factors in the process of viral shedding, and neonates born under such conditions tend to develop developmental abnormalities even if they do not have a congenital infection.[74] Extremely premature neonates, who are more vulnerable and prone to infection, are also at risk of neurodevelopmental complications even if infected after birth.

4.3 Breastfeeding

The fact that the CMV infection and period of shedding is commonly asymptomatic, means that people are usually unaware of the infection.[75] As people do not take the appropriate precautions to minimize the risk of transmission, this makes it easier for the virus to be transmitted through contact of bodily fluids such as breastfeeding.

Viral culture analyses and PCR have shown an association between lactation and reactivation of the CMV virus, as well as viral shedding through breast milk occurring in over 50% of seropositive women.[76,77] Various studies investigating the

transmission rates from mothers with a positive breastmilk viral culture to their nursing infants, have reported them to vary between 40% and 60%[78,79,80]. Nevertheless, as with intrapartum transmission, acquiring the CMV virus postnatally has not been commonly associated with problems in the healthy newborn at term. When weighing the undoubted benefits of breastfeeding against the risk of conducting the virus at this stage, there is no recommendation against breastfeeding in seropositive women.[81]

The recommendation changes when dealing with more complicated cases such as preterms, immunocompromised newborns or those with low birth weight. These cases have been linked to a more severe form of infection ranging from mild neutropenia to a disease that can become life threatening.[76,82] Efforts are being made with regards to potentially eliminating CMV viral activity from breast milk without removing its nutrients.[83,84] However as the precise conditions in which this could be successfully achieved are yet to be determined. Since the recommendations about breastfeeding very low birth weight (VLBW) and premature infants born to CMV-seropositive mothers are unclear, breastfeeding in these cases should be extensively discussed with a specialist [85]

5.0 Clinical presentation

5.1 Adults

CMV infection in the otherwise healthy host usually ranges from being clinically inapparent to fairly mild. The virus has an incubation period of up to two months and commonly presents with a non-severe flu-like illness with symptoms such as a sore throat, a fever and muscle aches. However, even in the immunocompetent adult, a primary infection can manifest in a more severe manner presenting with a mononucleosis type syndrome.[86,87] In such cases lymphadenopathy, splenomegaly, extreme fatigue, lymphocytosis and even hepatitis are the predominant clinical features.[88] The most likely clinical situations in which CMV is diagnosed is upon investigation for a non-EBV infectious mononucleosis syndrome, a presentation of acute hepatitis or a sudden deterioration of the immunocompromised host (i.e HIV positive and transplant recipients). Evidence shows that approximately 5 to 7 percent of mononucleosis cases are caused by CMV.[89] More specifically, in an older but large

study of 494 patients with infectious mononucleosis, 73 patients tested negative for a heterophile antibody test ruling out EBV. Of those 50% were CMV positive, indicating that CMV is not uncommon in this scenario and should always be investigated. [90] Even though it is expected for the CMV virus to attack multiple vital organs in the immunocompromised host, various less common sites of infection have been also witnessed in the immunocompetent adult such as the lungs, bowel, vasculature, eyes, kidneys, adrenals, pancreas and more. Of those the most frequently encountered are the lung and bowel.[91,92] CMV has been shown to have been the culprit for cases of colitis and severe community acquired pneumonia (CAP) usually requiring hospitalisation. Less severe cases of CMV pneumonia have been described but are likely to be missed due to potential unavailability of sensitive molecular diagnostic tests (i.e PCR) or due to mislabeling of the pathogen as something more common, such as influenza or adenovirus.[93] In any case once the most common causes have been excluded, CMV should be considered in order to weigh the risks and benefits of offering the appropriate antiviral therapy commonly. [94,95] . Table 1 below adapted from Cunha et al, 2009 depicts some of the sites of CMV involvement and clinical features in the immunocompetent host.[92]

Common Sites of CMV Involvement	Clinical Features	Uncommon Sites of CMV Involvement	Clinical Features
Lung	Severe CAP	Kidney	CMV viruria
Liver	Increased serum transaminases (AST/ALT)	Adrenals	Adrenalitis
Spleen	Splenomegaly	Salivary glands	Sialitis
Gastrointestinal tract	Segmental/pancolitis Colitis	Pancreas	Pancreatitis
Central nervous system	Encephalitis[a]	Esophagus	Esophageal ulcers Esophagitis
Hematologic	Leukopenia Relative lymphopenia Atypical lymphocytes Thrombocytopenia Aplastic anemia Increased procoagulant activity	—	—
Multisystem involvement	FUO	—	—

Table 1. CMV spectrum of infection: preferred organ involvement in immunocompetent patients. Adapted from Kunha et al, 2009[92]

In contrast to the less recognised manifestation of the virus on the immunocompetent host, the morbidity and mortality caused by CMV on the immunocompromised host has been extensively studied. Disease manifestations vary extensively depending on the patient's history and level of immunosuppression and can occur due to primary infection, reactivation of a latent virus or acquisition of CMV through blood products and transplanted organs. Dissemination of the virus in those patients commonly leads to a life-threatening disease due to multisystem involvement and extensive organ damage.

5.2 Children

5.2.1 Acquired perinatal infection in term infants.

As previously discussed perinatal non-congenital infection can be acquired during birth, through breastmilk or through the transfusion of blood products.[96,97] Since the incubation period for a perinatal infection begins after the first 4 weeks of life, confirmation of the absence of congenital infection, requires a negative PCR of urine or saliva in the first two weeks of life. Even though the amount of viral shedding in perinatal cases is less than in congenital cases, it becomes chronically established and persists for years. [98]

Term healthy infants with an acquired perinatal infection usually remain asymptomatic and do not seem to face the risk of neurodevelopmental abnormalities. This is probably due to the presence of maternal anti-CMV antibodies which are transferred to the fetus through the placenta and are proportional to maturation and GA.[99] Nevertheless, case of mild hepatitis, mild pneumonitis and blood count abnormalities have been documented even at term.[100,101] Isolated mildly deranged liver function tests are more common but usually resolve within 3 to 6 months and frequently remain unnoticed. [102]

5.2.2 Acquired perinatal infection in preterm infants

Premature infants, those with very low birth weights (< 1500g) or sick term infants have been reported to be more susceptible to infections and their consequences.[103] These infants can develop a wide range of clinical manifestations, the more

immediate of which are neutropenia, hepatosplenomegaly, thrombocytopenia and even sepsis in 15% of cases which can lead to death.[104] A recent study also identified a potential association between preterm infants with acquired CMV and spontaneous gastrointestinal perforation when compared to controls (13% vs 2%, respectively).[105] In 1979, Ballard et al described a syndrome of post-transfusion CMV infection in premature infants presenting with worsening respiratory function, hepatosplenomegaly, hemolytic anemia and blood count abnormalities.[106] However, the rates of this have greatly decreased due to the use of seronegative blood or leukocyte reduction techniques.

When a symptomatic postnatal infection is present it usually becomes apparent between 35 and 60 days of life, however a debate exists regarding the ability of early postnatal infection to mimic congenital infection and cause long-term abnormalities.[105,107] It is suggested that if long-term damage is possible it is usually small.[108,109]

Such abnormalities involve sensorineural hearing loss, chorioretinitis, neuromuscular impairments and learning difficulties. [110]

5.2.3 Acquired infection in immunocompetent children

An acquired CMV infection in healthy children or adolescents does not commonly lead to a symptomatic disease. Nevertheless there is always a small percentage of cases which can have a more severe presentation. As with adults, the infection can cause a mononucleosis syndrome with a negative monospot test presenting with fever, lymphadenopathy, hepatosplenomegaly and deranged transaminases. [86] Even more rare are cases of pneumonitis, myocarditis, encephalitis and Guillain-Barré syndrome.[111]

5.2.4 Acquired infection in immunocompromised children.

Due to the weaker or diminished cell-mediated immunity, immunocompromised children and adolescents are at higher risk of experiencing a more aggressive form of the CMV infection.[112] This group of patients typically involves children with congenital or acquired immunodeficiencies (i.e SCID, HIV), children undergoing iatrogenic immunosuppression due to receival of transplanted organs or

hematopoietic stem cells, and those suffering from malignancies receiving chemotherapy.[113] Symptoms and clinical outcome depends on the underlying condition causing the immunosuppression, but malaise, fever, neutropenia, and elevated transaminases are almost universal. Organ donor recipients infected with CMV frequently experience complications such as acute graft rejection.[114], Depending on the transplanted organ involved, CMV is associated with organ specific effects such as pneumonitis in lung, hepatitis and colitis in liver or myocarditis and coronary artery disease in heart transplants.[115,116]

Chapter 2 : Congenital CMV infection

2.1 Epidemiology of cCMV

Congenital CMV (cCMV) infection is the commonest congenital infection. Even though prevalence varies considerably among different study populations, the prevalence ranges from 0.5 to 2.5 %, with 40.000 neonates born with the infection annually in the US.[117,118] The level of maternal seroprevalence reflects and determines the prevalence of primary CMV infection during pregnancy, which is approximately 1-2% in the western world. This is different to the annual risk of a previously seronegative woman acquiring a primary CMV infection, which has been calculated as 5.9% in the US.[36]

2.1.1 Ethnicity, Race and Socioeconomic status

Regarding ethnicity and race, numerous studies are consistent in showing that they have a definite influence on seroprevalence when controlling for factors such as income, education, area of residence or birth, family size and medical insurance.[4] Seroprevalence among the non-white populations has been measured to be roughly 25% higher than in white populations (summary PR ¼ 1.59, 95% CI ¼ 1.57–1.61), and has been even calculated to almost 100% in some of the non-white groups.[74,39,119] Furthermore, epidemiological data from the United States, demonstrate that seroprevalence is higher in non-Hispanic blacks and Mexican Americans that in non-hispanic whites.[46,120]

The incidence of CMV infection differs between countries and socioeconomic backgrounds. In more developed countries the prevalence is lower in comparison to developing countries. This difference is highlighted when looking at the prevalence of CMV in children. Childhood seropositivity is described as up to 95% in countries like subSaharan Africa and south America as opposed to under 20% in the UK or certain areas of the US (figure 4).[121]

Differences of seroprevalence among socioeconomic backgrounds also become very obvious when looking at the variability of seroprevalence within areas of the same city or country (figure 4).[121] Unsurprisingly, the prevalence of CMV has been found to be 15–25% higher among people living in poor areas, in low-income households with crowded conditions.[34,46,86]

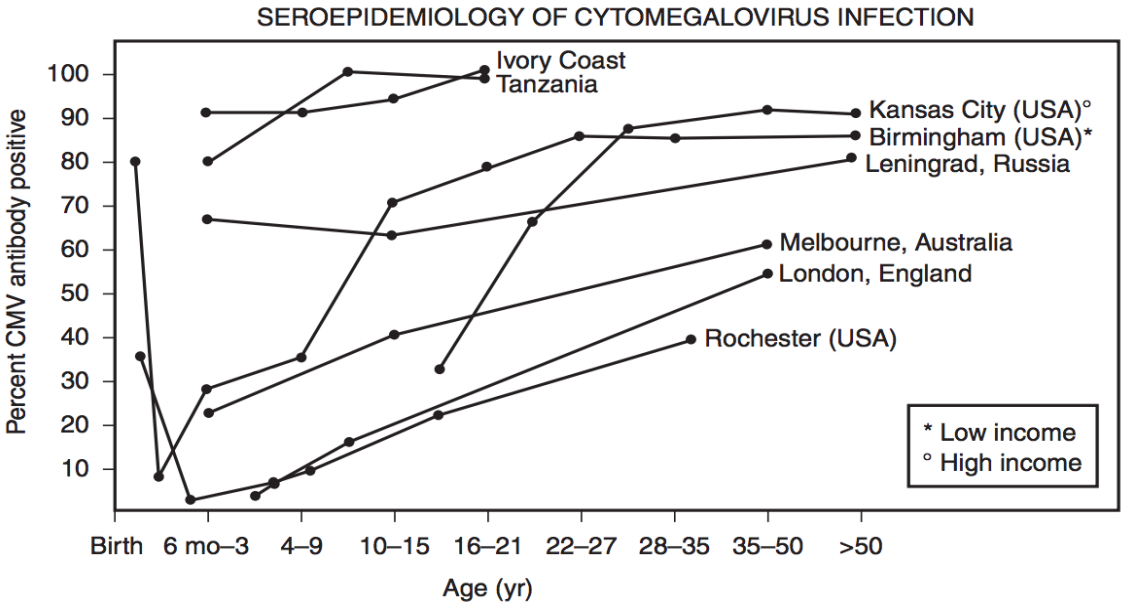


Figure 4. Age-related prevalence of antibodies to cytomegalovirus in various populations. Adapted from Nahmias AJ et al, 1981[121]

2.1.2 Maternal Seroprevalence

With CMV being the leading infectious cause of congenital disease, it is important to recognise the major role of women in the transmission of the virus. We can anticipate that the incidence of congenital CMV within a population, reflects prevalence of maternal seropositivity as well as the amount of viral reservoir in the area. When high rates of seroprevalence exist within a population, the incidence of primary infection, reinfection or reactivation of the virus is higher and therefore a pregnant woman is more likely to come into contact with a CMV-infected person.[122] This increases the risk of in- utero transmission from the mother to the fetus which then leads to a higher incidence of congenital CMV infection. [123] The effect of socioeconomic status, ethnicity and development of one's country on maternal seroprevalence is depicted in multiple studies, where seropositivity is shown to be much higher in countries like subSaharan Africa, India and south America (figure 5).[122]

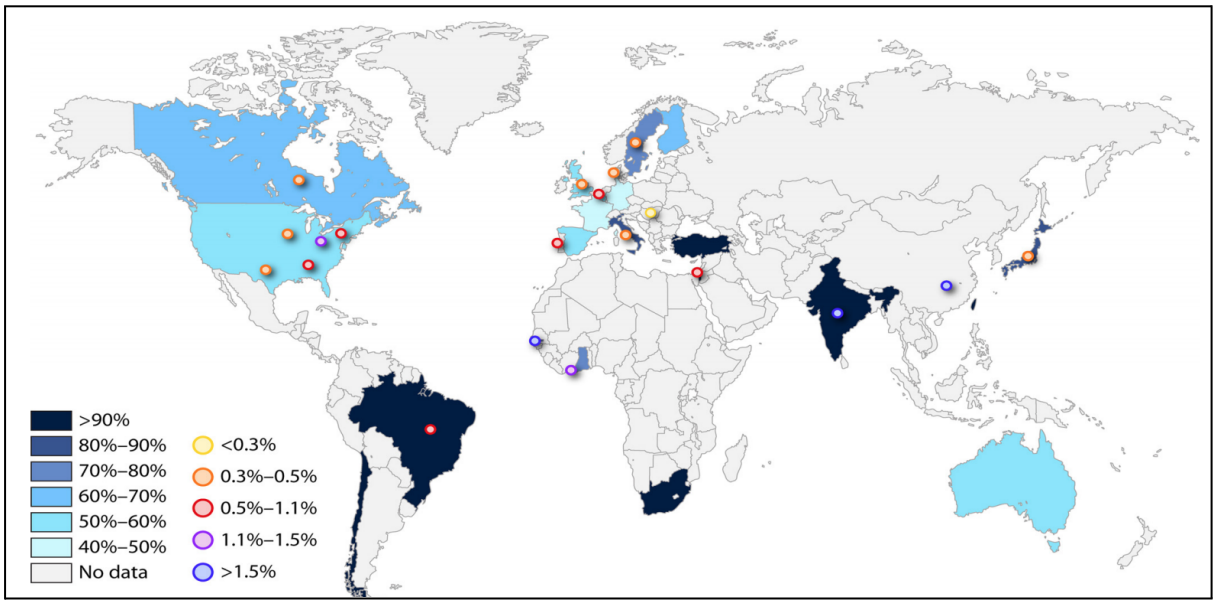


Figure 5. Worldwide CMV seroprevalence rates among women of reproductive age and birth prevalence of congenital CMV infection. Adapted from Cannon et al, 2010[39]

For years there has been global underreporting of congenital cytomegalovirus (CMV) probably due to the limited availability of testing in low- and middle-income countries (LMICs), as well as the absence of systematic testing in countries across all income levels. The existing guidelines for managing moderate to severe symptomatic cCMV recommend a 6-month course of valganciclovir. However, the role of valganciclovir in lower income countries is constrained by both safety and cost implications[124]. Consequently, children who are at higher risk of having cCMV are also the ones with limited access to appropriate treatment options. Furthermore, children in LMICs are less likely to undergo routine developmental and hearing screenings and may face challenges in accessing necessary interventions if deficiencies are identified in these areas. As a result, addressing the issue of cCMV in LMICs remains a critical unmet public health need. In 2021, a very important systematic review and meta-analysis by Ssentogo et al was published, demonstrating a 3 -fold burden of infection in LMICs compared to higher income countries (HICs). Specifically their analysis (figure 6) revealed that the factors significantly associated with cCMV prevalence were LMICs, higher maternal seroprevalence, higher population-level HIV prevalence, and younger maternal age. Temporal trend analysis showed that cCMV prevalence rate has remained unchanged for the past 60 years (figure 7) [1].

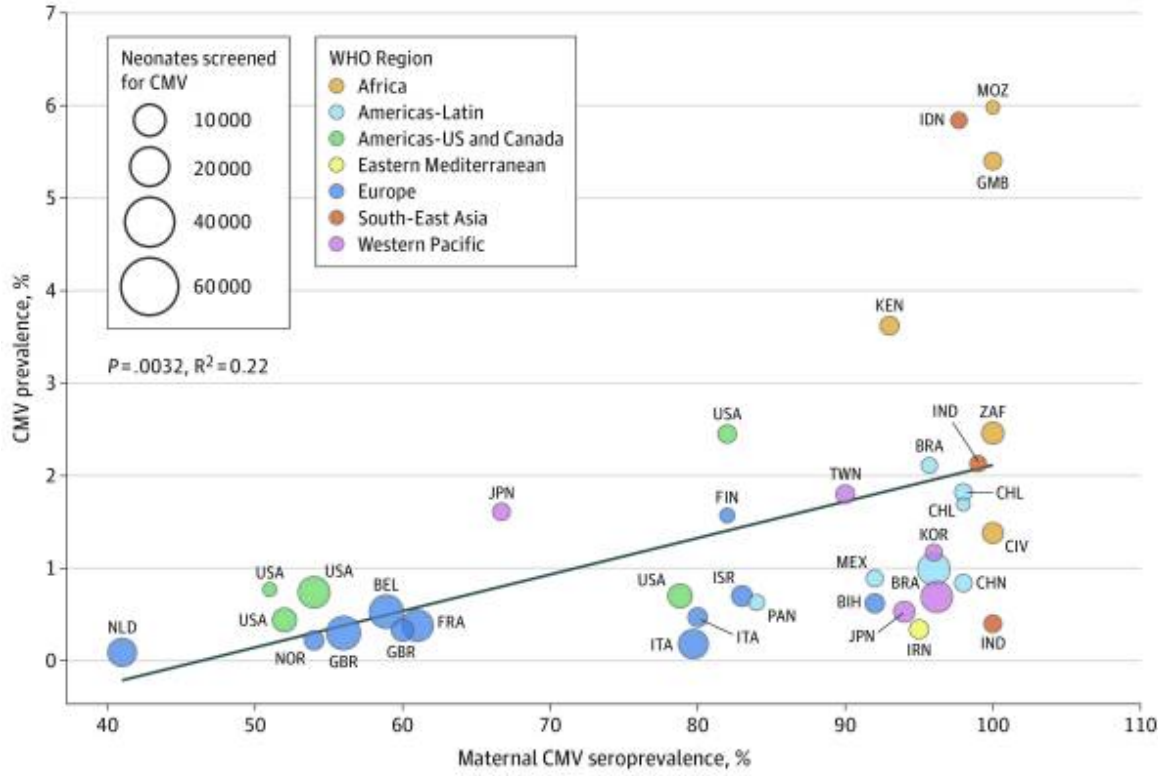


Figure 6. Correlation between maternal CMV seroprevalence and congenital CMV. Adapted from Ssentogo et al[1]

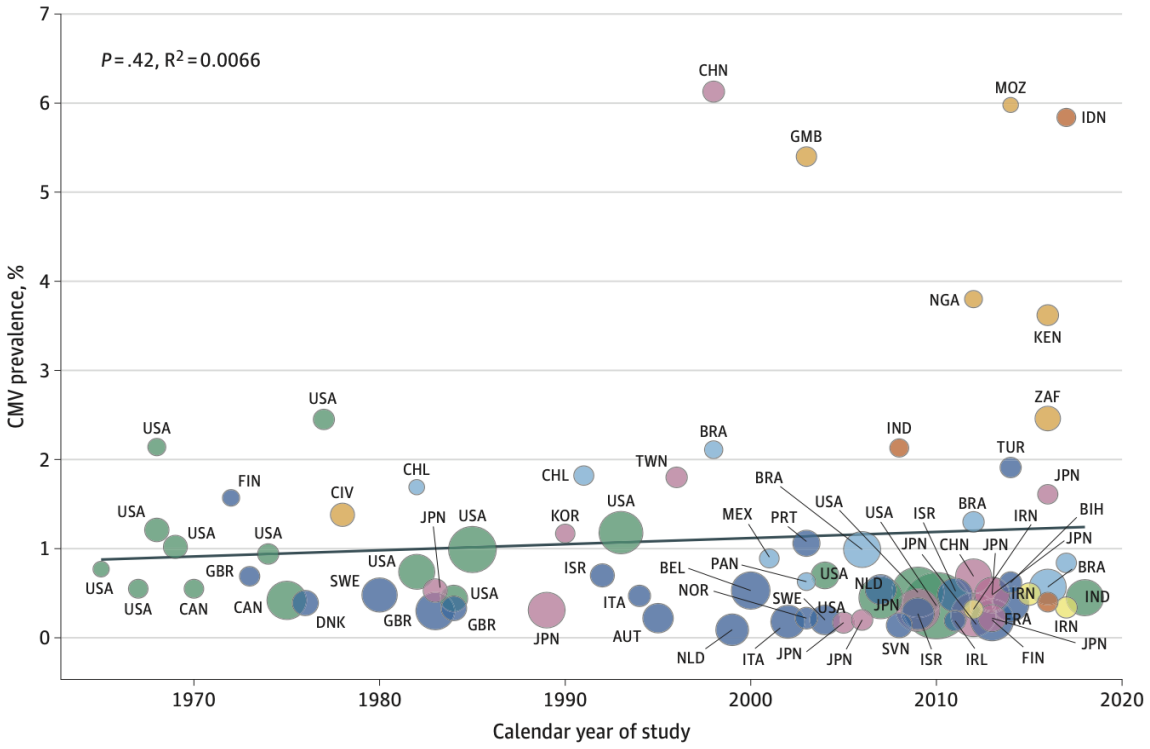


Figure 7. The prevalence of congenital CMV over time. Adapted from Ssentogo et al[1]

This leads to a significant gap in the detection and reporting of congenital CMV cases on a worldwide scale.It has been estimated that in industrialized countries, only 50% of women between the ages of 12-49 are seronegative which translates to a rate of up to 5% in CMV births per year.[33,125] These numbers are greatly reduced when it comes to areas of low maternal seroprevalence, where the rate of congenital CMV infection is approximately 0.6%.[47] As expected, a large variety of epidemiological patterns of congenital infections exist around the world, depending on the maternal ethnic, racial and socioeconomic background.[126] Given that a congenital disease could be the cause of severe neurodevelopmental consequences, educating the people at risk on the existence of congenital CMV infection, the likelihood of reactivation and reinfection by the virus, and simple methods of prevention is of vital public health importance.

2.2 Transmission mechanisms of congenital CMV infection

CMV can be transmitted transplacentally to the fetus leading to congenital CMV infection. Efforts are being made through the use of animal models in order to elucidate the complexity of the viral invasion through the placental barrier. However, the exact mechanism by which the virus is transmitted to the fetus is yet to be determined. Nevertheless, understanding the fundamentals of placental immunology is essential in order to comprehend how CMV crosses the placental barrier. The decidua contains maternal leukocytes, while the chorionic villi contain fetal macrophages called Hofbauer cells[127]. During the first trimester, a significant proportion of decidual cells consist of different types of leukocytes, including uterine natural killer (uNK) cells, macrophages, dendritic cells, and T lymphocytes[128]. Among these, uNK cells are the most abundant placental leukocytes early in pregnancy and play a crucial role in remodeling spiral arteries for proper placental development. CMV replication occurs in the decidua in humans, and the initial defense against CMV involves decidual CD8+ effector memory T cells and natural killer (NK) cells. In cases of maternal viral infection, uNK cells primarily restrict the spread of CMV into the fetal circulation, which explains the low rate of vertical transmission during the first trimester[129]. Essentially, the placenta's immune function varies throughout gestational time and in response to primary CMV infection.

In the first trimester, uterine NK cells (uNK) play a vital role in remodeling spiral arteries and protecting against viral infections that could infiltrate the chorionic villi. As pregnancy progresses into the second and third trimesters, the number of uNK cells decreases, rendering the placenta more susceptible to primary CMV infection. We know that 30-40% of vertical transmission cases occur during the first two trimesters, compared to approximately 70% in the third trimester[130]. When exposed to viral pathogens, Hofbauer cells become polarized and release pro-inflammatory cytokines, attracting peripheral immune cells to the infection site and triggering placental inflammation[131]. This process may cause damage to the placenta and potentially compromise the placental barrier allowing for viral invasion (figure 8).

The inflammatory and immune response in its turn weakens the blood brain barrier causing infection of neuronal progenitor cells (i.e astrocytes). The infection of progenitor cells leads to impaired differentiation, maturation and migration and eventually causes damage to the susceptible developing brain (figure 9) [132].

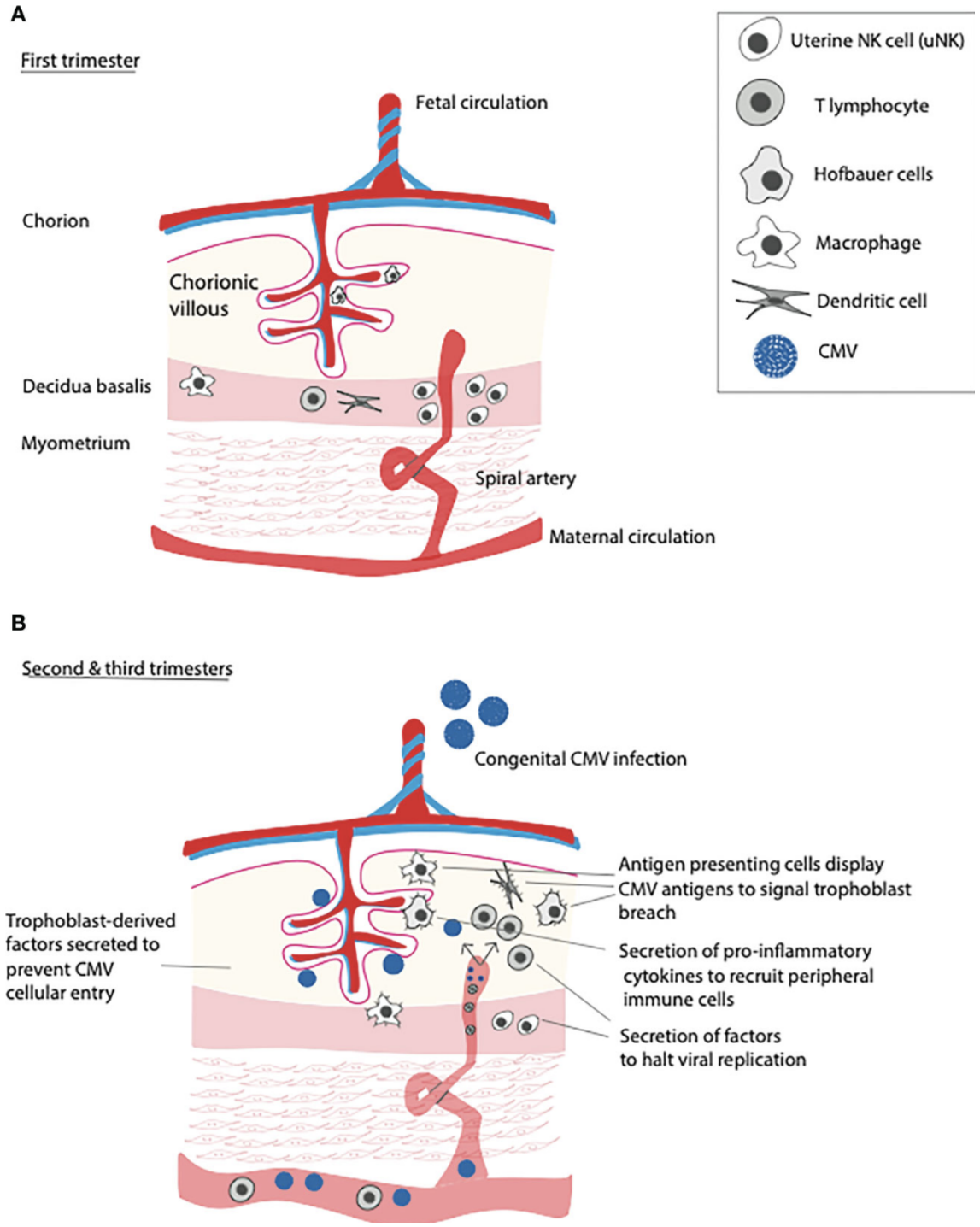


Figure 8. Placental Immune Function Modulation during Gestational Time and in Response to Primary CMV Infection. Adapted from Kirschen and Burd, 2023[133]

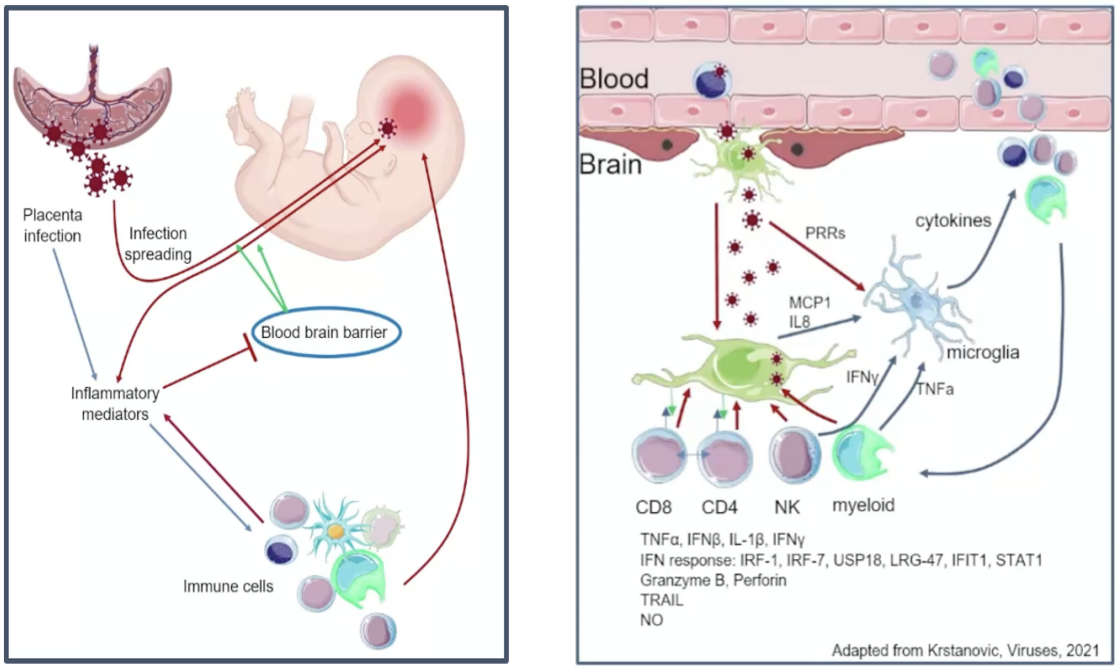


Figure 9. Immunological response during transplacental transmission affecting blood brain barrier. Adapted from Krstanovic, Viruses, 2021

2.3 Primary vs. Non-primary maternal infection

During pregnancy, when the mother experiences a primary CMV infection, a congenital fetal infection occurs in 30-40% of cases.[134] Of those affected, roughly 10-15% will be symptomatic at birth whereas 10% of the initially asymptomatic neonates will go on to develop neurological sequelae later in childhood. [135] It is important to note the cCMV can occur as a result of both maternal primary and non-primary infection (secondary infection). Women with a latent CMV infection who are seropositive prior to conception, can experience a secondary infection from reactivation of the latent virus or reinfection with a new viral strain.[136] Despite the pre existing notion that maternal immunity does not pose a risk to the fetus, the opposite has been recently discovered. More precisely, even though the exact rate of CMV transmission following a secondary maternal infection is unknown, it is estimated that at least 50% of congenital infections are related to non-primary infections. With regards to symptomatic cCMV disease, the risk is indeed smaller with secondary

infection, however, when long-term abnormalities do occur, they can be of equal severity.[68,137,138]

Furthermore, an ongoing debate exists regarding the risk of intrauterine transmission in each trimester. Up till recently it was thought that rates of transmission increase with gestational age meaning that they are lower during the first trimester of pregnancy, approximately 20%, and close to 70% during the third.[139,140] Nevertheless, some of the more recent studies show a close to equal rate of transmission throughout pregnancy, with the exception of the first 2 weeks where the risk of transmission is very low. [141,142]

Despite the lower transmission rates implicated in early pregnancy, both the risk and severity of neurological damage and hearing loss are much higher.

Looking into other risk factors for primary maternal infection, a study investigated the risk of primary CMV in the first versus second pregnancy. Data showed that women who are seronegative during their first pregnancy and conceive again within 2 years have a 19-fold greater risk of primary infection in the second pregnancy.[36,143] This indicates that the mother's older child is the most likely source of infection. Furthermore, studies have demonstrated that the shorter the interval between each pregnancy the higher the risk of congenital CMV infection, and that those fetuses have a 5-fold risk of experiencing any associated sequelae. [143,36] This was also shown to be the case even when women were seropositive during their first pregnancy, which raises suspicion that in those cases, reinfection with a new viral strain could be involved.

Finally, a French study illustrated that each of the two types of maternal infection (primary vs. non-primary) is associated with a different high risk socio demographic group, with regards to transmitting CMV to the growing fetus. Young maternal age was shown to be a risk factor for congenital disease in the background of both primary and non-primary infection. Unexpectedly, results showed that the risk of cCMV following a maternal primary infection was higher in parous women from higher income backgrounds. On the other hand, when it came to cCMV following a secondary infection, women from lower socioeconomic backgrounds were more at risk in transmitting the virus. [138]

Identifying the high risk group of mothers who are more likely to transmit CMV transplacentally to their fetus is extremely valuable as it could encourage healthcare professionals to screen them for CMV prenatally, and educate them on simple hygiene preventative measures to minimize their exposure. [144]

2.4 Clinical presentation of cCMV

In the majority of cases, congenital CMV is a subclinical infection with no long-term effects on the growing child. However, in 15 to 20% of cases, the infection becomes symptomatic with a wide range of hematological and neurodevelopmental abnormalities. It is important to emphasize that 10-15% of the initially asymptomatic newborns will go on to develop permanent sequelae later on in childhood.[4] This means that the majority of affected infants will not be identified through clinical examination at birth. This common absence of symptoms both for the pregnant mother and newborn means that there is a large group of symptomatic children who are not promptly diagnosed and therefore miss the opportunity for an early therapeutic intervention.

Congenital CMV is characterized by its multisystem involvement and its clinical presentation ranges from completely asymptomatic to severe life-threatening disseminated disease commonly referred to as cytomegalovirus inclusion disease (CID).[67] When the disease becomes symptomatic, common clinical and laboratory findings at birth include microcephaly, hepatosplenomegaly, jaundice, petechiae, haemolytic anemia, thrombocytopenia and deranged liver function tests such as elevated transaminases or conjugated hyperbilirubinemia.[38,145] These are usually transient abnormalities which resolve within the first year of life. However, when the CNS is involved, the clinical features tend to be more disabling and permanent.[67]

The most common long-term sequelae related to cCMV is sensorineural hearing loss (SNHL) which can be unilateral or bilateral as well as fluctuate in severity in 50% of cases.[5,146]More specifically, congenital CMV is considered the most common cause of infectious hearing loss and can be present at birth or develop at a later stage before the age of 5 years.[34]

Other features related to CMV fetal injury involve loss of vision related to chorioretinitis, optic atrophy, and strabismus.[147] Similarly to SNHL, chorioretinitis can

be absent at birth and develop insidiously in the first years of life making it more difficult to manage. Neurodevelopmental abnormalities of variable degree include motor deficits, learning disability, seizures and mental instability. Less prominent but possible are ataxia, hypotonia, behavioral problems or even an autistic type disorder. [148]

In a small percentage of cases, intrauterine infection with CMV can complicate the progression of the pregnancy itself. It has been shown to cause chorioamnionitis, leading to preterm birth, intrauterine growth restriction, and even stillbirth or neonatal death. [148]

Nevertheless, the precise prevalence of symptomatic congenital CMV infection is difficult to determine. The great variability of clinical features have contributed to the absence of a universal definition for symptomatic infection. Currently trying to categorize the disease into “symptomatic” vs “asymptomatic” has become less meaningful as we are moving towards a new approach which underlines the importance of creating a diagnostic scale of cCMV using different biomarkers. Such biomarkers involve clinical, laboratory and neuroimaging findings which can be used to assess the severity of infection and manage the patient accordingly.[149]

Recently two articles were published with expert consensus recommendations on prevention, diagnosis and therapy of cCMV, which clinically categorized the infection into the four following categories: 1) Asymptomatic congenital infection, 2) Asymptomatic congenital infection with isolated sensorineural hearing loss, 3) Mildly symptomatic congenital disease and 4) Moderately to severely symptomatic congenital CMV disease.[124] Mildly symptomatic disease (3) involves the presence of one or two transient manifestations occurring in isolation (i.e hepatomegaly, thrombocytopenia) whereas moderately to severely symptomatic disease (4) refers to multiple extracerebral transient manifestations or CNS long-term involvement. Severe disease also involved the presence of neuroimaging abnormalities such as as calcifications, moderate to severe ventriculomegaly, cysts, white matter changes, cerebral or cerebellar hypoplasia, hippocampal dysplasia, neuronal migration abnormalities[149]

Despite the relatively high prevalence of the virus in the community and potentially detrimental effects to the fetus, cCMV is still greatly underrecognized.[122] The great

variability of clinical manifestations, the common absence of clinical features at birth even in severe cases and lack of effective therapy, have contributed to a tendency of neglect on this matter.[150] Importantly, medical professionals tend to not screen for CMV prenatally and fail to educate pregnant women on the existence of congenital CMV, the risks to the fetus and the measures they can take to minimize primary infection or reinfection.[151] This underlines the urgency for a change in perspective on this matter within the medical community and the need of implementation of a universal screening programme for congenital CMV.

2.5 Diagnosis

2.5.1 Prenatal

Despite the extent of cCMV underecognition, the infection can be diagnosed both prenatally and postnatally with high accuracy. During pregnancy, the gold standard for identifying a cCMV infection is amniocentesis, a procedure which can be technically performed from 15 weeks gestation onwards. Amniotic fluid is used to perform CMV DNA polymerase chain reaction (PCR) with a sensitivity and specificity ranging between 80-100%.[152,153] The positive predictive value (PPV) is close to 100% whereas the negative predictive value (NPV) is 81%.[154]

The accuracy of PCR to detect cCMV however, is affected by the timing of the procedure. Various studies investigating the most appropriate time for amniocentesis have emphasized the importance of allowing time for fetal extraction of the virus.[155,156] More specifically, it usually takes approximately 6-8 weeks from maternal seroconversion to the establishment of placental infection and viral detection in the amniotic fluid.Therefore, even though an early first trimester infection can be detected prior to 21 weeks gestation, the sensitivity can be as low as 45% at this stage and thus a negative result cannot exclude the presence of the infection.[152]

Cases exist where the transplacental passage of the virus is delayed up to 19 weeks after maternal primary infection, occurring within the late second or early third trimester.[157] These cases are tricky since the infection can be easily missed even with a delayed amniocentesis. However, as mentioned earlier, an intrauterine infection occurring in advanced gestational age is very unlikely to cause long-term sequelae.[6] Nevertheless, the latest consensus on prenatal diagnosis states that

amniocentesis should be ideally performed after 20-21 weeks gestation with a sensitivity of 85-95% when fetal urination is established or at least 6-8 weeks after confirmation of maternal seroconversion.[124]

Even though amniocentesis is the gold standard method for diagnosis, it carries a risk of various complications such as rupturing of membranes, fetal injury and missacariage in a very small percentage of cases estimated around 0.2-0.3 %.[158] Finally, some have suggested the performance of PCR in maternal blood prior to amniocentesis.[157] The rationale behind this is to avoid the risk of accidental viral inoculation to a non-infected fetus. However maternal CMV blood PCR has been shown to be negative in a substantial percentage of cases where the fetus was actually infected, resulting in misleading parental reassurance.[159]

2.5.2 Postnatal

When suspicion for congenital infection is present due to known maternal infection or abnormalities seen in fetal US, post-natal testing is also recommended to confirm the diagnosis. As previously mentioned, a non-congenital infection in the neonatal period becomes apparent after the first few weeks of life due to viral shedding occurring from 3-6 weeks onwards.[155] On the contrary, a congenitally infected infant is shedding the virus at birth. Congenital CMV is therefore most accurately diagnosed when a positive test occurs in the first three weeks of life.[155] The sooner the testing is performed the more reliable it is in differentiating between an intrauterine infection and a neonatal infection occurring after birth. If this initial time period is missed, it is usually impossible to differentiate between the two, especially when maternal serostatus during pregnancy was not recorded.

Up until recently, the gold standard for diagnosing cCMV in neonates had been viral culture of urine and saliva specimens.[160] However, in the last decade, studies have compared the traditional method of viral culture to the use of real-time PCR showing excellent results with regards to PCR. When correctly timed, PCR sensitivity (93-100%) and specificity (100%) has been shown to even surpass that of viral culture.[48,161] However, due to the hypersensitivity of PCR, the possibility of false-positives do exist. Therefore the combination of PCR and viral culture or any other molecular test is optimal to confirm a diagnosis. [162]

When comparing these two tests in aspects other than sensitivity or specificity, PCR is faster, allowing for viral identification within 24 hours while simultaneously being less expensive compared to viral culture.[163] More specifically, the use of saliva specimens, either air dried or in viral transport medium, which are easier to collect than urine make the procedure even easier to perform.[164] These results have lead to the recent consensus recommending the use of urine or saliva PCR as the new gold standard for identifying cCMV at birth.[165]

As an alternative to real-time PCR and potential neonatal screening tool, studies have investigated the use of dried blood spots (DBS) from Guthrie cards which have become standard practise at birth. Although, the rates of CMV DNA detection have been unpleasantly low and therefore the isolated use of DBS was not recommended as a reliable diagnostic method, more recent data have shown that the sensitivity is acceptable and estimated 86%[166]. Therefore, DBS may be used for universal screening while importantly it is very useful in cases of children with delayed presentation of sequelae, where a positive DBS sample could confirm the diagnosis of a congenital infection.

Even though prevention of CMV transmission from the mother to the fetus has not been yet achieved, early perinatal diagnosis is important. Having access to a rapid and reliable diagnostic method such as saliva PCR is vital, as it could become suitable for large scale perinatal screening for cCMV in the future, in order to identify children at risk for severe disease.[164] It can aid parents in preparing for the possibility of an affected child and even used to trigger the increase in frequency and intensity of fetal monitoring. Furthermore, as the vertical transmission rate of CMV is 40% and not 100%, prenatal testing can be used to reassure parents and assist them in the decision making process of a potential termination of pregnancy.

2.6 Screening

2.6.1 Prenatal Screening

In order to decrease the prevalence, severity and burden of congenital CMV disease, screening strategies can be used in various stages, starting from the preconceptual period to the postnatal period. Currently during the prenatal period there are two main opportunities to detect cCMV; 1) Maternal universal screening for CMV antibodies or when maternal flu-like symptoms appear during pregnancy and 2) In the context of abnormal US fetal findings. Even though maternal CMV antenatal screening during pregnancy is offered in some countries such as Israel and Australia, it is not recommended at a universal level. [124,167] The cost-effectiveness, practicality and overall benefit of routine repeated maternal antibody screening during pregnancy is questionable and has not been established in many parts of the world. This is mainly attributed to the unavailability of an intervention that could prevent viral transmission to the fetus, predict disease severity or successfully treat infected newborns at birth.[168] Furthermore, some experts argue that since over 50% of infants with cCMV are born in resource-limited areas or to previously seropositive women, there is only a limited amount of cases who could benefit from such testing. Nevertheless, since maternal infection is usually clinically silent and antibody testing cannot always uncover the origin and timing of the infection, women at higher risk of transmitting the virus to their fetus are easily missed. In clinical practice, the two common reasons to perform antibody testing during pregnancy is when a pregnant woman presents with a flu-like illness or when fetal US depicts abnormalities which could indicate the presence of a congenital infection. [169]

With regards to antibody testing, the presence of CMV IgG positive antibodies in a pregnant woman who tested CMV IgG negative preconceptually, can confidently validate the presence of a primary infection. However, when a preconceptual antibody measurement is not available, the presence of both CMV IgG and CMV IgM antibodies are required but can only suggest a primary infection.[170] The reason behind this is that IgM antibodies can also become detectable in the setting of reinfection or reactivation of the virus and therefore their presence does not purely imply a primary infection.[171]

In cases of uncertainty it is very important to examine the IgG antibody avidity assay. Avidity is a marker of overall stability of the antibody-antigen complex. The longer the time since the first presentation of the antigen to the body, the more mature the antibodies become. Therefore a high IgG avidity in the first trimester signifies an older infection of over 18 weeks and indicates that the infection has occurred preconceptually. [172,173]

However, even with the aid of antibody avidity, there are still challenges that need to be overcome. Firstly, there are cases where the measured avidity does not represent the timing of an infection and it can therefore be falsely low or high. [174] Even commoner is the case where the IgG has an intermediate value, which in the first trimester, cannot differentiate between a preconceptual or early-pregnancy infection. In this scenario, a CMV PCR is performed in maternal blood. Even though a negative result can eliminate the possibility of a primary infection in the last 4 weeks with a 80% sensitivity, a positive result does not confirm a recent infection due to the possibility of a prolonged presence of viral DNA in the blood.[146] In conclusion, the combination of CMV IgG, IgG avidity and IgM testing can aid us in recognising women with a primary infection in majority of cases, however when the results are found in the gray area, the exact time period in which the infection has occurred cannot always be identified, making it hard to assess the risks to the growing fetus, and deciding about treatment, creating anxiety to both the parents and the physicians.[175]

Despite the challenges in identifying the exact timing of CMV infection during pregnancy, the importance of prenatal screening has been recently highlighted after the published results of a recent study examining the efficacy and safety of using valacyclovir in early pregnancy showing optimistic results with fetal infection being lower in the treatment group with valacyclovir (odds ratio, 0.318 (95% CI, 0.120-0.841); P = 0.021)[176].

2.6.2 Neonatal Universal vs Targeted Screening

Neonatal screening is currently of utmost importance in order to identify neonates at risk of long-term sequelae and initiate the appropriate care without delay. The rationale behind the use of neonatal screening lies on the recent evidence regarding therapeutic options that could improve the clinical outcome of severe cases. In 2015, Kimberlin et al. conducted a randomized controlled trial (RCT) of antiviral therapy concluding that a 6-month treatment plan with vangacyclovir starting within 30 days from birth improves hearing and developmental outcomes in the long-term (95% CI).[177] This supports the benefit of early diagnosis and risk stratification of newborns with cCMV. Today a debate is ongoing regarding the concept of universal vs targeted screening for cCMV. Even though cCMV meets most of the criteria for universal screening based on the world health organization (WHO) guidelines, it has still not been implemented in most countries [178]. Universal screening with the use of saliva or urine PCR, would identify all congenitally infected newborns who would benefit from closer follow up and antiviral treatment when deemed appropriate. A recent study in France evaluated the feasibility and effectiveness of universal newborn screening through saliva testing in 15,341 neonates and identified 63 cases of cCMV, 62% of which had no signs of congenital infection at birth or known maternal history and signs of CMV during pregnancy, making it unlikely for them to have been diagnosed on time for any intervention[179]. Furthermore, even in cases where antiviral treatment is not required, newborn screening for cCMV is still calculated as cost-effective under a wide range of assumptions and could offer large net savings by providing focused care to symptomatic cases.[180] However, a high level of skepticism still exists when it comes to universal screening, therefore it is reasonable to suggest targeted screening as a superior and more economically viable alternative.

Targeted screening concerns testing only high risk neonates for cCMV in order to minimize the costs involved in universal screening, but still identify the majority of infected neonates in need of treatment and follow up. Since cCMV is known to cause SNHL, targeted screening for cCMV mainly entails testing newborns who have failed the newborn hearing screening or who are small for gestational age (SGA) indicating intrauterine stress. In countries with established newborn hearing testing, this is an established and feasible practise[181]. However, even though newborn hearing

screening has been adopted by numerous healthcare systems around the world, due to the limited financial resources within the greek national healthcare system (ΕΣΥ), it is only routinely performed by private hospitals and only in a minority of cases in the public hospitals (i.e prolonged exposure to iv aminoglycosides). Importantly, since hearing loss is not the only neurological deficit related to cCMV and can appear in later stages (infancy), hearing tests still fail to identify an important number of initially asymptomatic newborns who will develop cCMV-related disease in the future.[182]

This highlights the importance of exploring other biomarkers at birth which could play a role in cCMV diagnosis and become part of a targeted screening programme. Recently, a lot of research is focusing on neuroimaging such as neonatal US, as a potential powerful tool in identifying those at risk. Nevertheless, one of the major difficulties in reaching a conclusion regarding the clinical significance of US abnormalities, is that most abnormalities seen on a neonatal cerebral scan (i.e periventricular calcifications and migrational defects) are not specific to cCMV but are commonly observed in other pathologies. Therefore finding clinically relevant abnormalities specific to cCMV is a challenging process.

Overall, it is evident that infected newborns with an early diagnosis will benefit from a series of multidisciplinary interventions that will assist their development, improve language skills and minimize potential neurodevelopmental impairment.[183,184]. These findings suggest that implementation of newborn cCMV screening programs is warranted.

2.6.3 LSV

In 1985, Grant et al. first described “branched linear echogenicities” and calcifications (figure 10) in the area of the thalamus and basal ganglia on cerebral sonograms of neonates with congenital infections (TORCH).[185] A few years later in 1987, Teele et al performed post-mosterm histopathological examinations on 4 out of 12 neonates with similar findings. The diagnoses of those 12 infants were: cytomegalovirus infection (5), rubella (2), congenital syphilis (1), trisomy 13 (3) and suspected but not confirmed congenital infection (1)[186].

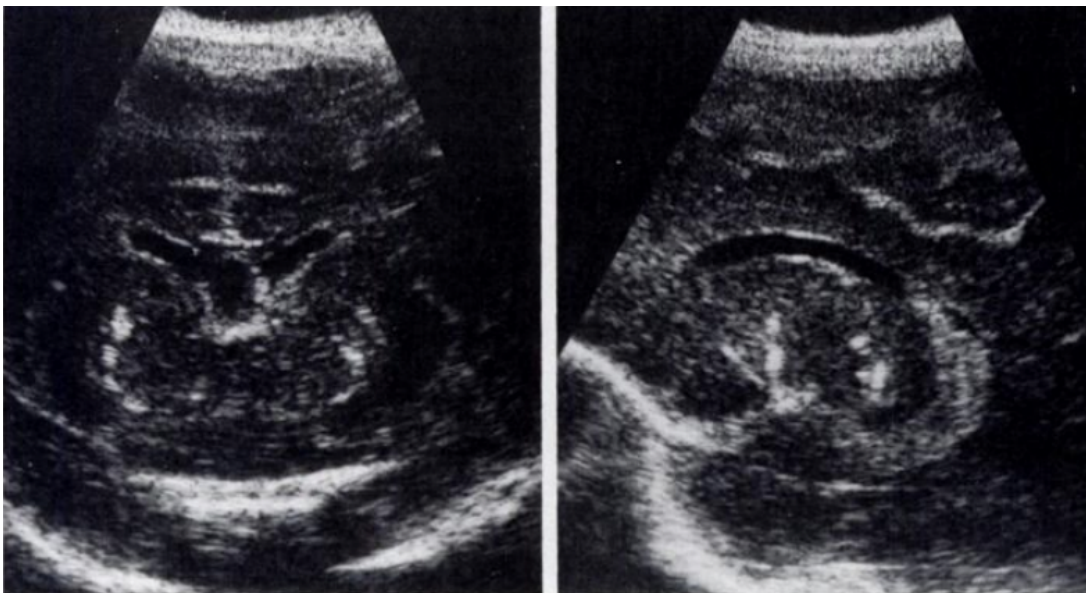


Figure 10. Lenticulostriate vessels in patients with CMV infection. Adapted by Teele et al, 1987 [186]

The pattern of the linear echogenicities seen on the ultrasound was paralleled with the branching pattern of the lateral lenticulostriate arteries arising from the middle cerebral artery, and the medial lenticulostriate arteries arising from the A1 segment of the anterior cerebral artery (figure 11) [187].

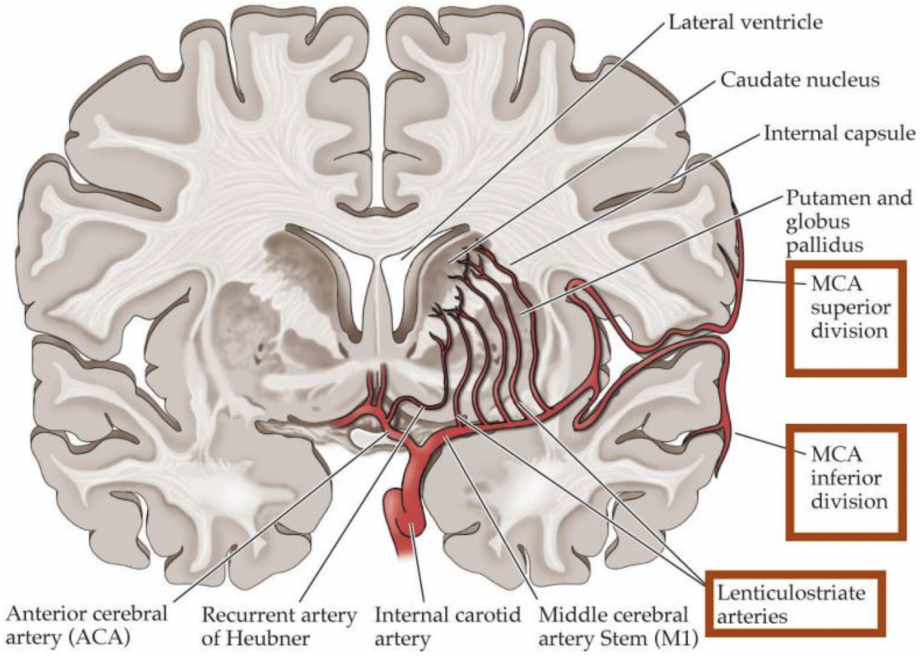


Figure 11. Anatomy of lenticulostriate arteries. Adapted from Sinauer Associates inc. 2002[188]

The histopathological studies in some of these patients demonstrated hypercellular thickening and deposition of amorphous basophilic material and was then described as a “mineralising vasculopathy”[186] (Figure 12). For the first time a relationship between the hyperechogenic lines and a possible underlying vascular pathology was made.

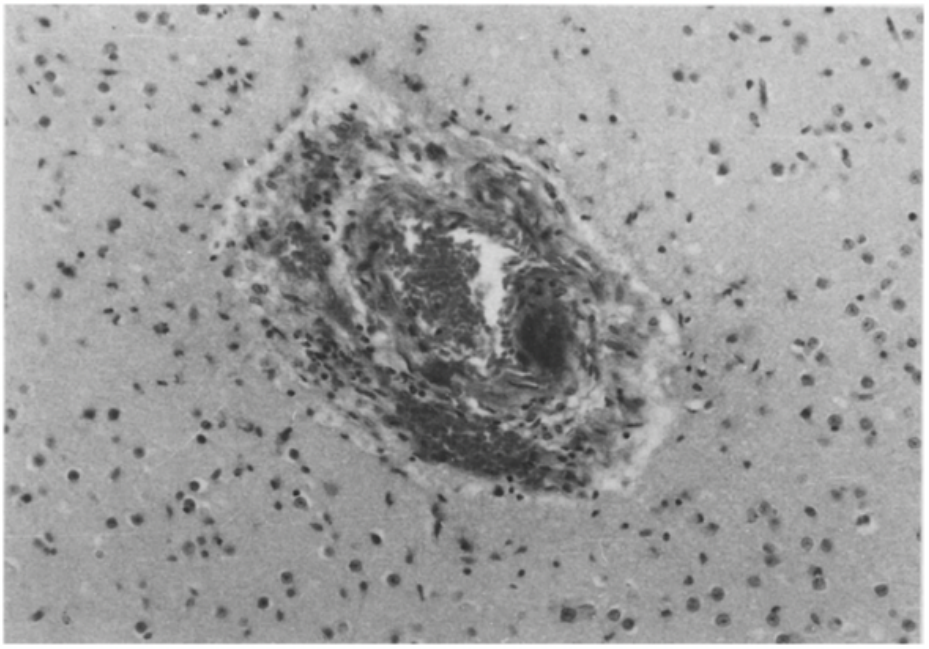


Figure 12. Arterial focal globular basophilic deposits. Adapted by Cabanas et al [189]

Furthermore Doppler Flow Studies (figure 13) of these vessels also reported arterial signals within the hyperechogenic linear findings, supporting the thesis that LSV could indeed represent a vasculopathy [189,190].

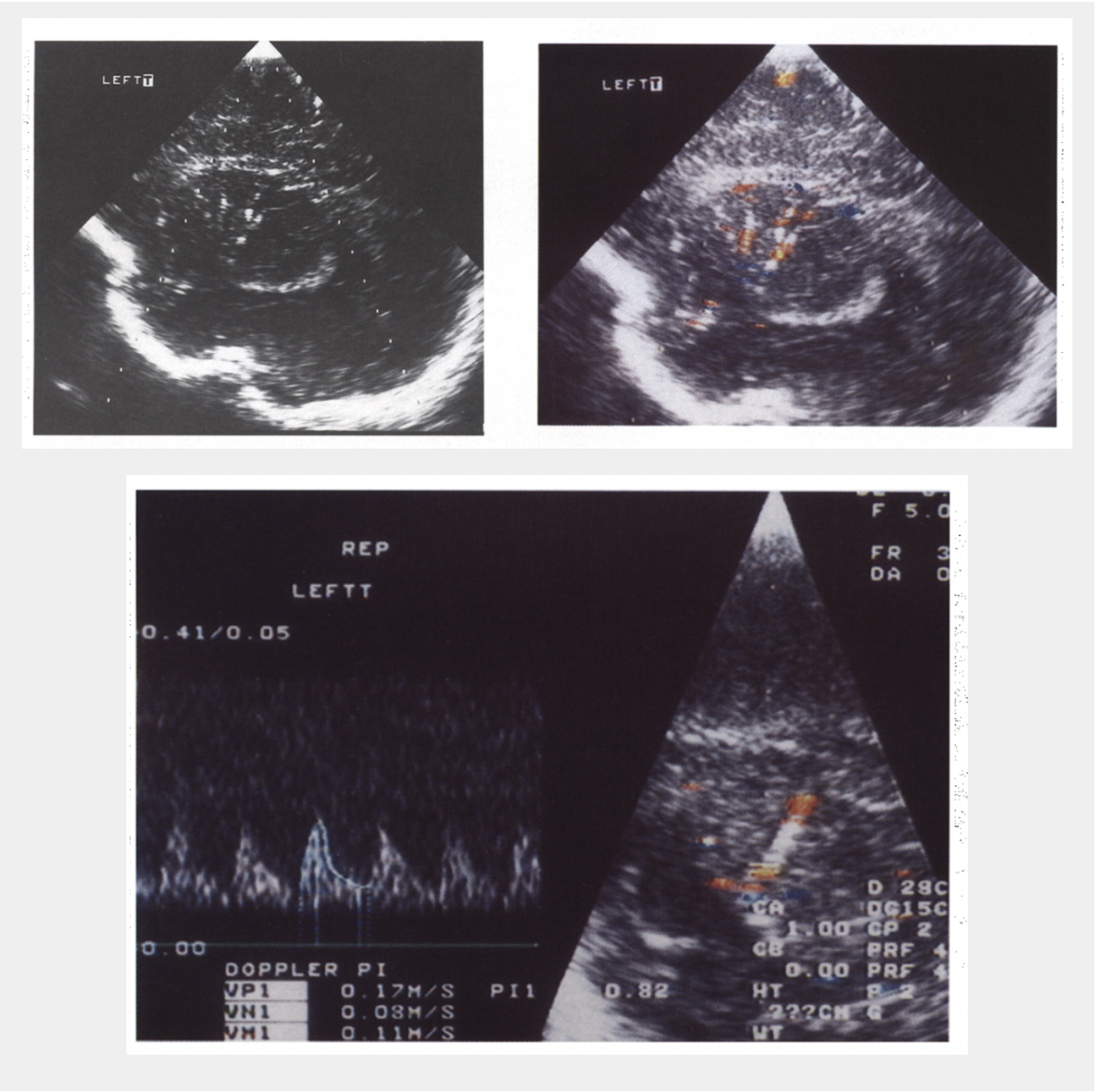


Figure 13. Doppler flow imaging studies demonstrating blood flow velocity wave within echogenic lenticulostriate arteries. Adapted by Cabanas et al, 1994 [189]

Furthermore, under normal circumstances, the vasculature supplying the deep gray nuclei of the basal ganglia and thalamus is indistinct on cranial US of a newborn infant. However, in the presence of a fetal insult, an inflammatory vascular response could lead to the vessels becoming echogenic and visible on cerebral US.

Therefore in light of these findings, LSV is currently defined as the linear bright echogenic structures in the basal ganglia and/ or thalamus of a small percentage of infants undergoing cerebral ultrasound, referred to as lenticulostriate vasculopathy (LSV).[191] Even though LSV has been described in neonates with a wide range of non-infectious conditions (i.e chromosomal abnormalities, maternal diabetes, drug exposure) (table 2)[186,192], the term LSV progressively became a synonym to "congenital infection" and more specifically cCMV.

Infectious causes
Cytomegalovirus
Rubella
Syphilis
Human immunodeficiency virus
Toxoplasmosis
Varicella
Bacterial meningitis
Rotavirus
Non-infectious causes
Hypoxic–ishemic encephalopathy
Cerebral infarction
Trisomy 13
Trisomy 21
Maternal alcohol use
Maternal drug use
Maternal autoimmune diseases
Maternal diabetes
Hypoglycemia
Congenital malformations, including heart disease
Inborn errors of metabolism
Congenital hypothyroidism
Twin-to-twin transfusion syndrome
Unknown/idiopathic
Prematurity

Table 2. Neonatal conditions associated with lenticulostriate vasculopathy. Adapted by Cantey et al, 2015 [193]

Multiple studies over the past few decades have reported the presence of LSV in neonates with cCMV [194]. One of the larger studies performing head US on 113 newborns with CMV found LSV in 40% of cases[195]. Even though various retrospective studies suggest an association between LSV and delayed neurodevelopment, the neurological sequelae of LSV and its clinical significance has yet to be determined [196,197] Furthermore, recent technological advances in the use of doppler imaging could have played a role in enhancing echoes of deeper thin vessels which we might mistakenly assess to be pathological, leading to overdiagnosis[198] .

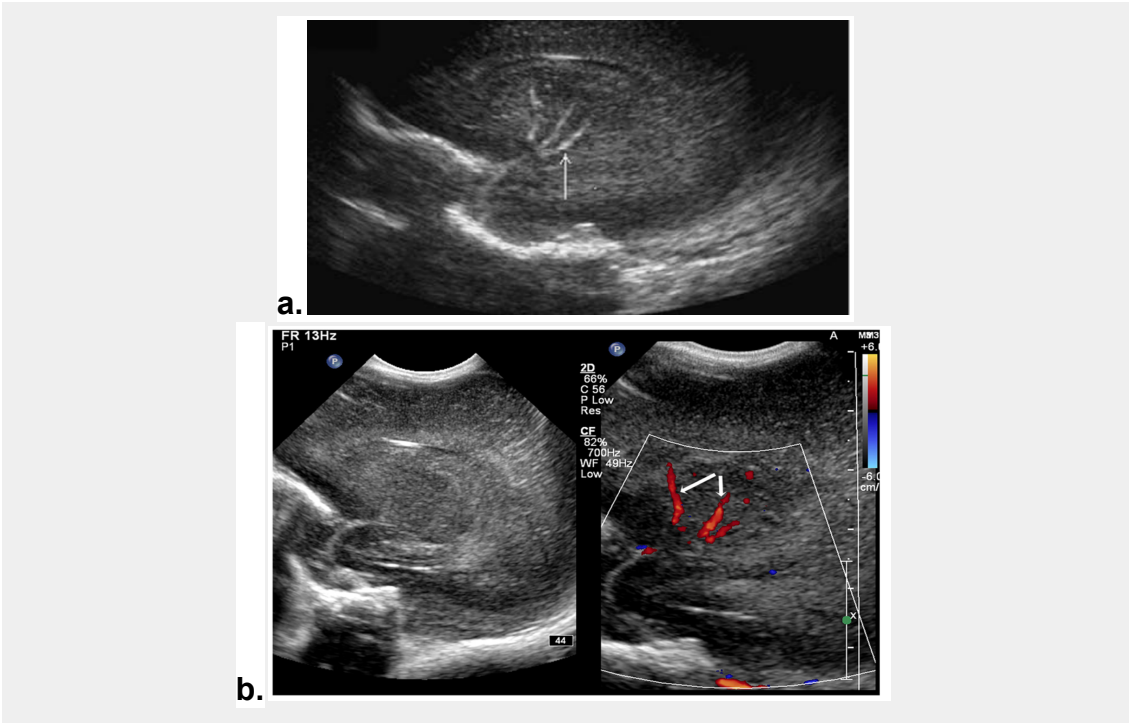


Figure 14. Lenticulostriate vasculopathy visualization with improved imaging modalities. Adapted byKandasamy et al, 2006 [199] and Koral et al, 2015 [200]

The following table (table 3) summarizes some of the most relevant studies performed aiming to evaluate the role of LSV and its relation to CMV. It is evident that over time the association between LSV and CMV is being questioned. Each color represents a different study design (Blue: retrospective, Orange: Prospective, Green: Reviews)

Title	Author	Year	Study Design	Cases	Aim	Results	Overall view on LSV importance
Sonographic Lenticulostriate Vasculopathy in Infants: Some Associations and a Hypothesis	Wenge	1995	Retrospective	Neonates undergoing US for neurological issues	Describe the causes of infantile LSV as demonstrated by sonography and propose the pathogenesis of these findings	LSV is a nonspecific marker of a previous insult to the developing brain, and the special hemodynamics of the fetal brain plays an important role in its pathogenesis.	(+) non specific marker of cerebral insult
Neonatal lenticulostriate vasculopathy: further characterization.	Markhoul	2003	Prospective case-control	LSV neonates	Compare the perinatal and neonatal clinical characteristics of neonates with LSV with matched controls and to summarise all published reports of LSV	Except for more multiple births, neonates with LSV did not display more adverse findings than their matched controls.	(-) LSV did not display adverse findings
Is Routine TORCH Screening Warranted in Neonates with Lenticulostriate Vasculopathy?	Jong	2009	Prospective	LSV neonates	Determine the relationship between LSV and congenital infections, as diagnosed by TORCH serology and viral culture for cytomegalovirus (CMV).	1) Routinely applied efforts to diagnose congenital infections in cases presenting with LSV have a poor yield. 2) Routine TORCH screening in neonates with LSV cases should only be regarded as mandatory once well-designed studies demonstrate a clear diagnostic benefit.	(-) more studies are needed to conclude
Lenticulostriatal vasculopathy - a marker for congenital cytomegalovirus infection?	Dusanovic	2011	Prospective	CMV neonates	(1) Whether there are differences in distribution and presence of TFCD flow between congenital CMV infection positive and negative group of children with LSV (2) Could US and TFCD findings of LSV be an indication for further investigation of possible congenital CMV infection, because of their variable and often adverse neurodevelopmental outcome?	1) Although LSV presents nonspecific marker for intracranial infection (ICI), all infants presenting with LSV should be evaluated for possible ICI 2) Thus, the Doppler findings of LSV in infants require a detailed examination, monitoring and follow-up of neuromotor outcome.	(+) non specific marker of intracranial infection, but follow up is required
Is lenticulostriated vasculopathy a sign of central nervous system insult in infants with congenital CMV infection?	Amir	2011	Prospective	CMV neonates	Investigate whether LSV as a single abnormal finding in neonates with congenital cytomegalovirus (cCMV) infection is a sign of central nervous system (CNS) involvement.	1) LSV is a common finding in infants with symptomatic congenital CMV infection and is a sign of CNS involvement. 2) LSV is a possible marker of high risk for sensorineural hearing loss in infants with congenital CMV infection.	(+) LSV is a possible marker of SNHL in CMV
Lenticulostriate vasculopathy in extremely low gestational age newborns: Inter-rater variability of cranial ultrasound readings, antecedents and postnatal characteristics	Soman	2014	Prospective	LSV neonates (extremely low GA < 28)	Evaluate the inter-rater reliability of cranial ultrasound readings of LSV, and to explore relationships with potential antecedents and developmental correlates in extremely low gestational age newborns	1) Positive agreement on the presence of LSV was low, as was the kappa value, an index of inter-rater reliability. 2) Infants with high illness severity scores and their correlates were at increased risk of developing LSV, while those who develop LSV appear to be at increased risk of motor dysfunction.	Low positive agreement on LSV (+) motor dysfunction in general
Lenticulostriated vasculopathy is a high-risk marker for hearing loss in congenital cytomegalovirus infections	Bilavsky	2015	Prospective	CMV neonates	Investigate the relationship between LSV and hearing loss in 141 infants with cCMV infection.	LSV was common in infants with cCMV infection and may serve as a sign of CNS involvement and further hearing deterioration.	(+) SNHL in CMV
The etiology of lenticulostriate vasculopathy and the role of congenital infections	Cantey	2015	Review	n/a	We propose screening infants with LSV for congenital cytomegalovirus infection and ensuring that perinatal screening included appropriate testing for syphilis, human immunodeficiency virus, and rubella immune status. Large, prospective observational studies are needed to determine the incidence of LSV and the relative contribution of infectious and non-infectious conditions to LSV in the neonate.	• LSV was associated with congenital infections, but can also be seen in an increasing number of non-infectious etiologies. • Indiscriminate testing for all congenital infections is unlikely to be cost-effective. • Infants with LSV should be screened for congenital CMV infection. • Additional testing should be limited to situations where additional history or clinical findings support another infectious diagnosis, or when more common etiologies have all been excluded.	(+) associated with CMV
Lenticulostriate Vasculopathy in Brain Ultrasonography Is Associated with Cytomegalovirus Infection in Newborns	Hong	2015	Retrospective	LSV neonates	Lenticulostriate vasculopathy is associated with various disorders, in particular cytomegalovirus infection, which can cause neurological consequences. We wanted to evaluate the association of lenticulostriate vasculopathy and cytomegalovirus infection. We retrospectively collected data on lenticulostriate vasculopathy from 858 neonatal ultrasonography scans.	Lenticulostriate vasculopathy on neonatal ultrasonography is useful for predicting cytomegalovirus infection, particularly in severe lenticulostriate vasculopathy. When severe lenticulostriate vasculopathy is associated with a brain structure anomaly, cytomegalovirus infection should be considered. The outcomes for the cases in which cytomegalovirus infection was associated with other brain structure anomalies were significantly worse than the outcomes in cases associated with lenticulostriate vasculopathy only.	(+) for CMV but especially when other cerebral anomalies were also present
Imaging Patterns of Sonographic Lenticulostriate Vasculopathy and Correlation with Clinical and Neurodevelopmental Outcome	Joo Jin	2015	Retrospective	LSV neonates	To evaluate the relationship between the imaging patterns of lenticulostriate vas- culopathy (LSV) and clinical outcomes.	High-grade sonographic LSV and absent color Doppler flow on lenticulostriate vessels were significantly associated with neurodevelopmental delay.	(+) LSV associated with neurodevelopmental delay
Lenticulostriate vasculopathy in neonates: Perspective of the radiologist	Koral	2015	Review	n/a	To examine the issues associated with the use of cranial US and the diagnosis of LSV, including alternative imaging, clinical abnormalities and the significance of LSV on cranial US.	Despite improvements in cranial US technology and increased familiarity and recognition of LSV in neonates, the significance of this observation remains as elusive as it was when first described 30 years ago. Prospective studies are needed	(+/-) LSV role is unclear
Lenticulostriate vasculopathy in neonates: Is it a marker of cerebral insult? Critical review of the literature	Soman	2015	Review	n/a	Review of the literature on LSV due to: 1) The clinical significance of Lenticulostriate vasculopathy (LSV) remains uncertain despite its recognition 30 years ago. 2) Diagnosis of LSV can be subjective, leading to false negatives/positives. 3) Increase in the reported incidence of LSV, may be attributed to a growing awareness of this finding during neonatal cranial US and advancements in US technology. 4) Limited prospective studies have evaluated the presence, significance, and diagnosis of LSV, yielding conflicting results. 5) The associated risk factors and clinical relevance of LSV on cranial ultrasound are still unclear.	1) LSV is suggestive of a cerebral insult, but it is considered a non-specific marker of perinatal and/or neonatal brain injury that may manifest with a delay. 2) LSV is not a specific marker for congenital infection or chromosomal abnormality. 3) Conflicting observations regarding the occurrence and relationship of LSV to various pathologic events before and after birth stem from the wide variation in its definition and recognition on neonatal cranial ultrasound. 4) A clearer definition of LSV on cranial ultrasound is necessary to improve diagnostic reliability and enhance understanding of its pathogenesis and long-term neonatal outcome.	(+/-) requires more studies unclear
An Electronic Health Record Investigation of Lenticulostriate Vasculopathy Features	Franowicz	2017	Prospective case-control	LSV neonates	Review of electronic health records of infants with and without LSV to investigate whether physician dictations indicated symptoms which could reflect subtle basal ganglia injury	Our findings provide some evidence that LSV is associated with an increased risk of early signs of abnormal development, possibly relating to signs of subtle basal ganglia injury. Historically LSV has been considered incidental. The associations identified here suggest that LSV findings are worthy of further study.	(+/-) requires more studies unclear
Is lenticulostriated vasculopathy an antiviral therapy prognostic finding in infants with congenital cytomegalovirus infection?	Giannattasio	2017	Prospective	CMV neonates	To assess the role of LSVin predicting neurodevelopmental and hearing outcomes in infants with cCMV infection.	Although LSV is a common MUS finding in infants with cCMV infection, its presence is not predictive of an adverse outcome. Our data suggest that MUS as a single neuroimaging investigation is unreliable in selecting candidates to antiviral therapy, mainly in presence of LSV as isolated finding.	(+) LSV is not a reliable finding to predict neurological impairment and need of antiviral therapy
Lenticulostriate Vasculopathy and Neurodevelopmental Outcomes in Preterm Infants: A Systematic Review	Robinson	2017	Review	n/a	The objective of this study was to perform a systematic review of all studies that report neurodevelopmental outcomes at 12 months' corrected age or later for preterm infants (< 32 weeks) who are diagnosed with lenticulostriate vasculopathy (LSV) on cranial ultrasound.	The available results raise some concern for future impairments in preterm infants with LSV, but they are confusing and inconclusive. There is insufficient evidence about the neurodevelopmental implications of LSV in preterm infants to inform counseling of parents.	(-) conflicting results, insufficient evidence
Lenticulostriate vasculopathy in preterm infants: a new classification, clinical associations, and neurodevelopmental outcome	Soman	2018	Prospective case-control	LSV neonates	To examine the inter-rater reliability for the diagnosis of LSV on cranial ultrasound (US), determine the risk factors associated with LSV and its progression, and examine neurodevelopmental outcome.	Establishment of well-defined stages of LSV improves the reliability of the diagnosis and allows identification of neonates with progression of LSV. Although LSV was associated with BPD, it was not associated with congenital CMV infection.	(-) LSV not associated with CMV
Hypernephrogenicity of lenticulostriate vessels: A poor prognosis or a normal variant? A seven year retrospective study	Fabre	2018	Retrospective	LSV neonates	Our study primarily describes the perinatal data and long-term follow-up of newborns with lenticulostriate vessel hyperechoic degeneration. Secondly, it describes the cerebral imaging data as a function of perinatal factors and neuro- development follow-up of these newborns.	From the results of our study, it appears that a low-grade LSV could be considered as a normal variant. There are no unifying diagnostic criteria for LSV on cerebral ultrasound. With a cerebral MRI, the use of ADC values in basal ganglia may well underscore the importance of such data in predicting long-term outcomes.	(-) probably a normal variant, especially when low-grade
Lenticulostriate vasculopathy in routine brain ultrasonography in infants: one step	Henjic	2021	Review	n/a	The aim of this review was to provide a better understanding of LSV ultrasound finding, as well as the need for further laboratory and imaging examinations in infants.	Therefore, although 35 years have passed since the first publication of LSV, there is still no consensus among experts on the clinical significance of isolated LSV, but caution is certainly needed given the fact that most infants with congenital CMV are asymptomatic.	still unclear
Lenticulostriate Vasculopathy in Very-Low-Birth-Weight Preterm Infants: A Longitudinal Cohort Study	Hong	2021	Retrospective	LSV neonates with VLBW	Our study aimed to determine the prevalence, presentation, and evolution of LSV, and the perinatal risk factors associated with LSV among very-low-birth-weight (VLBW) preterm infants.	LSV may be a nonspecific marker of perinatal insult to the developing brains of preterm infants. Prolonged postnatal oxygen usage may predispose VLBW preterm infants to late-onset LSV development. The long-term clinical impacts of LSV should be clarified.	non specific marker of brain insult
Placental pathology associated with lenticulostriate vasculopathy (LSV) in preterm infants	Soman	2021	Prospective	LSV neonates	Examine the frequency and type of placental abnormalities in neonates with LSV.	The association between LSV and LGA placenta may indicate a shared vascular response to an adverse perinatal environment.	LSV associated with LGA placentas

Table 3. Summary of studies on LSV and its association to CMV.

2.7 Prevention and Treatment

2.7.1 Hygiene techniques

Educating women of childbearing age on cCMV and the hygiene techniques that minimize the risk of infection is vital. Studies have shown that hygiene counseling has a major impact on preventing maternal infection, especially when a seronegative woman is in close contact with a toddler at home or at work.[201] A study compared the outcome of seronegative pregnancies in two groups. In the first group, the mothers were educated on CMV and hygiene measures during the first trimester whereas in the second group they were not. The results showed a 1.2% seroconversion rate in the informed group whereas a 7.6% rate in the non-informed group.[144] Since some of the women are already infected in early pregnancy without being aware of it, the earlier the timing of education the lower the seroconversion rates. Therefore an important aspect of maternal screening is to identify seronegative women and educate them on preventative measures as part of their preconception counseling. Apart from behavioral tools to prevent infection, there are some therapeutic options targeting the prevention of fetal infection in a known seropositive mother. However their effectiveness is still debatable.

2.7.2 HIG

Even though the role of the humoral and T-cell mediated immune responses to both primary and secondary CMV infection are not fully understood, the role of the immune system in protecting the fetus from becoming infected has become a topic of increasing interest in the last two decades. The knowledge that previously seropositive mothers show lower rates of intrauterine transmission in comparison to women who seroconvert during pregnancy, enhances the prospect that an immunological therapeutic agent could aid in the prevention of congenital infection.[2024] More specifically, the use of hyperimmune globulin (HIG) to prevent congenital CMV infection or to reduce the severity of symptomatic infection has been extensively studied. A few observational studies, showed a decrease in congenital infection in the treatment group with HIG compared to the placebo group.[203,204,205] Distinctly, Nigro et al demonstrated a decrease from 40% transmission in the untreated group to 16% when providing a HIG dose of 100U/kg intravenously

monthly.[203] However, this optimistic result was not confirmed by two randomized, double blinded trials who did not show any significant difference between the HIG and placebo group. [206,207]

Even though these results were not encouraging, the debate on HIG was re-invigorated on the basis that the CMV IgG half life is 11 days, which seems to be 9 days less than previously calculated.[208,209] This triggered the formation of a new study on 40 women with confirmed primary infection who were given bi-weekly 200U/kg administrations of HIG instead of 100U/kg monthly, in which CMV transmission occurred at a rate of 7.5% (95% CI, 1.6-20.4%).[208] This was significantly decreased when compared to a historic cohort of 108 women where transmission was reported at 35.2% (95% CI, 26.2-45.0%). The authors positively discussed the increase in administration frequency combined with higher doses and finally recommended the potential targeting of women with very recent primary infections in future studies. Therefore since more RCTs are needed to confirm these results and control for various parameters, the most recent recommendation is that HIG should not routinely be administered to women with primary CMV in pregnancy until further data become available.[124]

2.7.3 Antivirals

Prenatal

The use of antivirals to prevent intrauterine CMV transmission (secondary prevention) or decrease the chances of symptomatic infection (tertiary prevention) is currently recommended in the cases of primary infection during the first trimester with abnormal imaging. Various antivirals such as ganciclovir which can very successfully inhibit viral replication have been shown to be teratogenic and hence are not recommended for use in pregnancy.[210] To date, the most promising antiviral for the use during pregnancy, is valacyclovir. Valacyclovir inhibits the DNA-polymerase and can also inhibit viral replication to a satisfactory level (reference needed). In 2016, a multicenter, open-label, phase II study examined the use of oral valacyclovir in 41 pregnancies with confirmed fetal congenital infection with a primary endpoint the lowering of symptomatic neonates. Even though the study was not randomized and the use of a historic comparator group was lacking,

results were encouraging showing that high dose valacyclovir can improve the outcome of moderately symptomatic infected fetuses. More specifically, the use of valacyclovir increased the proportion of asymptomatic neonates from 43% without treatment, to 82% with treatment, while at the same time showing a tolerable safety profile.[211] This prompted the conduction of a double-blinded, randomized control trial (RCT) who tested valacyclovir (dose scheme) on 90 pregnant women with a recent seroconversion preconceptually or in early pregnancy, split into treatment and placebo group. According to the results of the study, 11.1% of treated women were CMV positive on amniocentesis versus 29.8% in the placebo group with odds ratio of 0.29 (95% [CI]: 0.09-0.90) for vertical CMV transmission.[212] Furthermore a case-control study of 65 women and matched controls also demonstrated that fetal infection was lower in the treated group (odds ratio, 0.318 (95% CI, 0.120-0.841); P = 0.021)[176]. These studies indicate that valacyclovir can be effective in improving the outcome of CMV infected pregnancies, however a recent study on the cost-effectiveness of valacyclovir prophylaxis compared universal maternal screening with subsequent valacyclovir prophylaxis (through 21 weeks' gestation) for those with primary infection versus the usual care (i.e., no routine antibody screening, but amniocentesis if abnormal mid-trimester ultrasound)[213]. They assumed a 35% risk of cCMV after primary maternal infection and a 71% risk reduction with valacyclovir but concluded that universal first-trimester serologic screening followed by valacyclovir treatment is not cost-effective under these assumptions. This data necessitates meticulous evaluation, considering that the treatment approach should be tailored to each individual case based on its specific severity.

Postnatal

Infants with symptomatic cCMV are treated with the antiviral intravenous drug ganciclovir, or its oral pro-drug valganciclovir. Two randomized controlled trials (RCTS) of treatment in symptomatic neonates showed moderate benefit in terms of preservation of hearing, and improved neurodevelopmental scores at 24 months of age[177,214]. From the two studies. the most recent RCT by Kimberlin et al. in 2015 included in a total of 96 neonates (0.196 effect size) with symptomatic CMV disease. Neonates were assorted into two groups, 47 neonates received vangacyclovir for a total of 6 months and 49 were started on vangacyclovir for 6 weeks and then continued with a placebo drug until the 6 month period was completed. It was concluded that a 6-month treatment plan with vangacyclovir starting within 30 days from birth improves hearing and developmental outcomes in the long-term (95% CI).[177] Therefore comparison of 6 weeks versus 6 months of valganciclovir demonstrated greater efficacy with longer treatment. In both studies infants were started on treatment before 1 month of age. Whether starting later reduces efficacy of treatment remains uncertain. However, when making treatment decisions, it is crucial to carefully consider the potential side effects of antivirals, including the risk of severe neutropenia.

It is worth mentioning that since the last stricter therapeutic guidelines in 2017 which suggest only treating symptomatic neonates, we are slowly moving away from the binary definition of “symptomatic” or “asymptomatic” . This has occurred because many of the cCMV patients are found in a “gray zone” regarding their clinical presentation. This larger spectrum of symptomatic infection has multiple therapeutic implications which therefore means that we are found in a transition period with regards to whom we treat.

Chapter 3: Biomarkers for symptomatic CMV infection

3.1 Prognostic tools of symptomatic cCMV

Since congenital CMV screening at different stages has not been implemented by the majority of countries, identifying clinical and biochemical biomarkers related to disease severity is of vital importance. As discussed in previous sections, the nature of maternal infection and gestational age in which fetal infection occurs have been implicated as factors affecting the severity of disease. More specifically, primary maternal infection with transmission in the first trimester have been reported to increase the risk of adverse events by various studies.[215,156] Further studies however who demonstrated that first trimester infections were associated with higher risk of sequelae, also recorded a substantial amount of neurological and SNHL cases in second and third trimester infections.[216,217] Moreover, recent studies have shown that infection is more common in later pregnancy and frequency is also high following non-primary infections and should not be overlooked.[218] Data from a recent meta-analysis including 6 studies (n=722 neonates) demonstrating that risk of vertical transmission of CMV increases as pregnancy progresses (table 4)[130]. Since we cannot exclude a severe case of cCMV following a non-primary and late infection, we need to investigate other markers of disease severity in order to address the issues at an early stage and majorly decrease the burden of disease.

	Transmission rate	SNHL or neurodevelopmental impairment if fetus is infected	SNHL or neurodevelopmental impairment if transmission is unknown
First trimester	36.8% (95% CI, 31.9–41.6)	22.8% (95% CI, 15.4–30.2)	8.4%
Second trimester	40.3% (95% CI, 35.5–45.1)	0.1% (95% CI, 0–0.8)	0%
Third trimester	66.2% (95% CI, 58.2–74.1)	0% (95% CI, 0–2.1)	0%

CMV, cytomegalovirus; *SNHL*, sensorineural hearing loss.

Chatzakis. Timing of primary maternal cytomegalovirus infection and rates of vertical transmission and fetal consequences. Am J Obstet Gynecol 2020.

Table 4. Timing of primary CMV infection and rates of vertical transmission and long-term sequelae. Adapted from Chatzakis et al, 2020[130]

3.2 Amniotic Fluid viral load

Various studies have investigated the use of amniotic fluid (AF) viral load as a prognostic marker of cCMV related disease.[219,220] In the previous decade, a number of studies suggested that a high CMV DNA load in amniotic fluid could predict symptomatic congenital infection at a prenatal stage,[123] whereas others did not find enough evidence to support this fact.[221] More specifically, studies have discussed various important limitations. Firstly, most of these studies had small sample sizes which made it difficult to assess the significance of those findings. Moreover, they did not use a clear threshold value above which symptomatic disease was more likely, and did not adjust for significant variables such as gestational age and time period from maternal infection, both of which have been shown to potentially influence clinical outcome.[220] Furthermore, when interpreting HCMV DNA quantification in AF samples, one has to consider the absence of a universal PCR quantification standard. This is essential as it is expected that assays used from various studies differ in multiple ways such as specimen type or nucleic acid extraction procedure.[222] Therefore samples will have undergone considerable interlaboratory alterations, making it difficult to universalize the predictive threshold value of CMV disease and to use it as a targeted therapeutic guide.[223] Due to vast recognition of this problem, the World Health Organisation (WHO) has recently proposed the standardization of references for CMV PCR which will be key in future studies evaluating the reliability of AF as a prognostic tool for disease severity.[222] Furthermore, recent investigations have revealed a positive correlation between the duration of infection and the increase in amniotic viral load. Consequently, the latest recommendations highlight the limited reliability of amniotic fluid as an indicator of disease severity.

3.3 Neuroimaging

The most promising prognostic tool for disease severity is prenatal and/or neonatal imaging. Since universal screening for cCMV is not established, clinical suspicion for cCMV is raised in two common clinical scenarios. The first is when there is known maternal CMV infection or maternal flu-like symptoms during pregnancy and the second, when cerebral abnormalities are seen on prenatal imaging. Since awareness regarding the importance of early diagnosis has increased, more studies

are interested in the use of both US and MRI as both prenatal and postnatal tools to predict symptomatic infection. Recent advances in the field of magnetic resonance imaging (MRI) have facilitated the visualization of fetal brains and enabled the discovery of important lesions that could be crucial in obstructing normal development. More specifically, through the use of prenatal conventional T1- and T2-weighted MRI we have access to early white matter (WM) abnormalities, maturation and migration defects which can be also used to reveal information on the timing of infectious insults.[224]

During the prenatal period, ultrasound is routinely performed. A series of cerebral abnormalities have been associated with cCMV infection such ventriculomegaly, periventricular echogenicity, subependymal cysts, temporal cysts, calcifications and more (figure 15). However at this early prenatal stage, only severe cases of cCMV commonly demonstrate significant abnormalities to raise suspicion. Isolated findings on fetal ultrasound have rarely been associated with severe symptomatic disease.

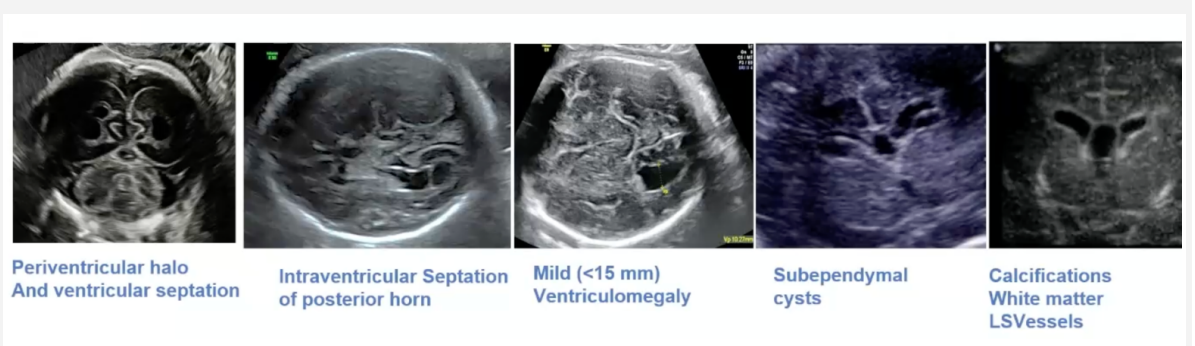


Figure 15. Common mild abnormalities observed on fetal cUS. Adapted from Lereuz-Ville et al, 2016

In the past, MRI scans were typically limited to cases of symptomatic congenital cytomegalovirus (cCMV) infection or when abnormal brain ultrasound results were detected. However, recent studies have underscored the complementary nature of these two imaging techniques. Keymeulen et al. conducted a study demonstrating that 20% of cCMV children with normal cranial ultrasound exhibited abnormal MRI findings. Among these cases, 91% were classified as symptomatic, with 40% being designated as severely symptomatic based solely on MRI lesions [225,226]. Another

study found that 36% of children who developed sensorineural hearing loss (SNHL) by the age of 3 had normal ultrasound but grade 4 MRI results [225,226].To better assess imaging findings identified by fetal or neonatal MRI, efforts have been made to establish a scoring system, with higher grades (3 & 4) significantly associated with long-term neurological sequelae (Table 5).

Cannie et al–2016 Fetal MRI		Alarcon et al. 2016– Neonatal US and MRI	
Score	**Findings**	**Score**	**Findings**
1	Normal findings	0	None of the following abnormalities
2	Isolated frontal or parieto-occipital periventricular increased signal intensity on T2 weighted sequence	1	Single punctate periventricular calcification, LSV, caudothalamic germinolysis, ventriculomegaly (excluding severe) and/or focal/multifocal white matter signal abnormality on MRI
3	Isolated temporal periventricular increased signal intensity on T2 weighted sequence	2	Multiple discrete periventricular calcifications, paraventricular germinolytic cysts, occipital horn septations, severe ventriculomegaly, diffuse white matter signal abnormality and/or temporal lobe involvement
4	Cysts and/or septa in the temporal and/or occipital lobe		
5	Migrational disorders, cerebellar hypoplasia	3	Extensive calcifications, brain atrophy, abnormal gyration, cortical malformation, dysgenesis of the corpus callosum and/or cerebellar hypoplasia

Table 5. Scoring system for fetal and neonatal MRI abnormalities in cCMV. Adapted by Cannie et al [227]and Alacron et al [228]

Similarly to neonatal MRI, fetal MRI has also gained importance as a predictive tool for sequelae in cCMV infection, particularly in cases of known first-trimester infections with a high risk of neurological sequelae. Fetal MRI offers advantages over neonatal MRI as it can be performed at an earlier stage and is non-invasive for both the mother and fetus. If fetal imaging can provide comparable information to neonatal imaging, fetal MRI may serve as a screening tool, reducing the need for neonatal MRI. In this scenario, neonatal imaging would be reserved for a subset of children at a higher risk. It is evident that both fetal and neonatal brain MRI currently significantly impact decision-making regarding the treatment and follow-up of symptomatic and asymptomatic cCMV newborns[229] .

Specific Part

Chapter 4.0 Aims and thesis outline

While extensively reviewing the literature on congenital CMV it is clear that there is a gap of knowledge on biomarkers that could guide targeted screening and diagnosis as well as predict the severity of infection at an earlier stage. Over the recent years there has been a lot of discussion on the key role of neuroimaging in diagnosing CNS involvement in cCMV, making it the most promising predictive biomarker to date. However, there are still a lot of challenges to overcome regarding access to imaging, interpretation, grading and assessment of relevant abnormalities.

Our main focus of interest in this thesis was to explore the role of neuroimaging as a biomarker of cCMV. To address this matter we first elected to study the role of prenatal imaging. A) We conducted a literature review and meta-analysis of the published literature, aiming to investigate the role of prenatal imaging in predicting postnatal clinical outcome in fetuses with known congenital CMV. More specifically, we analyzed reports on fetal MRI and fetal ultrasonography (US) and reviewed their ability to detect clinically meaningful cranial abnormalities.

Based on the important finding that MRI has a vital complementary role in identifying cerebral defects related to cCMV, we aimed to further explore the use of MRI in cCMV diagnosis. Given the difficulties associated with performing neonatal MRIs we decided to compare its diagnostic accuracy to fetal MRIs which are more easily performed. B) We conducted a study (case-series) on patients with known cCMV who had undergone both fetal and neonatal MRIs during their perinatal assessment and compared the reported findings. Finally, recognising the current importance of targeted screening and early diagnosis, C) we conducted a prospective case control study in order to explore the clinical significance of lenticulostriatal vasculopathy (LSV) in relation to cCMV. LSV refers to a cerebral finding seen on cerebral neonatal US and appears as linear echogenicities in the area of the thalamus. In the last few decades LSV has been associated with congenital infections and cCMV. We aimed to study LSV and to explore whether it could be an informative diagnostic and screening tool for congenital CMV infection.

4.1 Systematic Review and Meta-analysis

Being interested in biomarkers of disease severity and specifically in the presence of cranial abnormalities as biomarkers of disease, we performed a systematic review and meta-analysis aiming to gather and examine all evidence on the ability of prenatal imaging (MRI and/or US) to predict the presence of neurodevelopmental deficits in fetuses with confirmed cCMV during pregnancy.[2]

We included both retrospective and prospective cohorts investigating, qualitatively or quantitatively, the ability of prenatal diagnostic imaging to predict a poor postnatal clinical outcome in fetuses with confirmed cCMV infection. A total of 26 studies were included which translated into a total of 1226 neonates with confirmed cCMV. Our first aim was to identify the nature and frequency of cerebral abnormalities detected by fetal US and MRI. Even though the studies reported numerous abnormalities, the commonest cranial abnormalities identified by both imaging modalities in fetuses with confirmed cCMV were: ventriculomegaly, periventricular abnormalities, temporal cysts and other parenchymal lesions.

Our second objective was to examine any significant association between any of the fetal cranial abnormalities and symptomatic cCMV disease. We performed a meta-analysis to assess the association between each abnormality and poor clinical outcome independently. Our goal was to identify subtle findings seen in early gestation, which could be vital in guiding fetal monitoring, rigorous postnatal follow up or even termination of pregnancy if decided by the parents. With regards to fetal US, only microcephaly, a rather late and obvious clinical sign, was shown to be strongly clearly correlated to poor clinical outcome. Regarding fetal MRI, a meta-analysis on the association between cranial abnormalities and outcome did not show clear clinical correlation.

Our third aim was to collect all attempts to compare the ability of MRI and US to detect clinically meaningful cranial abnormalities antenatally. Even though no specific abnormality was significantly associated with clinical outcome, fetal US and MRI seem to be complementary to each other with regards to diagnosing cerebral abnormalities and predicting clinical outcome of congenitally infected fetuses. More specifically, out of 14 studies using both imaging techniques, 7 reported on those scans in detail. Reports showed that MRI was significantly better at identifying

temporal cysts and other parenchymal lesions than US, whereas fetal US was better at recognizing extracerebral abnormalities such as hyperechogenic bowel (Table 6). However, the clinical significance of such lesions when they present as an isolated finding remains unclear.[230] Thus great care needs to be taken when interpreting MRI results to avoid a misleading over diagnosis.

Abnormalities	Total number of fetuses with abnormality	US only	MRI only	US and MRI	P-value
Ventriculomegaly	19	5	7	7	0.77
Microcephaly	8	1	4	3	0.38
Periventricular Abnormalities	20	5	14	1	0.06
Temporal cysts & other parenchymal lesions	29	3	25	1	3×10^{-5}
Subependymal cysts	12	3	7	2	0.34
Migrational defects	2	0	2	0	0.50
IVH	2	2	0	0	0.50
Other CNS abnormalities	12	2	1	9	1.00
Cerebellar hypoplasia	1	0	1	0	1.00
LSV	2	2	0	0	0.50
Uterine abnormalities	6	6	0	0	0.03
Non-CNS abnormalities	16	11	0	5	0.001

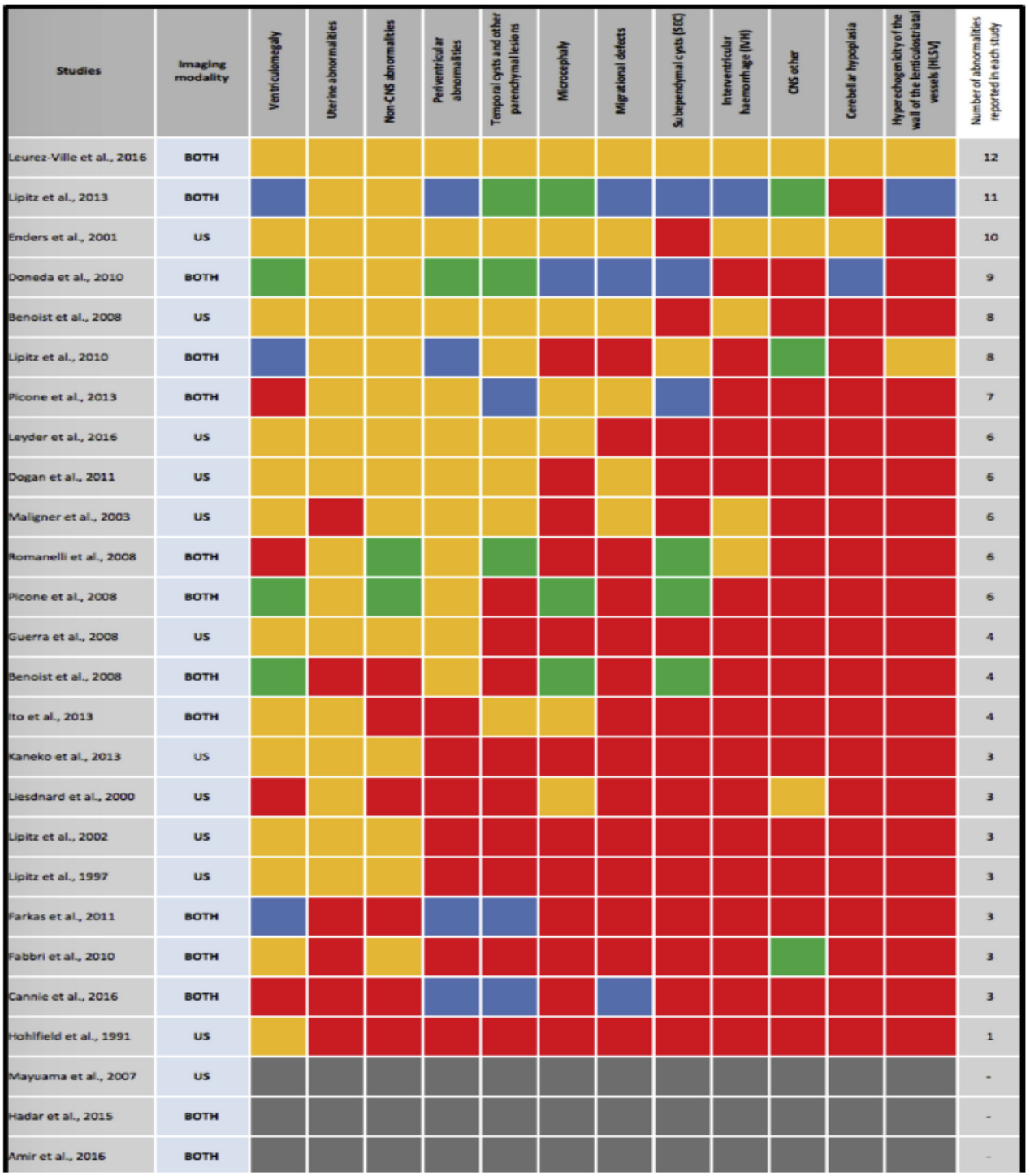
Table 6. Abnormalities identified by 7 studies performing both imaging techniques and reporting on individual abnormalities. Two-sided p-value calculated using McNemar's Exact Test. Adapted from Kyriakopoulou et al, 2020[2]

Furthermore, the complementary value of the two imaging techniques was highlighted by the fact that in none of the 7 studies did fetal US and MRI identify the same abnormalities (figure 16). Therefore in the presence of US cranial abnormalities, clinicians are encouraged to perform a prenatal MRI scan as it is a non-invasive method that could provide additional information in cases with increased suspicion of disease. Of the 14 studies using both imaging modalities, only three[231–233] attempted to qualitatively compare their ability to predict clinical outcome (Table 7). However, they presented conflicting opinions regarding the sensitivity, specificity, positive and negative predictive values (PPV and NPV) of MRI and US to identify clinically significant findings. One study even reported that the MRI's PPV and NPV of predicting poor outcomes were both 100%. Such high numbers need to be further investigated.

Study	Imaging	Number of fetuses with cCMV	Number with poor outcome	Timing of imaging	Time of outcome ascertainment	Sens	Spec	PPV	NPV
Benoist et al, 2008[232]	US	39	*	Every 2 weeks from diagnosis	Birth	86%	85%	71%	94%
	MRI	39	*	Within a week of one of the US scans	Birth	41%	93%	67%	80%
Doneda et al, 2010[231]	US	36	*	Between 20-34 weeks	Up till 8 years	33%	79%	29%	83%
	MRI	36	*	Within a week of one of the US scans	Up till 8 years	83%	63%	36%	94%
Hadar et al, 2010[233]	US	155	*	Every 3-4 weeks from diagnosis	Up till 6 years	86%	85%	71%	94%
	MRI	155	*	At 32-34 weeks	Up till 6 years	43%	91%	67%	80%

Table 7. Efficiency of prenatal US vs. MR Imaging in Predicting CMV Infection–related Postnatal Symptoms compared by 3 studies. Adapted from Kyriakopoulou et al, 2020[2]

Importantly, the combination of normal fetal US and MRI in cases of congenital CMV infection was shown to have a high negative predictive value for poor postnatal clinical outcome. This is of great value since it can be used to reassure worrying parents who could be facing the dilemma of TOP. The most significant finding of our study was the vast heterogeneity regarding fetal cranial imaging practice used to date. Importantly, articles differed in study design, eligibility criteria, definitions of poor clinical outcome, timing of the scans, amount of detail collected from scans and follow up time of neonates. Even though our calculations of heterogeneity (64.71% for univariable analysis and 57.01% for multivariable analysis) indicate a moderate level of heterogeneity, the 95% confidence intervals of heterogeneity are wide. It becomes apparent that the increase of awareness regarding cCMV and the development of guidelines in clinical practice is of urgent importance. Furthermore it highlights the need for consensus regarding fetal imaging in pregnancies with suspected or confirmed congenital CMV. This will enable accumulation of comparable data necessary for identifying those at risk, consulting parents and assessing future therapeutic interventions.



Colors; Red. None of the imaging modalities identified abnormality; **Blue.** Both imaging modalities identified abnormality; **Yellow**. Only fetal US identified abnormality; **Green.** Only fetal MRI identified abnormality;

Figure 16. Abnormalities reported by fetal US and fetal MRI in all 26 studies.

Adapted from Kyriakopoulou et al, 2020

4.2 Fetal vs Neonatal Imaging (case-series)

After performing our systematic review and meta-analysis it became clear 1) that targeted neonatal screening for cCMV is of vital importance and that 2) MRIs can play an important role in identifying relevant abnormalities that are not visible by ultrasonography. We therefore decided to focus our research towards these two fields of interest and conducted two concurrent studies. The first was targeted towards the use of neonatal MRIs in the diagnosis of cCMV abnormalities. Even though neonatal MRIs are being successfully used around the world when neurological involvement is suspected, multiple limitations and ethical issues are related to accessibility (MRI machine & anaesthesiology team), as well as regarding the common practice of sedating newborns undergoing an MRI. On the contrary, fetal MRI is less invasive and provides more time for decision making.We became interested in the correlation between fetal imaging and neonatal imaging. More specifically we were interested in exploring whether late fetal MRIs could provide similar information to neonatal MRIs. In this retrospective case series, we included cases of children with confirmed cCMV who had undergone both fetal and neonatal MRIs. Our aim was to (1) compare the ability of fetal versus neonatal MRIs to recognize cerebral abnormalities related to cCMV infection; (2) record the cerebral abnormalities in the fetal and neonatal MRI of each cCMV case; (3) compare the concordance in reporting of abnormalities by two blinded esteemed radiologists within each category.

4.2.1 Study population and setting

We performed a single-center retrospective cohort study on a convenience sample of asymptomatic newborns (n=10) with confirmed congenital CMV infection who had undergone both fetal and neonatal MRIs. Despite the lack of official guidelines in Greece, most obstetricians test for CMV in the first prenatal visit. Maternal primary CMV infection diagnosis was made post either seroconversion or detection at the first prenatal of CMV-IgM with a low IgG avidity and/or CMV viremia. All mothers underwent amniocentesis that confirmed fetal infection. Fetal MRI was performed due to high risk of developing symptomatic cCMV disease with seroconversion

occurring prior to the 20th week of pregnancy, although no CNS findings were noted in fetal ultrasound. A confirmed case of congenital CMV was defined in newborns with a positive urinary CMV PCR (polymerase-chain-reaction) in the first 2 weeks of life. The MRI images from the 10 cases were sent to four esteemed consultant radiologists with expertise in fetal and neonatal imaging. The radiologists were blinded to the clinical outcome of the child, as well as to the other radiologist's interpretation of the scan. An MRI was recorded as "abnormal" in the presence of any of the following abnormalities: Gyral abnormalities, ventricular dilatation, ventricular adhesions, subependymal cysts, calcifications, white matter T2- weighted signal hyperintensity (parieto-occipital), white matter T2 hyperintensity (temporal), temporal horn dilatation, cerebellar abnormalities, ventricular adhesions-temporal/occipital lobe, temporal/occipital lobe cysts, microcephaly, microencephaly, cerebellar hypoplasia).

4.2.2 Results

Concordance within each MRI category (Fetal & Neonatal)

We first evaluated the concordance in reporting of abnormalities within each imaging category and then we compared the two (fetal vs. neonatal). In Fetal imaging the overall concordance between the 2 radiologists was high. Specifically, for 9 out of 14 categories of abnormalities the concordance was 100%. The findings with the least concordance were "gyral abnormalities" (50%), and "white matter T2 abnormal hypersensitivity (Temporal)" (60%). Similarly to fetal MRIs, the concordance within neonatal images was also high. Specifically for 10 out of the 14 categories of abnormalities the concordance was calculated at 100%, meaning that the radiologists agreed on the presence of those abnormalities throughout the 10 cCMV cases. For the remaining four categories of abnormalities (white matter T2 abnormal hyperintensity-temporal, temporal horn dilatation, cerebellar abnormalities, cerebellar hypoplasia) the concordance rate between the two radiologists interpreting the scans was still high at 90% (Table 8), indicating no difference in reporting between the two radiologists (p= 1). Images representing the concordance and discordance between radiologists regarding fetal and neonatal images are available (figure 17)

MRI	FETAL MRI					NEONATAL MRI		
Abnormalities	Concordance (%)	Abnormality reported within concordance group		Non Concordance group	Concordance (%)	Abnormality reported within concordance group		Non Concordance group
		Present (%)	Not present (%)	Inconclusive (%)		Present (%)	Not present (%)	Inconclusive (%)
Ventricular Dilatation	10 (100)	2 (20)	8 (80)	0(0)	10 (100)	0 (0)	10 (100)	0 (0)
Calcifications	10 (100)	0 (0)	10 (100)	0 (0)	10 (100)	2 (20)	8 (80)	0 (0)
Abnormal White Matter T2 Hyperintensity (Parieto-occipital)	10 (100)	4 (40)	6 (60)	0 (0)	10 (100)	2 (20)	8 (80)	0 (0)
Temporal Horn Dilatation	10 (100)	0 (0)	10 (100)	0 (0)	10 (100)	5 (50)	5 (50)	0 (0)
Cerebellar Abnormalities	10 (100)	0 (0)	10 (100)	0 (0)	10 (100)	0 (0)	10 (100)	0 (0)
Temporal/Occipital lobe cysts	10 (100)	3 (30)	7 (70)	0 (0)	10 (100)	5 (50)	5 (50)	0 (0)
Microcephaly	10 (100)	0 (0)	10 (100)	0 (0)	10 (100)	2 (20)	8 (80)	0 (0)
Microencephaly	10 (100)	0 (0)	10 (100)	0 (0)	10 (100)	2 (20)	8 (80)	0 (0)
Cerebellar Hypoplasia	10 (100)	0 (0)	10 (100)	0 (0)	10 (100)	0 (0)	10 (100)	0 (0)
Ventricular Adhesions	8 (80)	2 (20)	6 (60)	2 (20)	10 (100)	0 (0)	10 (100)	0 (0)
Subependymal Cysts	8 (80)	2 (20)	6 (60)	2 (20)	9 (90)	0 (0)	9 (100)	1 (10)
Ventricular Adhesions Temporal/Occipital Lobe	8 (80)	1 (10)	7 (70)	2 (20)	9 (90)	2 (20)	7 (70)	1 (10)
Abnormal White Matter T2 Hyperintensity (Temporal)	6 (60)	4 (40)	2 (20)	4 (40)	9 (90)	2 (20)	7 (70)	1(10)
Gyral Abnormalities	5 (50)	0 (0)	5 (50)	5 (50)	9 (90)	0 (0)	9 (100)	1(10)

Table 8. Agreement between two accredited radiologists regarding the presence or absence of abnormalities in fetal and neonatal MRIs. Adapted from Kyriakopoulou A. et al 2023[3]

Overall, the concordance between the two fetal MRI reports for each cCMV case was high (90%) with 9 out of 10 MRIs being classified as “abnormal” by both radiologists. For neonatal MRIs, the concordance rate was overall even higher compared to the fetal MRI reports. MRI reports were concordant for each one of the cases (100%).

Concordance between each MRI category (Fetal vs. Neonatal)

We then compared the two categories (fetal vs neonatal MRI) with regards to the reported abnormalities and whether the radiologists reported the scans to be normal or abnormal (scan outcome) (Table 9). In 7 out of 10 cases, both were reported abnormal by all 4 radiologists, with a positive predictive value of fetal vs neonatal MRI calculated at 70% (7/10, 95% CI, 35-92). In 2 cases (20%) (case 2 and 5), the fetal MRI was deemed abnormal by both radiologists, but the neonatal were considered normal. In 1 case (case 7), the radiologists evaluating the fetal MRI disagreed on whether the scan was overall “normal” or “abnormal”, but both radiologists examining the neonatal MRI agreed that the neonatal scan was normal. An image representing the concordance and discordance between radiologists regarding fetal vs neonatal images is shown below (Figure 17).

Patient	MRI	Scan Outcome	Neonatal Antiviral Treatment
1	Fetal	Abnormal concordant	Yes
	Neonatal	Abnormal concordant	
	Fetal vs Neonatal	**Abnormal fetal + neonatal**	
2	Fetal	Abnormal concordant	No
	Neonatal	Normal concordant	
	Fetal vs Neonatal	**Non-concordant**	
3	Fetal	Abnormal concordant	No
	Neonatal	Abnormal concordant	
	Fetal vs Neonatal	**Abnormal fetal + neonatal**	
4	Fetal	Abnormal concordant	Yes
	Neonatal	Abnormal concordant	
	Fetal vs Neonatal	**Abnormal fetal + neonatal**	
5	Fetal	Abnormal concordant	No
	Neonatal	Normal concordant	
	Fetal vs Neonatal	**Non-concordant**	
6	Fetal	Abnormal concordant	Yes
	Neonatal	Abnormal concordant	
	Fetal vs Neonatal	**Abnormal fetal + neonatal**	
7	Fetal	**Non-concordant**	No
	Neonatal	Normal concordant	
	Fetal vs Neonatal	**Non-concordant**	
8	Fetal	Abnormal concordant	No
	Neonatal	Abnormal concordant	
	Fetal vs Neonatal	**Abnormal fetal + neonatal**	
9	Fetal	Abnormal concordant	Yes
	Neonatal	Abnormal concordant	
	Fetal vs Neonatal	**Abnormal fetal + neonatal**	
10	Fetal	Abnormal concordant	No
	Neonatal	Abnormal concordant	
	Fetal vs Neonatal	**Abnormal fetal + neonatal**	

Table 9. Concordance between the radiologic findings of fetal and neonatal MRIs for each patient. Adapted from Kyriakopoulou A. et al 2023[3]

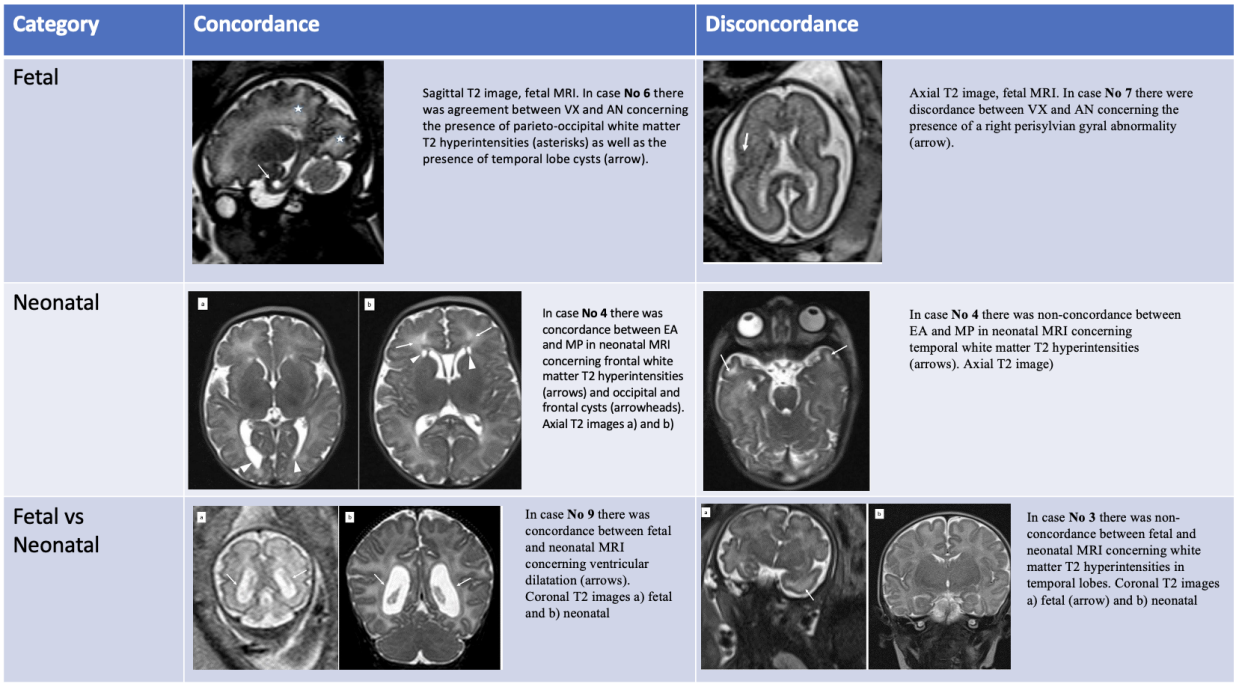


Figure 17. Images representing the concordance and discordance between radiologists in fetal, neonatal and fetal vs neonatal MRIs. Adapted by Kyriakopoulou A. et al 2023[3]

In conclusion, despite this being a small descriptive study, the observations indicate that fetal MRI could potentially replace neonatal MRI and provide important information pointing towards CNS involvement in infected fetuses. Larger prospective studies of cCMV patients are needed in order to further compare fetal and neonatal MRIs as well as to establish the optimal gestational age at which it should be performed. Importantly we have learned that an abnormal fetal MRI could play a future role in screening for patients in high - risk for neurodevelopmental issues planning postnatal follow - up.

4.3 LSV vs CMV

As previously discussed, following our systematic review of the literature, we became interested in targeted neonatal screening. Due to the recent literature discussing the presence of lenticulostriate vasculopathy (LSV) as an important finding related to cCMV, we decided to investigate this further. More importantly, we performed a case control study in newborns to examine whether LSV is an informative diagnostic tool in the case of cCMV.

Objective

In this study, our aim was to prospectively investigate the role of LSV on cerebral neonatal US of premature neonates, as a potential diagnostic tool for cCMV infection.

4.3.1 Methods

Selection of Cases and Controls and Data Collection

We conducted a prospective multicenter case-control study in two tertiary hospitals in Athens, Greece (Attikon General Hospital and Iaso Maternity Hospital). Our aim was to assess whether the presence of LSV on cerebral US of premature neonates is associated with congenital CMV infection and can be used as a diagnostic tool. We defined cases as premature neonates (≤36 weeks) born between January 2019 and September 2022 who underwent cerebral ultrasound within the first 3 weeks of life, and in which LSV was detected. Each case was matched with one control based on gestational age at birth (+/- 3 days). Controls were selected at random from the same neonatal unit, based on the closest gestational age to the matched case. Newborns above the age of 36 weeks were excluded. Furthermore, since congenital CMV infection is diagnosed best by identification of the virus in the urine or saliva before the age of 3 weeks, neonates with LSV on cerebral US above the age of 3 weeks were excluded. Once the case and control was identified, urine specimens were collected by a urine bag (1-3mls) and stored at −80°C to maintain viral DNA integrity. For each participant we recorded the following data: somatometric measurements, day of US imaging, other cerebral and extracerebral US findings, 1st blood results (FBC, LFTs when available), maternal medical history, IVF, pregnancy

related issues and demographic data (post code, insurance type, parental occupation).

Even though we gathered numerous characteristics during the study, we defined the following variables as the most relevant and included them in the final statistical analysis: presence of congenital cytomegalovirus (cCMV) infection, somatometric variables (head circumference, body length, and body weight), sex, intrauterine growth restriction (IUGR), small or large for gestational age (SGA/LGA), presence of any other cerebral findings on cerebral ultrasound (with each abnormal finding independently examined in relation to LSV), maternal gestational diabetes, and gestational hypertension. Furthermore, we note that given that the study encompassed newborns with a GA ranging from 27^{+1} to 36^{+6} weeks, all somatometric variables were adjusted for gestational age and reported as Z-scores[234].

Ultrasound assessment

Ultrasound over the anterior and posterior fontanel and asterion (six standard quasi-coronal views and five sagittal views)[235] was performed by an experienced paediatric radiologist using high frequency transducers (7.5 and 10 MHz LOGIQ V2 and VIVID i by GE Healthcare). No additional neuroimaging was conducted.

DNA Extraction and CMV Assay analysis

The molecular detection of cytomegalovirus (CMV) in urine samples was performed using one of two Real Time Polymerase Chain Reaction (PCR) applications:

1. The Artus CMV QS-RGQ kit from QIAGEN was used for quantitative detection of CMV DNA in clinical samples. The kit targets the Major Immediate-Early (MIE) gene and has a detection limit of 79.4 copies/ml to 10^8 copies/ml. Genetic material was extracted from urine samples using the DSP virus/pathogen mini kit, and the Real Time PCR assay was performed using the QIAsymphony QS-RGQ automatic analyzer and Rotor Gene Q MDx thermocycler, both from QIAGEN.
2. The Simplexa Congenital CMV Direct kit from Diasorin Molecular was used for qualitative molecular detection of the UL83 gene of CMV in urine samples without prior genetic material extraction. The assay has a detection limit of 713 IU/ml to

3.96x10^8 CMV DNA IU/ml and was performed using the Laison MDX thermocycler from Diasorin Molecular.

Statistical Analysis

In this study, the sample size was determined based on the estimated incidence of lenticulostriate vasculopathy (LSV) and the desired precision of the results. Calculations indicated that a sample size of 360 participants would be needed to evaluate LSV incidence among premature neonates, considering a wide range of 5% to 20% as reported in previous studies, and aiming for a narrow 95% confidence interval with a half-width of 2% to 4%. However, due to practical constraints and a time limit of three years for the study, a total of 166 participants were enrolled, including 83 cases with LSV and 83 controls without LSV. Although the final sample size was smaller than initially calculated, it was determined that this number would still provide valuable insights into the association between LSV and the presence of congenital cytomegalovirus (CMV) infection. The reduced sample size resulted in a power closer to 65%, which is acceptable for the study objectives. Despite the adjustment in sample size, rigorous statistical methods were applied to analyze the collected data, and the study findings are expected to contribute to the understanding of LSV as a potential diagnostic tool for CMV in preterm neonates.

Our statistical analysis matched on the basis of gestational age in terms of completed full weeks. For example, a neonate of 36+1 weeks was classified under 36 weeks, as was a neonate of 36+6 weeks. Even though our study design paired each case to an individual control on the basis of gestational age ± three days, this approach improves statistical power with a minimal sacrifice in precision[236].

We computed descriptive statistics using medians and interquartile ranges, which are robust to right and left skew. We quantified differences between cases and controls using the standardized mean difference (SMD). SMD is commonly used to assess covariate balance between matched cases and controls because respective means may not be directly comparable, due to potential influence by the matched variables. It is calculated by taking the difference in means between the cases and

controls and dividing it by the mean standard deviation between cases or the controls. An SMD < 10% is considered ideal, and an SMD < 20% is considered acceptable balance[237]

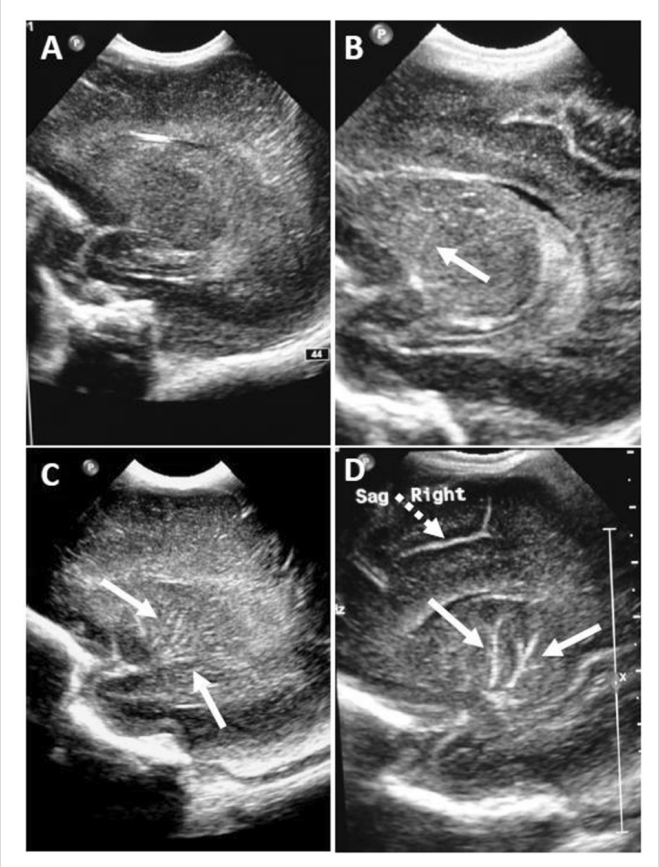
Gestational week-matched differences in numeric variables were tested using the Fisher-Pitman permutation test [238]. Unlike one-way Analysis of Variance (ANOVA), this test does not require the normality assumption [239]. Similarly, matched differences in categorical variables were tested using the Cochran-Mantel-Haenszel test [240]. Unlike McNemar's test, this allows for more than two strata, each of which can be of any size. Two-sided p-values were approximated using the non-parametric bootstrap with 10,000 Monte Carlo resamplings with replacement.

Univariable matched odds ratios were estimated using the conditional logistic regression (with exact estimation of the partial likelihood), stratifying by gestational week. Multivariable matched odds ratios were calculated using the same approach, while also adjusting for sex, gestational diabetes, and gestational hypertension. Two-sided p-values were computed using the likelihood ratio test.
All analyses were done in R 4.2.2 [241] using the packages coin [242] and survival [243].

4.3.2 Results

During the study period spanning from January 2019 to September 2022, a convenience sample was collected. As part of standard clinical practice, routine cerebral ultrasound (US) scans were conducted for all preterm neonates with a gestational age (GA) of ≤32 weeks, as well as for the majority of neonates with a GA between 32 and 36 weeks (non-inclusive). Prospective assessment and grading of Lenticulostriate Vasculopathy (LSV) were performed on the acquired images. It is worth noting that the majority of ultrasound examinations took place within the first week of the neonates' lives. Ultrasounds were conducted by paediatric radiologists with at least 10 years of experience in neonatal cerebral imaging. In order to minimize the substantial interrater variability reported in previous studies concerning LSV [244] the visible lenticulostriate vessels (LSV) were evaluated based on grading systems adapted from recent studies (Figure 18). Neonates were separated into four groups based on the following grading system: A: No LSV, B: mild (stage 1): one or

two thin branches seen; C: moderate (stage 2): two to three thin branches seen; D: severe (stage 3): three prominent thick branches seen [245–247].



A= normal (not visible); B=mild LSV (faint linear echogenicities corresponding to a lenticulostriate artery) ; C= moderate LSV (Thin hyperechogenic lenticulostriate vessels) and D = severe LSV (thick and echogenic equals sulci)

Figure 18. Images representing the grades of Lenticulostriate Vasculopathy (LSV). Adapted from Sisman et al, 2018. [247]

Descriptive statistics

Upon comparing the two groups, it was observed that congenital CMV infection was detected in 2 neonates from the control group (2.4%) and 3 neonates from the cases group (3.6%, p=0.677) (Table 10).

Within our cases, the severity of LSV was categorized into three groups: mild, moderate, and severe. Among the cases, 36 (43.4%) exhibited mild LSV, 41 (49.4%) showed moderate LSV, and 6 (7.2%) displayed severe LSV. The distribution of all variables studied among the three groups of LSV (mild; moderate; severe) can be seen in Table 11, Figure 19.

Additionally, all other cerebral findings were recorded. Our analysis indicated that our cases had a significantly higher occurrence of concomitant abnormalities compared to the control group. Specifically, 19 cases (22.9%) exhibited concomitant abnormalities, whereas 10 controls (12%) showed such abnormalities (p=0.046). Notably, periventricular echogenicity was the most common concomitant abnormality, observed in 12 cases (14.5%) compared to 5 controls (6.0%) (p=0.038). During the analysis of somatometric measurements, we classified a somatometric measurement as "abnormal" when it deviated by 2 standard deviations (SD) from the mean, corresponding to a Z-score of -2 or +2. Based on this assumption, we further examined the association between “abnormal somatometric measurements” for gestational age and the presence of LSV.

In terms of head circumference, a higher proportion of our cases displayed "abnormal head circumference" compared to the control group, however the p-value did not reach statistical significance [7 (8.4%) vs. 2 (2.4%), p = 0.0873]. A similar pattern was observed for body length, with a greater number of cases exhibiting abnormal measurements compared to controls [6 (7.2%) vs. 3 (3.6%), p = 0.492]. However, concerning weight, our data revealed a higher prevalence of abnormal weight among the control group [3 (3.6%) vs. 4 (4.8%), p = 0.6983].

When assessing the standardized mean difference (SMD) between the two groups (cases vs. controls), a substantial SMD (greater than 0.2) was observed for multiple

variables: z-length (0.403), z-weight (0.283), z-head circumference (0.350), abnormal head circumference (0.268), and other cerebral findings (0.450). In all of these variables, the cases exhibited larger weight, length, and head circumference compared to the controls.

Initially, a gestational week-matched univariable analysis was conducted to determine the differences in both numerical and categorical variables between the cases (with LSV) and controls (without LSV). Several variables were found to have a significant association with LSV.

Specifically, the variables that demonstrated a significant association with LSV included increased z-length ($p = 0.011$), increased z-head circumference ($p=0.019$), head circumference ($p=0.037$), the presence of other cerebral findings ($p=0.044$), and the total number of other cerebral findings ($p=0.046$). These findings indicate that higher z-length, z-head circumference, head circumference, and the presence of other cerebral findings were significantly associated with the occurrence of LSV.

Characteristic	Controls	Cases	SMD	p value
	N=83	N=83		
cmv (%)	2 (2.4)	3 (3.6)	0.071	0.677
male sex (%)	53 (63.9)	45 (54.2)	0.197	0.280
gestational hypertension (%)	5 (6.0)	7 (8.4)	0.093	0.553
gestational diabetes (%)	19 (22.9)	18 (21.7)	0.029	1.000
head circumference				
z-head (mean (SD))	0.24 (0.92)	0.57 (0.96)	0.350	0.019
abnormal head circumference (%)	2 (2.4)	7 (8.4)	0.268	0.087
weight				
z-weight (mean (SD))	-0.38 (0.97)	-0.11 (0.92)	0.283	0.083
abnormal weight (%)	4 (4.8)	3 (3.6)	0.060	0.698
body length				
z-length (mean (SD))	0.04 (1.01)	0.45 (1.01)	0.403	0.011
abnormal length (%)	3 (3.6)	6 (7.2)	0.160	
IUGR (%)	7 (8.4)	7 (8.4)	<0.001	1.000
birth weight for gestational age (%)			0.175	0.800
VSGA (<3rd percentile)	12 (14.5)	8 (9.6)		
SGA (<10th percentile)	3 (3.6)	2 (2.4)		
AGA (10th <> 90th percentile)	65 (78.3)	69 (83.1)		
LGA (>90th percentile)	3 (3.6)	4 (4.8)		
LSV grade (%)				
n.a.	83 (100.0)	0 (0.0)		
mild	0 (0.0)	36 (43.4)		
moderate	0 (0.0)	41 (49.4)		
severe	0 (0.0)	6 (7.2)		
other cerebral findings total (%)	10 (12.0)	19 (22.9)	0.289	0.044
other cerebral findings (%)			0.450	0.046
no other findings	73 (88.0)	64 (77.1)		
ventriculomegaly	1 (1.2)	2 (2.4)		
periventricular echogenicity	5 (6.0)	12 (14.5)		
SEH Bilaterally	2 (2.4)	3 (3.6)		
choroid Plexus Cyst	2 (2.4)	0 (0.0)		
second degree IVH	0 (0.0)	1 (1.2)		
ventricular Asymmetry	0 (0.0)	1 (1.2)		

Table 10. Descriptive Characteristics and Group Comparisons of Neonates with LSV (cases) and Neonates without LSV (controls)

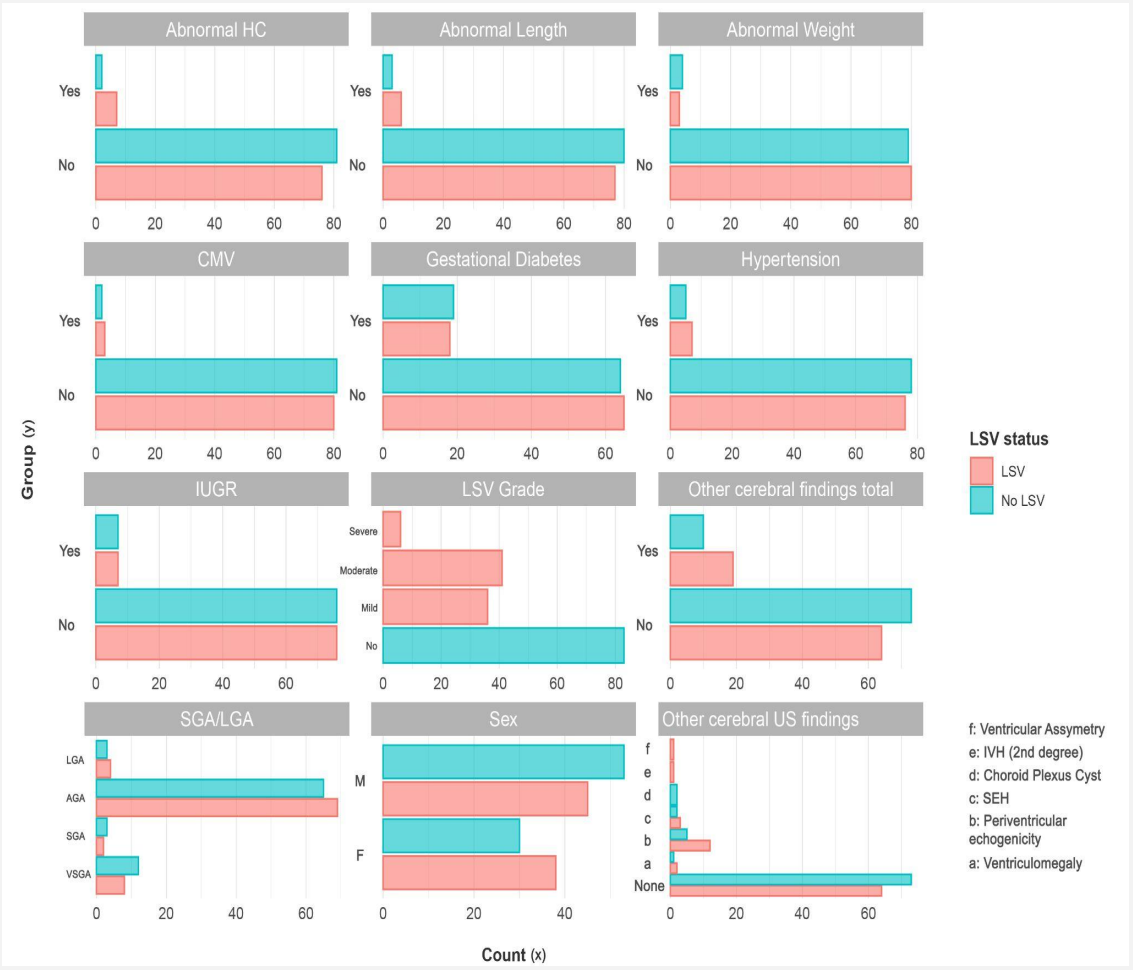


Figure 19. Comparison of Categorical Variables between Neonates with LSV (pink) and Neonates without LSV (blue)

Characteristic	Cases	LSV Grade		
		Mild	Moderate	Severe
	N=83	N=36	N=41	N=6
cmv (%)	3 (3.6)	1 (2.8)	2 (4.9)	0 (0.0)
male sex (%)	45 (54.2)	16 (44.4)	26 (63.4)	3 (50.0)
gestational hypertension (%)	7 (8.4)	5 (13.9)	2 (4.9)	0 (0.0)
gestational diabetes (%)	18 (21.7)	7 (19.4)	10 (24.4)	1 (16.7)
head circumference				
z-head (mean (SD))	0.57 (0.96)	0.45 (0.97)	0.71 (1.00)	0.26 (0.41)
abnormal head circumference (%)	7 (8.4)	2 (5.6)	5 (12.2)	0 (0.0)
weight				
z-weight (mean (SD))	-0.11 (0.92)	-0.40 (1.01)	0.16 (0.82)	-0.26 (0.43)
abnormal weight (%)	3 (3.6)	1 (2.8)	2 (4.9)	0 (0.0)
body length				
z-length (mean (SD))	0.45 (1.01)	0.16 (1.07)	0.77 (0.92)	-0.03 (0.54)
abnormal length (%)	6 (7.2)	1 (2.8)	5 (12.2)	0 (0.0)
IUGR (%)	7 (8.4)	5 (13.9)	2 (4.9)	0 (0.0)
birth weight for gestational age (%)				
VSGA (<3rd percentile)	8 (9.6)	7 (19.4)	1 (2.4)	0 (0.0)
SGA (<10th percentile)	2 (2.4)	1 (2.8)	1 (2.4)	0 (0.0)
AGA (10th <> 90th percentile)	69 (83.1)	28 (77.8)	35 (85.4)	6 (100.0)
LGA (>90th percentile)	4 (4.8)	0 (0.0)	4 (9.8)	0 (0.0)
other cerebral findings total (%)	19 (22.9)	6 (16.7)	9 (22.0)	4 (66.7)
other cerebral findings (%)				
no other findings	64 (77.1)	30 (83.3)	32 (78.0)	2 (33.3)
ventriculomegaly	2 (2.4)	2 (5.6)	0 (0.0)	0 (0.0)
periventricular echogenicity	12 (14.5)	4 (11.1)	6 (14.6)	2 (33.3)
SEH Bilaterally	3 (3.6)	0 (0.0)	1 (2.4)	2 (33.3)
choroid Plexus Cyst	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
second degree IVH	1 (1.2)	0 (0.0)	1 (2.4)	0 (0.0)
ventricular Asymmetry	1 (1.2)	0 (0.0)	1 (2.4)	0 (0.0)

Table 11. Distribution of Characteristics among Subgroups of Neonates with Lenticulostriate Vasculopathy (LSV): Mild, Moderate, and Severe Cases.

Matched Univariable analysis (Conditional Logistic Regression)

To calculate the univariable matched odds ratios, a conditional logistic regression was performed, grouping the data based on gestational week (Table 12, Figure 20). The variables that showed a significant correlation with LSV were increased z-body length (odds ratio [OR] = 1.51, 95% CI 1.09 - 2.08, p = 0.013), increased z-head circumference (OR = 1.52, 95% CI 1.07 - 2.16, p = 0.020) and the presence of other cerebral findings (OR = 2.80, 95% CI 1.08 - 7.26, p = 0.034). Furthermore, when examining individual cerebral findings separately, LSV was associated with the presence of periventricular echogenicity (OR, 4.39; 95% CI 1.25-15.45;P, 0.021).
We note that an odds ratio of 4.06 (95% CI 0.82 - 20.22, P = 0.087) was calculated for the association between an "abnormal head circumference" (z-score of +2 or -2) and LSV, however the p-value did not reach statistical significance (P = 0.087).

We performed a focused analysis only on the severe LSV cases which did not differ from the aforementioned analysis of the whole LSV group. It revealed a significant association between severe LSV and increased z-length (odds ratio: 1.49, 95% confidence interval: 1.04 - 2.13, P = 0.029) as well as with an increased z-head circumference (odds ratio: 1.71, 95% confidence interval: 1.11 - 2.63, P = 0.015) (table 13) .

Matched Univariable Conditional Logistic Regression						
Predictors	**Odds Ratios (OR)**	**SE**	**Z-scores**	**P-values**	**CI (low)**	**CI (high)**
z-length	1.51	0.16	2.492	0.013	1.09	2.08
z-head	1.52	0.18	2.317	0.02	1.07	2.16
Periventricular echogenicity	4.39	0.64	2.305	0.021	1.25	15.45
Other cerebral findings total	2.80	0.49	2.12	0.034	1.08	7.26
z-weight	1.35	0.17	1.718	0.086	0.96	1.89
Abnormal head circumference	4.06	0.82	1.711	0.087	0.82	20.22
Sex	0.70	0.31	-1.161	0.246	0.38	1.28
SEH Bilaterally	2.83	1.09	0.954	0.34	0.33	23.85
Abnormal length	1.97	0.73	0.921	0.357	0.47	8.29
Ventriculomegaly	2.31	1.23	0.681	0.496	0.21	25.69
LGA	1.79	0.91	0.64	0.522	0.3	10.62
Hypertension	1.48	0.61	0.639	0.523	0.45	4.89
Abnormal weight	0.63	0.8	-0.571	0.568	0.13	3.03
CMV	1.58	0.92	0.499	0.618	0.26	9.61
IUGR	1.10	0.56	0.179	0.858	0.37	3.29
Gestational diabetes	0.94	0.38	-0.15	0.881	0.44	2.01
SGA	0.96	1.03	-0.037	0.97	0.13	7.3
Choroid Plexus Cyst	0.00	6319.37	-0.003	0.998	0	Inf
Second degree IVH	284,000,000	8944.37	0.002	0.998	0	Inf

Table 12. Conditional Logistic Regression Analysis of Variables in Neonates with Lenticulostriate Vasculopathy (LSV)

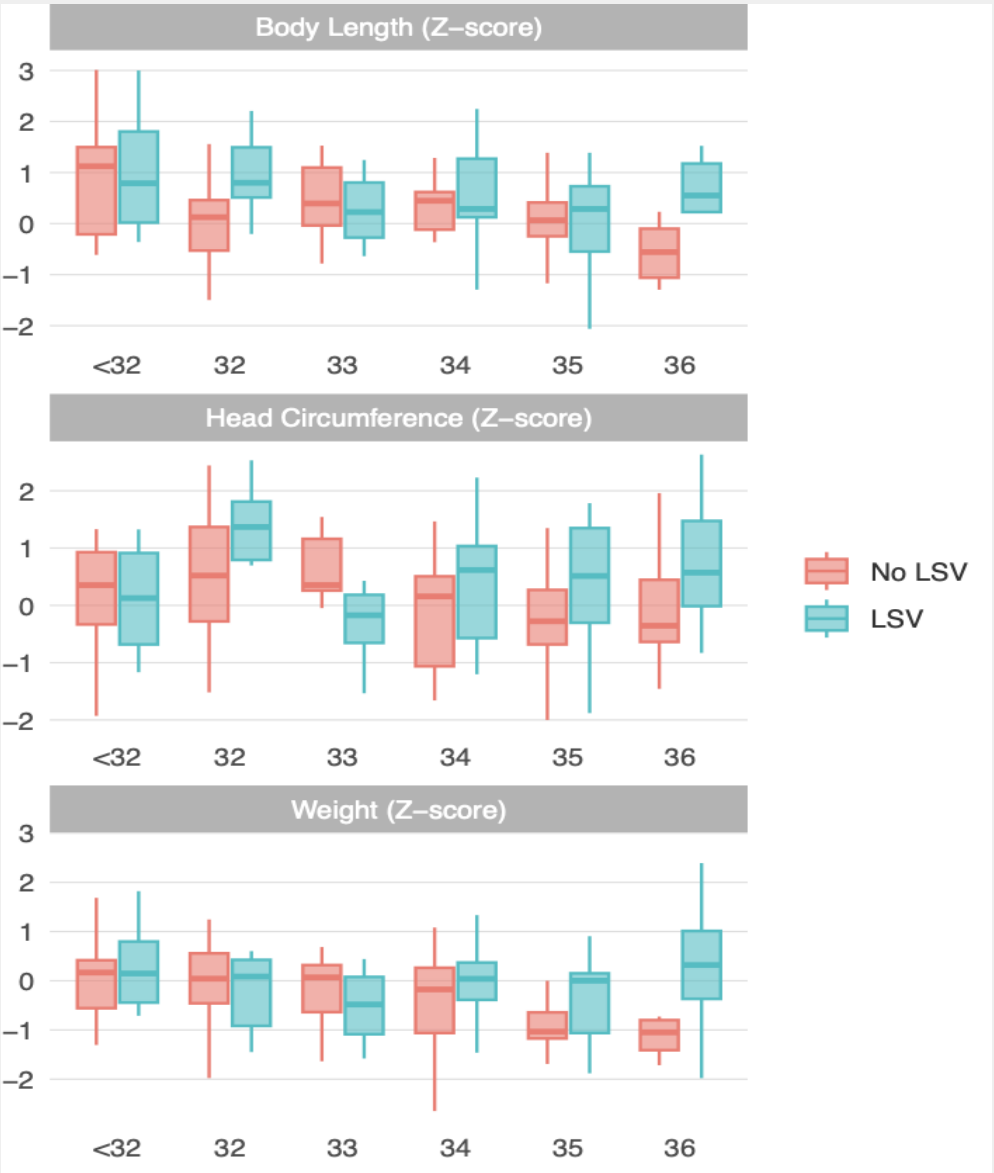


Figure 20. Matched Logistic Regression analysis demonstrating the distribution of z-length, z-weight, and z-head circumference in Neonates with LSV and Controls Across Different Gestational Ages

Severe Matched Logistic Regression						
Predictors	**Odds Ratios (OR)**	**SE**	**Z-scores**	**P-values**	**CI (low)**	**CI (high)**
z-head	1.71	0.22	2.439	0.015	1.11	2.63
z-length	1.49	0.18	2.179	0.029	1.04	2.13
Other cerebral findings total	2.48	0.54	1.673	0.094	0.86	7.16
Periventricular echogenicity	3.07	0.71	1.583	0.113	0.77	12.32
z-weight	1.35	0.19	1.56	0.119	0.93	1.98
Abnormal head circumference	2.96	0.82	1.325	0.185	0.59	14.79
CMV	4.41	1.19	1.249	0.212	0.43	45.3
Gestational diabetes	0.56	0.47	-1.216	0.224	0.22	1.42
SEH Bilaterally	3.85	1.29	1.043	0.297	0.31	48.62
LGA	3.41	1.26	0.977	0.329	0.29	40.03
Abnormal weight	0.47	0.85	-0.893	0.372	0.09	2.47
Hypertension	1.64	0.63	0.781	0.435	0.47	5.68
Sex	0.81	0.35	-0.604	0.546	0.41	1.6
Abnormal length	1.60	0.77	0.604	0.546	0.35	7.28
IUGR	1.43	0.65	0.551	0.581	0.4	5.08
SGA	0.65	1.04	-0.412	0.681	0.09	4.99
Ventriculomegaly	211,000,000	10733.1	0.002	0.999	0	Inf
Choroid Plexus Cyst	0.00	10955.23	-0.002	0.999	0	Inf

Table 13. Conditional Logistic Regression Analysis of Variables in Neonates with Severe Lenticulostriate Vasculopathy (LSV)

Matched Multivariable - Logistic Regression

The multivariable matched odds ratios were calculated to assess the associations between various variables and LSV (table 14). The analysis revealed that the same variables remained significantly associated with LSV, even after adjusting for sex, gestational diabetes, and gestational hypertension. Specifically, z-length (OR 1.58, 95% CI: 1.13-2.21, P = 0.007), z-head circumference (OR 1.57, 95% CI: 1.10-2.26, P = 0.014), and the presence of other cerebral findings (OR 3.09, 95% CI: 1.17-8.15, P = 0.022) all showed significant associations with LSV.

Matched Multivariable Regression (adjust by sex , gestational diabetes, and hypertension)						
Predictors	**Odds Ratios (OR)**	**SE**	**Z-scores**	**P-values**	**CI (low)**	**CI (high)**
z-length	1.58	0.17	2.7	0.007	1.13	2.21
z-head	1.57	0.18	2.457	0.014	1.1	2.26
Periventricular echogenicity	4.86	0.65	2.423	0.015	1.35	17.48
Other cerebral findings total	3.09	0.49	2.285	0.022	1.17	8.15
z-weight	1.41	0.18	1.898	0.058	0.99	2.01
Abnormal head circumference	4.07	0.83	1.7	0.089	0.81	20.56
SEH Bilaterally	2.97	1.08	1.009	0.313	0.36	24.61
Abnormal length	1.91	0.74	0.871	0.384	0.45	8.15
LGA	2.05	0.94	0.765	0.444	0.33	12.8
Abnormal weight	0.58	0.81	-0.67	0.503	0.12	2.85
Ventriculomegaly	2.14	1.23	0.616	0.538	0.19	23.9
CMV	1.49	0.93	0.428	0.669	0.24	9.22
IUGR	1.10	0.57	0.167	0.867	0.36	3.35
SGA	0.97	1.05	-0.028	0.977	0.12	7.67
Choroid Plexus Cyst	0.00	6337.58	-0.003	0.998	0	Inf
Second degree IVH	220,000,000	8968.6	0.002	0.998	0	Inf

Table 14. Multivariable Logistic Regression Analysis of Variables in Neonates with Lenticulostriate Vasculopathy (LSV)

Unmatched Logistic Standard Regression (univariable & multivariable analysis)

In addition to the matched analysis, we conducted both univariable and multivariable unmatched analysis by adjusting for gestational age in a logistic regression (table 15)[236]. We aimed to assess the association between predictor variables and the presence of LSV when taking into account gestational age. The results of this analysis confirmed the findings obtained in the matched analysis, regarding the variables that demonstrated significant associations with LSV among the cases.

When taking gestational age into account, results revealed that increased body length (OR = 1.49, 95% CI: 1.10 - 2.06, p = 0.012), increased z-weight (OR = 2.45, 95% CI: 1.07 - 6.04, p = 0.041), increased head circumference (OR = 1.28, 95% CI: p = 0.028), increased z-head circumference (OR = 1.51, 95% CI: 1.07 - 2.16, p = 0.012), and the presence of other cerebral abnormalities in total (OR = 2.62, 95% CI: 1.07 - 2.16, p = 0.012) were all positively associated with LSV. Tables 11 and 12 respectively demonstrate the results of the univariable and multivariable unmatched analysis.

We additionally performed a focused unmatched analysis only on the severe LSV cases which showed a significant association with increased z-length (OR 2.17, 95% CI 1.44 - 3.47, P = 0.00), z-weight (OR 1.98, 95% 1.28 - 3.23, P = 0.003) and z-head circumference (OR 1.86, 95% CI 1.26 - 2.96, P = 0.007). Furthermore Importantly, severe LSV was significantly associated with neonates who were large for gestational age (LGA) (OR 24.80, 95% CI 2.3 - 670, P = 0.018) as well as those with abnormal head circumference (OR 6.30, 95% CI 1.23 - 47.5, P = 0.0038).

These consistent findings from both the matched and unmatched univariable analyses indicate that neonates with greater z-body length, z-weight, and z-head circumference are more likely to exhibit LSV as observed on cerebral ultrasound, when compared to the control group.

	Univariable			Multivariable Adjust by sex, gestational diabetes, gestational hypertension		
Predictors	**Odds Ratios (OR)**	**P-values**	**95 % CI**	**Odds Ratios (OR)**	**P-values**	**95 % CI**
z-length	1.49	0.012	1.1-2.06	1.58	0.006	1.15-2.2
z-head	1.51	0.021	1.07-2.16	1.58	0.013	1.11-2.29
Periventricular echogenicity	3.78	0.031	1.19-13.9	4.11	0.024	1.27-15.5
Other cerebral findings total	2.62	0.043	1.06-6.95	2.80	0.032	1.12-7.51
z-weight	1.37	0.065	0.99-1.95	1.45	0.041	1.02-2.09
Abnormal head circumference	3.92	0.099	0.9-27.3	3.94	0.099	0.89-27.4
Sex	0.67	0.209	0.36-1.25	n/a	n/a	n/a
SEH Bilaterally	3.05	0.277	0.41-27.9	2.99	0.285	0.41-27.1
Abnormal length	2.07	0.315	0.53-10.1	2.02	0.337	0.5-9.99
LGA	2.07	0.417	0.36-13.2	2.47	0.325	0.41-16.5
Ventriculomegaly	2.26	0.512	0.21-49.4	2.05	0.565	0.19-45.4
Hypertension	1.44	0.556	0.43-5.1	n/a	n/a	n/a
CMV	1.53	0.649	0.25-11.8	1.41	0.715	0.22-11.1
Abnormal weight	0.73	0.688	0.14-3.45	0.68	0.625	0.12-3.28
Gestational diabetes	0.92	0.834	0.44-1.95	n/a	n/a	n/a
SGA	0.96	0.969	0.11-7.22	0.99	0.991	0.11-7.61
IUGR	1.00	1	0.33-3.05	1.00	0.993	0.32-3.1

Table 15. Univariable & Multivariable Unconditional Logistic Regression Analysis of Variables in Neonates with Lenticulostriate Vasculopathy (LSV)

Severe Unmatched Logistic Regression						
Predictors	**Odds Ratios (OR)**	**SE**	**Z-scores**	**P-values**	**CI (low)**	**CI (high)**
z-length	2.17	0.22	3.483	0	1.44	3.47
z-weight	1.98	0.23	2.921	0.003	1.28	3.23
z-head	1.86	0.23	2.721	0.007	1.21	2.96
LGA	24.80	1.36	2.364	0.018	2.3	670.00
Abnormal head circumference	6.30	0.89	2.073	0.038	1.23	47.50
Periventricular echogenicity	3.69	0.7	1.868	0.062	0.95	15.60
Other cerebral findings total	2.66	0.56	1.749	0.08	0.89	8.18
Abnormal length	3.59	0.76	1.686	0.092	0.83	18.30
SEH Bilaterally	3.77	1.5	0.882	0.378	0.13	109.00
SGA	3.67	1.56	0.833	0.405	0.12	115.00
IUGR	0.54	0.83	-0.735	0.462	0.08	2.39
CMV	2.07	1.02	0.712	0.477	0.24	17.90
Hypertension	0.76	0.87	-0.324	0.746	0.1	3.73
Sex	0.92	0.4	-0.195	0.845	0.42	2.06
Gestational diabetes	1.03	0.46	0.068	0.946	0.41	2.49
Abnormal weight	0.95	0.9	-0.055	0.956	0.13	5.21
Choroid Plexus Cyst	0.00	1691.47	-0.009	0.993		2.30E+108
Second degree IVH	93,400,000	2399.54	0.008	0.994	0	
Ventricular Asymmetry	33,200,000	2399.54	0.007	0.994	0	
Ventriculomegaly	0.00	2399.54	-0.006	0.995		3.88E+205

Table 16. Unconditional Logistic Regression Analysis of Variables in Neonates with Severe Lenticulostriate Vasculopathy (LSV)

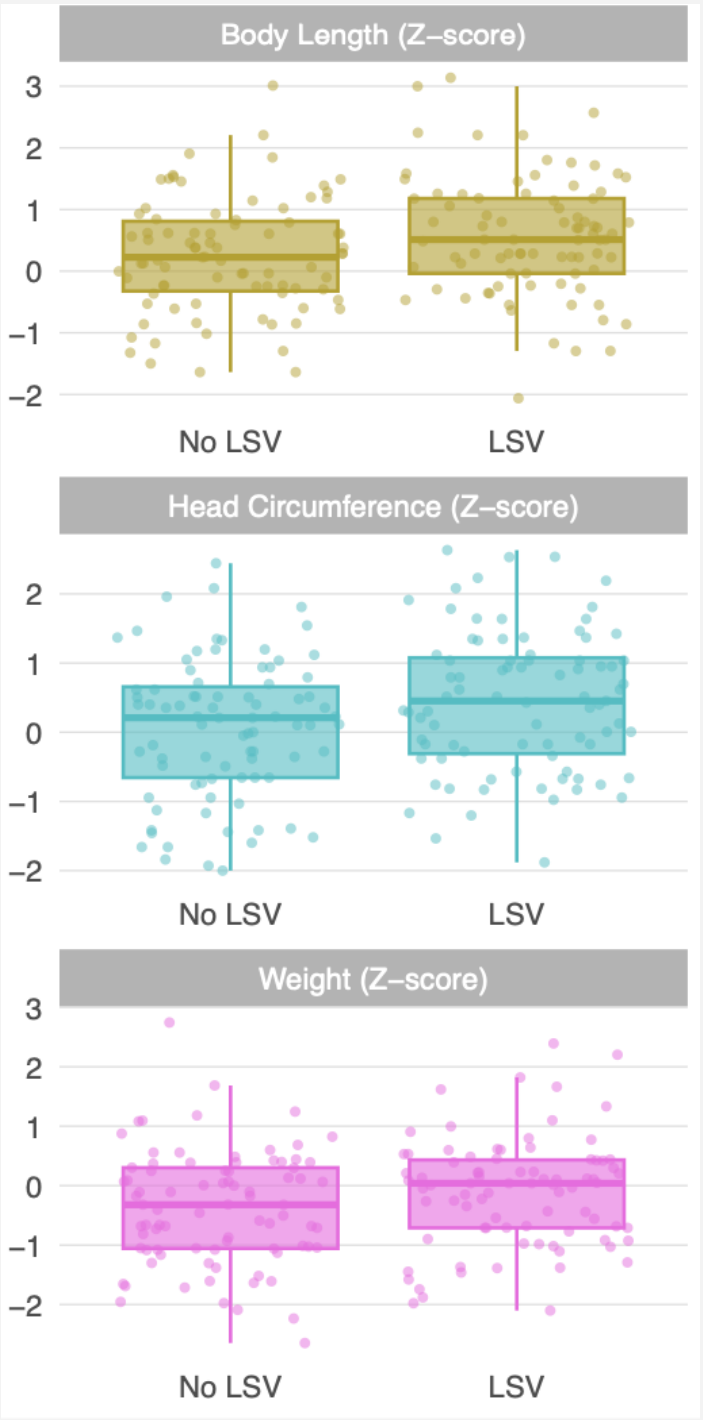


Figure 21. Distribution of z-length, z-weight, and z-head circumference in Neonates with LSV and Controls

		Cases (LSV) vs. Controls					Severe LSV vs. Controls	
Statistical analysis		Matched			Unmatched		Unmatched	Matched
		Univariable		Multivariable	Univariable	Multivariable	Univariable	
		Descriptive statistics	Conditional Logistic Regression		Logistic Regression		Logistic regression	Conditional Logistic Regression
variables	z-length	√	√	√	√	√	√	√
	z-head	√	√	√	√	√	√	√
	z-weight						√	
	Abnormal head						√	
	Other cerebral findings (total)	√	√	√	√	√		
	Periventricular echogenicity		√	√	√	√		
	LGA						√	

Table 17. Overview of variables found to be significantly associated with LSV at the 0.05 level of significance among the different types of analysis performed

4.3.3 Discussion

Despite cCMV being the most prevalent viral congenital infection, public awareness is low and numerous questions regarding prevention, diagnosis, prediction of disease severity and treatment interventions remain. Universal newborn screening programs for cCMV have not yet been implemented, resulting in a large proportion of infected children remaining undiagnosed, missing any opportunity for early intervention. Arguments against a universal screening programme arise from the high costs of screening every live birth for CMV and the absence of a definitive therapeutic intervention. Nonetheless, considering both the current limitations and the recent therapeutic advancements, a more targeted screening approach may be preferable until further progress is achieved to warrant universal screening. Given the neurotropism of the virus, neuroimaging has emerged as having a key role in CMV diagnosis but also in identifying abnormalities indicating CNS involvement, making it the most promising predictive tool of cCMV severity[248].

This thesis involved three separate studies under a common objective, which was aimed towards studying the role of neuroimaging abnormalities as biomarkers for cCMV. We were interested in examining whether prenatal neuroimaging could facilitate early recognition of severely affected fetuses to assist parental guidance and identification of newborns in need of prompt therapeutic intervention.

A systematic review and meta-analysis was initially conducted to evaluate the predictive capability of prenatal MRI and US in determining clinical outcomes in cCMV. Despite challenges in interpreting the heterogeneous findings across studies, a significant observation was the high negative predictive value of normal fetal US and MRI for adverse outcomes. Furthermore, the complementary role of the two imaging techniques in identifying relevant abnormalities was underlined. Since the majority of cCMV infected fetuses will have normal CNS imaging, this information can play a crucial role in reassuring parents and planning follow-up. In terms of cerebral abnormalities, microcephaly was the only finding exhibiting a strong correlation with neurodevelopmental impairment. Finally, we observed a high level of heterogeneity among the studies. The variety of different methodologies and most importantly lack of universal definitions regarding cCMV, diminished the ability to correlate specific findings and outcome. Our meta-analysis stresses the importance

of establishing international consensus regarding cCMV in order to promote a more homogenous clinical practice while also enabling systematic collection of data aiming towards discovering reliable biomarkers of disease severity.

Through our meta-analysis we became interested in the role of MRI in the diagnosis of symptomatic disease. MRI has been shown to have a vital complementary role to ultrasonography in identifying relevant cerebral defects. Recent studies have reported a significant number of cCMV cases with normal ultrasounds but abnormal MRIs. Especially when discussing identification of white matter changes and cortical malformations related to neuronal migration, MRI is clearly superior to US. In clinical practice, clinicians encourage doing a neonatal MRI, when US cranial abnormalities are identified. However, limitations related to accessibility, cost, and sedation hinder its widespread use. Conversely, fetal MRI is a less invasive procedure performed during the prenatal stage. It allows more time for decision-making while at the same time avoiding some of the negative implications (i.e. neonatal sedation) associated with neonatal MRI. We believe that studying the correlation of fetal and neonatal imaging might therefore be of great value. To our knowledge there is no study directly comparing the results between fetal and neonatal MRI in children with cCMV infection. Based on this premise, we performed our second study.

We conducted a small retrospective study (case-series) of 10 patients with known cCMV who had undergone both fetal and neonatal MRIs during their perinatal assessment and compared the reported findings. We specifically aimed to compare the diagnostic accuracy of fetal MRI to identify relevant cerebral abnormalities compared to neonatal MRIs. Despite the limited sample size, the observations indicated that fetal MRI could provide comparable information to neonatal imaging, indicating that it could potentially become an important diagnostic tool helping clinicians decide when to perform neonatal MRIs in high-risk patients. Of note, there were high levels of concordance between fetal and neonatal images with a positive predictive value (PPV) of 70% (fetal MRIs and Neonatal MRIs were concordant in 70% of cases). Surprisingly our study findings imply that fetal MRI not only identifies important findings, but may even overestimate cerebral abnormalities. Larger prospective studies are needed in order to compare the ability of fetal versus

neonatal imaging to identify cerebral abnormalities, as well as to establish the optimal gestational and neonatal age at which it should be performed.

Through our first two original studies we had the opportunity to recognise the importance of neuroimaging (MRI and US) in the diagnosis and prediction of neurological outcomes in infected infants. Nevertheless, both imaging techniques carry their own disadvantages, such as the MRI's indisputable limitation related to accessibility and cost. On the contrary, cerebral US is a non-invasive and cost-effective modality that holds particular promise due to its routine use in neonatal intensive care units worldwide. However, even though multiple cerebral abnormalities have been associated with congenital infections, they are not specific to cCMV and are commonly also observed in other pathologies. In order to make full use of the ultrasound's advantages and potential role in cCMV diagnosis it is imperative to identify abnormalities more distinctively associated with congenital cytomegalovirus (cCMV).

Over the past three decades, lenticulostriate vasculopathy (LSV) has garnered significant attention and since its first description in 1985 in cCMV neonates, it has often been used in association to cCMV. Numerous studies have documented an association between higher-grade cases of LSV and CMV infection, with reports arguing it can be used as a marker of CNS involvement and sensorineural hearing loss. Nonetheless, certain researchers argue that isolated lower-grade LSV cases might not serve as a reliable indicator of adverse outcomes or have long-term consequences. Despite the subject being studied for over three decades, experts have yet to reach a consensus regarding the clinical significance of LSV.

Our third and final study was a prospective 1:1 case-control study matched for gestational age, aiming to explore the potential role of LSV in congenital cytomegalovirus infection (cCMV) diagnosis. Our study included a total of 166 participants (83 cases and 83 controls). The primary objective was to investigate whether the presence of LSV, in conjunction with prematurity—a frequently observed consequence of congenital infections—could serve as a diagnostic tool for cCMV. Furthermore we also aimed to examine the relationship between other perinatal characteristics and LSV.

We performed both a matched and an unmatched analysis (univariable and multivariable) and found no statistically significant difference in the presence of congenital cytomegalovirus (CMV) infection between cases and controls. It is important to note that there were only 5 children with cCMV (3 in the cases and 2 in the control group). Therefore, our study did not have the power to identify an association between LSV and cCMV. Notably, neonates with LSV (cases), had a higher occurrence of concomitant cerebral abnormalities, and when analyzed against each separate abnormality, LSV was primarily associated with periventricular echogenicity. Regarding somatometric measurements (reported as Z-scores), an increased z-length and z-head circumference was associated with the presence of LSV. These associations remained significant in the multivariable logistic regression, even after adjusting for sex, gestational diabetes, and gestational hypertension, meaning that LSV was associated with increased head circumference and length irrespective of the presence of relevant confounders. Additionally, severe cases of LSV (n=6) were correlated with neonates who were classified as large for gestational age (LGA), and had a higher prevalence of abnormal head circumference (above or below 2 standard deviations from the mean) (Table 12)

Our results consistently demonstrate the surprising finding that neonates with LSV exhibited a non-pathological but statistically significant increase in body length, weight, and head circumference compared to their controls. To date, the pathophysiological mechanisms proposed for LSV have been associated with an inflammatory response following a cerebral insult and intrauterine stress. Consequently, one would expect to observe microcephaly in this context, a finding also associated with symptomatic CMV disease[249]. These contradictory findings prompted us to review the available literature on the pathophysiological mechanisms underlying the appearance of LSV.

The notion that LSV is associated with a vascular pathophysiological mechanism is supported by Doppler studies and histopathological examinations revealing thickened, hypercellular arterial walls with basophilic deposits in the basal ganglia and/or thalamic area[190,192,186]. However, it is important to note that these findings have not been consistently observed in all histopathological examinations of infants with LSV on cerebral US. Based on findings such as acute neuronal necrosis and

reactive gliosis, other mechanisms have been suggested to play a role such as hypoxic-ischaemic injury[250]. Additionally, advancements in Doppler ultrasounds have enabled improved imaging of deeper and thinner vessels, which may cause potential overdiagnosis of a non-pathological feature. Studies assessing the agreement between radiologists have shown low levels of inter-observer reliability, further complicating the interpretation of subjective findings, particularly when they are mild in nature[244]. Consequently, determining the clinical significance of such subjective findings can be exceptionally challenging.

Interestingly, in a recent study by Sisman et al[247] also found that neonates with stage 3 (severe) LSV had significantly higher birth weight when compared to controls and neonates with mild or moderate LSV. However, in this cohort authors did not report length or head circumference, neither offered a plausible pathogenic explanation for this finding. Moreover, the same group recently reported that neonates with severe LSV are more likely to have large for gestational age (LGA) placentas[251].

The presence of placentomegaly (thickness > 40mm) has been observed in congenital infections associated with placentitis[252]. However, the relationship between large for gestational age (LGA) placentas and larger neonates (LGA) remains unclear, lacking clear data to support our findings. Limited studies on gestational diabetes have reported the co-occurrence of LGA placentas and LGA neonates, but this has primarily been explored within the context of diabetes pathophysiology[253]. Therefore, caution should be exercised when generalizing these findings.

In their recent study, Sisman et al. mention the concept of "fetal programming" and its relevance to the relationship between prenatal insults, such as placental structural abnormalities and inflammation, and neonatal outcomes. They discuss potential mechanisms involving placental adaptations, including advanced villous maturation and compensatory growth, in response to stress. While the study itself does not investigate compensatory growth mechanisms in relation to intrauterine pathologies, the authors address this area of research that has not yet been extensively explored.

Another notable clinical observation in this study was the presence of LSV on cerebral ultrasounds of neonates who did not exhibit LSV in their previous scans. This finding suggests that LSV can develop and become detectable in the late

neonatal period. However, due to our study's exclusion criteria (neonates above 21 days of life were excluded in order to confidently diagnose congenital infection), we were unable to include those specific neonates in the analysis. The fact that LSV was commonly observed after the first 3 weeks of life has been reported by other studies and could imply that LSV represents a neonatal brain injury that may manifest with a delay[198].

In conclusion, this case-control study does not provide sufficient evidence to support the use of lenticulostriate vasculopathy (LSV) as a standalone diagnostic tool for congenital cytomegalovirus (cCMV) infection. This lack of evidence can be attributed to the limited sample size of our study. LSV was initially described in cases of congenital CMV infection over 30 years ago and has since been suggested to be associated with central nervous system (CNS) involvement and sensorineural hearing loss. Furthermore, some experts had suggested that asymptomatic cCMV infected infants with LSV should be offered antiviral treatment to improve hearing outcome. However, most previous studies investigating its significance were conducted with CMV-infected participants, leading to bias in evaluating the results.

Additionally, in a recent large prospective study it was indicated that although LSV is a common HUS finding in infants with cCMV infection, its presence is not predictive of an adverse outcome and that it is a rather unreliable finding in selecting candidates to antiviral therapy. Moreover, LSV has also been observed in various other conditions and is commonly found in healthy neonates without any signs of CNS pathology. Our findings, particularly the significant association of LSV with other cerebral abnormalities, suggest that LSV may represent a non-specific cerebral insult, the clinical significance of which is yet to be determined. Therefore, we recommend that when LSV is the only cerebral US finding in asymptomatic newborns this should not necessarily lead to further investigations such as MRI or to antiviral treatment. On the other hand it should not be disregarded in a newborn born post maternal PI early in pregnancy or in a nenoate with additional other mild symptoms. Therefore, based on the available data, the presence of LSV cannot serve as a marker prompting further diagnostic or therapeutic interventions in asymptomatic cCMV neonates. Larger prospective blinded studies are required to gain a better understanding of the significance of this finding.

Study Limitations

It is essential to acknowledge the limitations of all three study designs and data collection process. Our initial systematic review and meta-analysis encountered limitations associated with the heterogeneity of the included studies. Specifically the studies exhibited differences in terms of design, criteria, timing, and follow-up. Furthermore, the predominance of observational studies introduced potential biases and inconsistencies in reporting. The limited number of studies performing fetal ultrasound and MR at the same gestational age, hindered robust comparisons. The need of establishing standardized protocols and consensus regarding cCMV was underlined.

Regarding our second study (case-series), limitations involve the fact that it was a case series with a small sample size identified using convenience sampling which may have introduced bias. The retrospective design limited control over imaging timing, meaning that fetal and neonatal MRIs were not all performed at the exact same gestational age. Finally, even though our study was blinded, there was a checklist of CMV related abnormalities created for the radiologists to evaluate, which is not representative of everyday practice.

Our final prospective case control study exploring the role of CMV in LSV diagnosis also involved a number of limitations. First, our study only identified five individuals with cCMV. The limited number of cCMV cases did not allow for the statistical power necessary to adequately study the relationship between cCMV and LSV. . The decision to limit the study duration to three years was made to ensure the feasibility of data collection within the available resources and logistical constraints. Despite the reduced sample size, we believe that the enrolled participants adequately represent the target population of premature neonates with lenticulostriate vasculopathy (LSV) and controls without LSV. Second, the absence of multiple independent reviewers for each image may have influenced the accuracy and reliability of the diagnostic assessments, especially when identifying the milder cases of LSV. To strengthen the objectivity and validity of the interpretations, the inclusion of multiple radiologists would have been advantageous. However, through our sensitivity analysis we identified no statistical difference on the incidence of LSV between the different radiologists.

Finally, the study relied on the initial scan data and did not include any follow-up scans. This limited the ability to capture potential changes or developments in the diagnostic markers over time. Longitudinal assessment would have provided a more comprehensive understanding of the condition and its progression. Even though such data were not directly related to our specific aims, we look forward to future studies on disease progression.

To summarize, the main findings of this work include:

A) Normal fetal US and MRI have a very high negative predictive value for adverse outcomes in cCMV-infected fetuses. Since the majority of cCMV infected fetuses will have normal CNS imaging, this information can be used as a tool to reassure both parents and clinicians.

B) There is a high level of concordance between fetal and neonatal cerebral MRI and fetal MRIs do not underdiagnose abnormalities. This emphasizes the potential role of fetal neuroimaging to identify cerebral abnormalities and advise parents. Further studies need to establish the optimal gestational age at which it should be performed.

C) Our prospective case-control study suggests that the presence of LSV should not be included among potential findings triggering the screening of cCMV. Mechanisms associated with a potential vascular pathology have been proposed as a result of a perinatal insult and subsequent inflammatory processes, although alternative mechanisms have also been suggested. While LSV, particularly in severe cases, should not be disregarded, it currently lacks the ability to serve as a definitive diagnostic marker for CMV based on the available data.

D) LSV was associated with the presence of other cerebral findings on cerebral ultrasound, especially periventricular echogenicity. It was also associated with larger somatometric measurements (z-head circumference, z-body length) compared to matched controls and severe cases were linked to LGA neonates. Further studies are needed to understand the underlying pathophysiological mechanisms.

E) Routine CMV screening in neonates with LSV cases should only be applied once well-designed larger studies demonstrate a clear diagnostic benefit.

Future Research

It has become clear through both our research and work from other groups, that in children with cCMV, a normal fetal and/or neonatal MRIs has a high NPV for an adverse long term outcome (SNHL and neurodevelopmental impairment). Thus this is an important message for clinicians when consulting parents.

Future research should now focus on identifying the clinical relevance of specific findings on cerebral US and MRI in neonates with cCMV. This will be important both for anticipatory guidance as well as decision making on neonatal antiviral treatment.

Additionally, at present we are facing a different cohort of babies born post maternal antiviral treatment (ie fetal treatment) and thus new data will accumulate.

Further research is warranted to explore the underlying pathophysiological mechanisms of lenticulostriate vasculopathy (LSV) and its potential associations with adverse neonatal outcomes. Considering the recent evidence linking LSV to large for gestational age (LGA) placentas and our own findings of a correlation between LSV and increased z-head circumference, z-body length, and LGA neonates, additional investigation is needed to explore potential pathophysiological mechanisms that could connect these phenomena. To enhance the consistency and reliability of future studies, collaboration among researchers and the standardization of LSV grading systems should be prioritized to avoid false positives.

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