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Infants at high neurological risk for cerebral palsy: new pathways for early diagnosis and early intervention

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Table of contents

Chapter 1

General Introduction and Thesis' outline

1.1 Background

Cerebral palsy: Definition and Epidemiology

Cerebral Palsy (CP) is a nosographic term referred to a spectrum of pathological conditions affecting the developing brain and represents, by now, the most common cause of motor disability in childhood. Since the first description in 1861 by Little, the definition has changed over time, until the most recent revision in 2006 after the international workshop of Bethesda that gathered leading scientists in pre-clinical and clinical research around this medical condition. Cerebral palsy was then defined as *"a group of permanent disorders of the development of movement and posture, causing activity limitation, that are attributed to non-progressive disturbances that occurred in the developing fetal or infant brain. The motor disorders of cerebral palsy are often accompanied by disturbances of sensation, perception, cognition, communication, and behavior, by epilepsy, and by secondary musculoskeletal problems*" (1). A systematic review and meta-analysis by Oskoui and colleagues (2013)(2), aimed to define an overall prevalence of CP, reported 2,11 cases per 1000 live birth (95% CI 1.98-2.25); furthermore, stratifying by birth weight and gestational age group, the prevalence of CP reached the highest prevalence in infants born before 28 weeks (111.80 per 1000 live births; 95% CI 69.53-179.78) and in infants with birth weight 1000-1499 g (59.18 per 1000 live birth; 95% CI 52.06-66.01) (2). As for the risk factors for developing CP, pre-eclampsia, twin pregnancy, massive intrapartum haemorrhage, prolonged traumatic delivery, placental inflammation (e.g. chorioamnionitis) during the latter stage of pregnancy or labour, prolonged rupture of membranes, prematurity and low birth weight are considered the most significant (3). As for the etiology of CP, we can differentiate antenatal causes represented by congenital brain malformations, vascular events, maternal infections during the first and second trimester; perinatal causes, such as obstructed labour, antepartum haemorrhage, cord prolapse, severe infant hypoglycemia, untreated jaundice or severe neonatal infection and post-natal causes like cerebrovascular accidents or cerebral infections. Except for brain malformations that account for a 10% of cases, almost the 90% of CP results from brain injuries damaging a healthy tissue. All the cited causes generally predispose to pathological conditions of brain hypoxia and/or ischemia with different consequences in term of lesion patterns and clinical outcome according to the gestational age and consequently the degree of brain maturation at which the disruption occur. In preterm infants, periventricular white matter represents the most vulnerable site of disruption which results in "periventricular leukomalacia", the typical lesion pattern of premature brain with diverse degrees of severity. In full-term infants, the cerebral cortex is typically affected with subcortical and periventricular white matter involved at various degree according to vascular supply impairment (4).

Clinical manifestations of cerebral palsy are very heterogenic as for the type of movement disorder, the topography of the motor impairment and the neurological signs, the degree of functional ability and limitations. The main classification systems are addressed to define the degree of impairment and the activity limitation levels. As concern the impairment classification, CP are defined on the basis of the predominant type of movement disorder as spastic, dyskinetic, ataxic or hypotonic; the topography of the motor impairment which allow a distinction between hemiplegic, diplegic and tetraplegic forms is still employed clinically, although more recently, a simplified classification in unilateral or bilateral with an indication of upper and lower extremity function is preferred. As regard the activity limitation, the Gross Motor Function Classification system (GMFCS) is widely used (5) and consists in a rating system of patient's ambulatory function, the use of mobility aids as well as performance in sitting, standing and walking. The Manual Ability Classification System (MACS) was developed for describing the upper extremity performance in activities of daily living in subjects affected by CP (6).

Given the developmental nature of this complex disorder whose signs and symptoms emerge early in infancy, and persist through lifetime, an early diagnosis is mandatory to obtain a prompt referral to appropriate intervention pathway and try to achieve the best levels of functional outcome.

Early detection, early surveillance, early intervention:

The etiological investigations on the complex causal paths responsible for CP have represented an important research stream in the last decades. Above all, the achievements in the genetic field, seem of particular interest for etiopathogenetic purposes, although far from being conclusive. Beyond these important progresses in understanding the causal mechanisms of such a disabling disease, the absence of a cure, the early onset, and the long-life impact of symptoms as well as the suggestions from pre-clinical research that the infant brain is more keen to neuroplasticity, have implied more efforts in earlier detection of CP. Historically, in fact, the diagnosis occurred between age 12 and 24 months, but in 2017, Novak and colleagues systematically reviewed the literature to summarize the available data on the best diagnostic clinical tools and their reliability in order to achieve an earlier detection of CP (7). They found evidence that an accurate diagnosis of CP is possible before 6 months of corrected age and suggested that a combination of magnetic resonance imaging (MRI) (86%-89% sensitivity), General Movements Assessment (GMA) (98% sensitivity) or Hammersmith Infant Neurological Examination (HINE) (90% sensitivity) should be recommended to define a diagnosis or, at least, an "high-risk" condition to develop a CP. This statement implies, therefore, an earlier infants' access to specific surveillance services aimed to assess the functional limitations and the potential complications often associated to the motor impairment since the very first months of age. These include chronic pain, epilepsy, intellectual disability, hip displacement, scoliosis, behavior disorders, sensory impairments that should be prevented or at least managed to limit secondary major complications over time.

Beyond a careful clinical follow-up of the neurodevelopmental trajectory of infants with CP, the opportunity of an earlier diagnosis, allow also to take advantage of the so-called "sensitive periods", time windows in which the brain plasticity is at its highest expression, being more receptive to environmental stimuli than later in life (8). At this stage, evidence-based interventions should be delivered as soon as possible to maximize functional outcomes shaping the neural circuitries reorganization. A recent review by Novak and colleagues (9) analyzed the best evidence-based interventions available both in preventing and in managing cerebral palsy, providing a guidance both to clinicians and families in the decision-making. A section of this review was specifically addressed to early interventions that, although more limited in number, represent a rising area of scientific investment. Specifically, novel proposals of intervention based on active motor-learning and environmental enrichment principles have demonstrated promising improvements in motor and cognitive skills in infants with CP (10–13).

Early detection: the UCP diagnostic issue

In the last twenty years, many efforts have been made to ameliorate the diagnostic sensitivity for CP and build up appropriate care pathways laying on scientific evidence and translational models based on pre-clinical results. While optimal results have been achieved for the detection of the CP in general, in particular to discriminate pathological conditions from low-risk ones, some issues remain still open for the detection of specific subcategories of CP such as Unilateral Cerebral Palsy. In this category, pregnancy and labor may appear uneventful and first pathological motor signs could appear later, at about 5 months of age, when the motor skills become more complex mainly for the upper limb. Thus, the neuroimaging approach is mandatory to achieve a diagnosis of unilateral brain damage. In other cases, when a well-known asymmetrical or clearly unilateral lesion occur both in preterm and in full-term infants, the above mentioned clinical tools used to evaluate an infant at high neurological risk (GMA and HINE) seem to loose, at least in part, their diagnostic strength. Infants with milder presentation of CP could in fact report score in a normal range in standardized scales although the quality of movement could be abnormal. Some authors reported that some cases of UCP could be missed by General Movement Assessment despite its high sensitivity in detecting CP in general (14). Furthermore, HINE scores above the thresholds defined for the motor disorder were reported, in infants with UCP, leading to a misinterpretation of the results (15). Over the years, many authors recommended the evaluation of segmental movement asymmetries as a reliable qualitative sign of unilateral motor impairment (16,17) but dedicated standardized assessments are still lacking. As regard the neuroimaging, compelling

studies suggest that MRI diffusion techniques represent, to date, the gold standard for the diagnosis of perinatal stroke and a growing literature has focused on the detection of early neuroimaging markers for predicting the clinical outcome. The DTI acquisition in follow-up MRI has been recognized to be a reliable predictor of neurodevelopmental outcome, allowing the detection of abnormalities, namely asymmetries, in the descending corticospinal tracts (CST), definable at the levels of the posterior limbs of the internal capsule, cerebral peduncles, basis pontis and medullary pyramids (18,19). Advanced diffusion MRI techniques aimed to perform white matter analysis across the whole brain to estimate structural connectivity patterns both in the lesioned and non-lesioned hemispheres were recently explored as promising approaches for reliable correlations with clinical outcome. Mackey and colleagues recently proposed a new semi-quantitative system for scoring early ischemic changes on imaging and found a significant correlation between the score and the rate of cerebral palsy and epilepsy outcome in neonates with perinatal arterial ischemic stroke (20). Although supported by robust evidence, neuroimaging extensive application for diagnostic and prognostic purposes is still hindered by some limitations. For instance, some authors suggest that diffusion MRI technique reach the highest sensitivity in detecting CST abnormalities in follow-up neuroimaging acquisition, while, in the acute phase, at perinatal age, diffusion could be less sensitive in assessing the white matter damage extension because of the physiopathological mechanisms of tissue reorganization after vascular injury. Another important point concerns the type of lesion investigated. More recent works illustrated the effectiveness of advanced diffusion techniques associated with specific imaging scoring. However, these were focused on homogeneous samples in terms of lesion pattern and etiology (e.g. perinatal arterial ischemic stroke) and thus their results shouldn't be generalized to all the types of asymmetrical brain lesion. Finally, the application of advanced MRI techniques often requires a specific knowledge and expertise which is not always and easily available. Clinical assessment is then still recommended to be integrated in the evaluation of an infant at risk of UCP, electrophysiological assessments should be considered as well as a potential field of investigation of new reliable biomarkers.

Early intervention: the family-centered care as a neurobiological-based model for new infant habilitative programs

The most recent international recommendations on early intervention (EI) for infants at high-risk of CP aged 0-2 (21) strongly suggest the use of models based on the family-centered care paradigm (FCC) and stress the crucial role of parental engagement together with the need to support parental mental health and wellbeing to potentially augment the effects of personalized habilitative treatments. The emphasis given to early detection of CP, and consequently, the need to anticipate infants' referral to early rehabilitation services, has also steadily changed the role of the family in CP care pathways during the last decades. While the role of parents in ameliorating the care in this clinical population has long been supported in the NICU, awareness that families could play a central role in early habilitative processes, beyond neonatal care, has increased over time and only lately become a major focus of interest. The attention paid to early identification of children at risk of CP also shed light on the importance for early habilitative models to take into account the young age of the patients and the need of parental mediation to promote infants' active involvement. Therefore, the extent to which families participated changed over time: child-focused habilitative programs steadily moved toward models that emphasized the role of parents and the use of ecological environments in activities proposals (22). Novak and colleagues (2017) highlighted in their review work that the best outcome of an early intervention in infants at neurological risk can be achieved when the treatment is delivered at home, in natural settings and with an active parental involvement in the process (7). During early infancy, caregiving practices can facilitate and/or constraint motor development. Therefore, differences in how caregivers structure the environment and interact with their children affect learning of new motor skills and shape their motor developmental trajectory (23). The focus of rehabilitative interventions for children with or at risk of CP has gradually shifted from passive mobilization towards approaches based on motor learning-induced plasticity that emphasize the promotion of environmental enrichment to facilitate goal-oriented and child-initiated activities (10,11,21). In the last decades, evidence from pre-clinical studies on animal models has stressed the concept of "environmental enrichment" (EE) as a crucial promoter of

neuroplasticity, especially in the developing brain. These studies highly contributed, through structural, molecular and neurophysiological investigations, to strengthen previous behavioral intuitions that parental involvement had a beneficial effect in the infant development trajectory being itself a form of environmental enrichment. EE is indeed a combination of factors that produce stimulating environments and challenge the continuous adaptation to the surrounding through the exposure to (likely novel) sensory stimuli, greater social interactions, and voluntary physical activity (24). At first focused on physical enrichments, EE research on animal models has gradually introduced multisensory and social approaches. In humans, although the translation of EE paradigms is more challenging, in the context of CP, the role of social touch and parental caring strategies, in particular parent-infant interactions, as sensory stimulations have received more attention in early intervention programs (21,25). Among sensory modalities, touch is the first to develop and the first sense that builds the perception of our own bodies and the sense of self and the surrounding world (26). Social touch received from others is also one of the crucial components of early environmental enrichment and this is appreciable even prenatally (27). After birth, mothers stay in close proximity to their infants to provide them with physical comfort, and touch is one of the most used senses to establish the early caregiver-infant bonding (28). Affective and social touch is a powerful promoter of human development as well. Guzzetta and colleagues (2009) tested the effects of tactile stimulation in human preterm infants (exposed to impoverished environment) and rodent pups and found that tactile stimulation (or infant massage in the case of infants) accelerated the maturation of visual function and brain developmental activity, measured through the electroencephalogram (EEG) (29,30). Infants' social touch, and in particular touch by or with caregivers during early affective interactions, and also during mother-infant face to face interactions, is considered a means of regulation of infant physiological and emotional states (likely through the hypothalamic-pituitary-adrenal axis), and the application of the tactile stimulations, including kangaroo mother care and infant massage, has become a well-established procedure in neonatal intensive care units (NICUs) and a particularly relevant model of social environmental enrichment for very early intervention (29–33). Taken together, evidence seem to suggest that early intersubjectivity is one of the highest forms of social enrichment and early interactions may represent a valuable field of intervention for fragile infants and their parents. Care programs should then specifically focus on empowering early parent–infant transaction from birth onwards and on promoting parents' wellbeing in populations of infants at high neurological risk.

1.2 Purpose of the thesis

CP is a well-known neurodevelopmental disorder with a still quite high prevalence in child population, despite the advances in neonatal intensive care. An extensive literature supports evidence-based recommendations on the choice of the better diagnostic tools to achieve an early and accurate diagnosis for a prompt referral to habilitative services. Nevertheless, the possibility to achieve an early diagnosis has imposed some issues both diagnostic and rehabilitative. Particularly, the UCP detection and prognosis in infants with unilateral brain damage is still challenging before 6 months and early evidence-based intervention programs are still limited in number and efficacy.

The main aim of this PhD project was to address these issues with review studies, a study of feasibility for a protocol of early intervention and two observational studies on infants at high neurological risk of UCP according to neonatal neuroimaging examination.

The present thesis is composed of 2 sections. **Section 1** addresses the UCP diagnostic issue. First, a systematic review of the literature on the early clinical signs of congenital hemiplegia was conducted. Then, preliminary data of an observational longitudinal study on the natural history of UCP in a sample of infants referred to Infant Neurologic Section of IRCCS Fondazione Stella Maris Institute following the occurrence of a perinatal unilateral brain lesion is presented in order to evaluate potential early prognostic biomarkers and to speculate on the possible underlying physio-pathological mechanisms. Finally, interhemispheric difference in sleep spindles in infants with perinatal unilateral brain injury as a potential biomarker for the detection of the risk of UCP outcome was investigated. **Section 2** addresses the early intervention topic. A systematic

review on the available data on early parent-infant interactions in infants at high-risk of CP was first conducted. Then, a feasibility study of a home-based and parent-delivered intervention based on infant massage in infants at high risk of CP is presented.

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SECTION 1

Unilateral Cerebral Palsy: the challenge of early diagnosis

Chapter 2

Early motor signs in unilateral spastic cerebral palsy: a systematic review

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In preparation

2.1 Introduction

Unilateral cerebral palsy (UCP) is a long-lasting complex neurodevelopmental disorder which represents the most common outcome after the so called "perinatal stroke" whose prevalence has been recently estimated to be approximately in 1 in 1100 births. This umbrella term encompasses a group of cerebrovascular diseases generally occurring around the perinatal age (between the 20th week of gestation and the 28th day after birth) such as neonatal arterial ischemic stroke (NAIS), neonatal cerebral sino-venous thrombosis and neonatal hemorrhagic stroke (NHS), but also other kind of focal vascular injuries that clinically present themselves later in infancy or childhood such as periventricular venous infarction (PVI), arterial presumed perinatal ischemic stroke (APPIS) and presumed perinatal hemorrhagic stroke (PPHS) (1). Given the chronic nature of the disease and the possible involvement of different functions other than the motor one, (language, vision, cognition, emotion, and behavior) as well as the affection of the family wellbeing, an appropriate intervention is mandatory to achieve the highest level of autonomy for the patient in his everyday life. An extensive literature supports, so far, the neuroplastic potential of interventions based on the neurobiological principles of environmental enrichment and activity-dependent motor learning that reach their maximum effectiveness in a specific time-window corresponding to the first three years of life (2–8). Novak and colleagues in 2017 systematically reviewed the existing literature in order to define the best evidence for early diagnosis (before 5 months of age) and early intervention protocols in infants at risk of cerebral palsy (CP) in order to take advantage of neuroplasticity. Their results brought to the recommendation of applying a combination of standardized tools in population of high-risk infants to detect cerebral palsy, with high-quality evidence that GMs assessment or HINE combined with neonatal MRI achieve a high rate of accuracy (9).

Indeed, GMs assessment has shown an extremely high predictive value as to the development of CP in infants at neurodevelopmental risk. In infants with unilateral brain damage, *Fidgety movements* typically expressed between 9- and 20-weeks post-term age, seem to be very useful in the early identification of congenital hemiplegia, although their diagnostic and prognostic accuracy remain still limited as to topography and severity of the impairment (10). More specifically, the absence of typical bilateral fidgety movements was shown to strongly correlate with the development of CP, although a heterogeneous expression of the fidgety pattern was reported. In children with UCP, patterns of asymmetrical representation of fidgety movements, bilateral absence of fidgety movements or sporadic but bilaterally present fidgety movements were all reported, suggesting a large variability in the expression of early motor repertoire in UCP.

More traditional neurological examinations, such as the HINE, have also shown good predictive power in infants at risk. This is particularly true in those that are older than 6 months as the number of false positives and negatives is still high at an earlier age, and in particular for infants with unilateral brain damage at risk of developing UCP (11). More recently, attention has shifted from the global scores at the HINE to the possible presence and number of asymmetries, consistent with lesion side, in the various items of the assessment (12). A threshold number of 4 asymmetries was identified as a good cutoff to predict hemiplegia, but the age heterogeneity of the samples assessed does not allow to define the role of asymmetries in the specific age range between birth and 6 months post-term (13).

Understanding the variability of early motor signs in a population at risk of developing can contribute to clarifying potential mechanisms of plasticity after perinatal stroke as well as predicting the motor outcomes and maximize the functional recovery by means of personalized early interventions. The main aim of this review was to collect information on the predictive validity of the available clinical tools in assessing the risk of developing UCP after a perinatal brain injury, detected by mean of neuroimaging (ultrasound or brain MRI), within the first 6 months post-term.

The specific questions we addressed were:

- What is the predictive diagnostic accuracy of the available clinical assessment tools in a population of infants at risk of Unilateral cerebral palsy? To answer this question, we searched for prospective

papers exploring early signs in at risk populations and their predictive pawer as to the development of UCP.

- What were the early clinical characteristics of infants later diagnosed with unilateral cerebral palsy? To answer this question, we searched for both prospective and retrospective studies in which early motor signs in children later diagnosed with UCP were available.

2.2 Methods

Literature Search and selection of studies

A systematic literature search was performed in Janaury 2022, through the following electronic databases: PubMed/MEDLINE, EMBASE (OVID), Web of Science. No publication date limits were applied to the searches. The following search strategy, including both MeSH headings and keywords, was used: ("infant") OR ("baby") OR ("newborn") OR ("neonate") OR ("preterm infant") AND ("hemiplegia") OR ("congenital hemiplegia") OR ("congenital hemiplegic cerebral palsy" OR "congenital hemiplegias") OR ("unilateral cerebral palsy") OR ("unilateral spastic cerebral palsy") OR (hemiparesis)) OR (congenital hemiparesis) AND ("general movements") OR ("general movement assessment") OR ("general movement assessment gma") OR ("dubowitz neurological assessment") OR ("dubowitz neurological examination") OR ("hammersmith infant neurological examination") OR ("hammersmith infant neurological examination hine") OR ("fidgety movement assessment") OR ("neurological examination") AND ("brain stroke) OR ("cerebral stroke")) OR ("pediatric stroke") OR ("perinatal stroke) OR ("ischemic cerebral stroke") OR ("hemorrhagic cerebral stroke") OR ("hemorrhagic stroke") OR ("cerebral venous thrombosis") OR ("venous infarction") OR ("cerebral venous infarction") OR ("cerebral arterial infarction") OR ("intraventricular hemorrhage") OR ("middle cerebral artery infarction") OR ("periventricular leukomalacia") OR ("hypoxic encephalopathy") OR ("prematurity"). Slight changing in the choice of the keywords had to be done according to the databases' different query options.

The list of records was first checked manually for duplicates. Subsequently, two authors independently reviewed the remaining records by title and abstract. Finally, full-text articles were screened to select those meeting the inclusion criteria. Based on the bibliography of articles of interest, more publications were manually added if considered relevant for the topic.

Inclusion criteria

The selection of the articles was limited to peer-reviewed research papers published in English and limited to human studies. The following criteria for selection were established:

1) Study design: Longitudinal observational cohort studies (prospective or retrospective);
2) Sample population: infants born at term ore preterm with perinatal unilateral stroke or other clearly asymmetrical cerebral lesions assessed with cranial ultrasound or brain MRI;
3) Methods: neurological examination performed at 3 months or within 6 months of age (chronological or corrected) with clinical instruments standardized or not (i.e. general movement assessment GMA, Hammersmith Infant Neurological Examination)
4) Availability of data on the clinical outcome of the subjects examined, at any age after 6 months to understand if it was possible to establish a diagnosis of Unilateral Cerebral Palsy (UCP).

Perinatal unilateral brain injury or asymmetrical cerebral lesions were defined by the following condition: perinatal stroke both hemorrhagic and ischemic, cerebral venous infarction, asymmetrical intraventricular hemorrhage, periventricular leukomalacia, hypoxic-ischemic encephalopathy. As for the clinical data, studies including HINE and/or GM's assessment were selected as well as other clinical examination that allowed to understand the reader the presence or not of any neurological asymmetries.

Data extraction

Detailed information of all included articles was systematically extracted and collected in an electronic database. This included: title, authors and year, country, study design, sample size, demographic characteristics, instrumental data available, type of clinical assessment with the related results of the neurological assessment and data of the long-term motor outcome.

2.3 Results

The PRISMA flow chart in Figure 1 summarizes the whole selection process and exclusion criteria at each selection step. A total of 204 references from MEDLINE, EMBASE and WEB OF SCIENCE were initially identified, and 5 additional articles were found by hand searching the reference lists of the selected papers. Nineteen duplicates were removed, and 190 records were screened by two independent authors. Based on title 121 studies were excluded and, after reading the abstract, another pool of 39 papers were excluded because in language other than English or because they were review of the literature or their clinical population didn't include CP patients or assessments were performed at a developmental stage later than six months. Thirty full texts were screened, and 11 studies were excluded as they didn't provide specific data on UCP, didn't report early clinical data, or had neuroimaging data as the main focus.

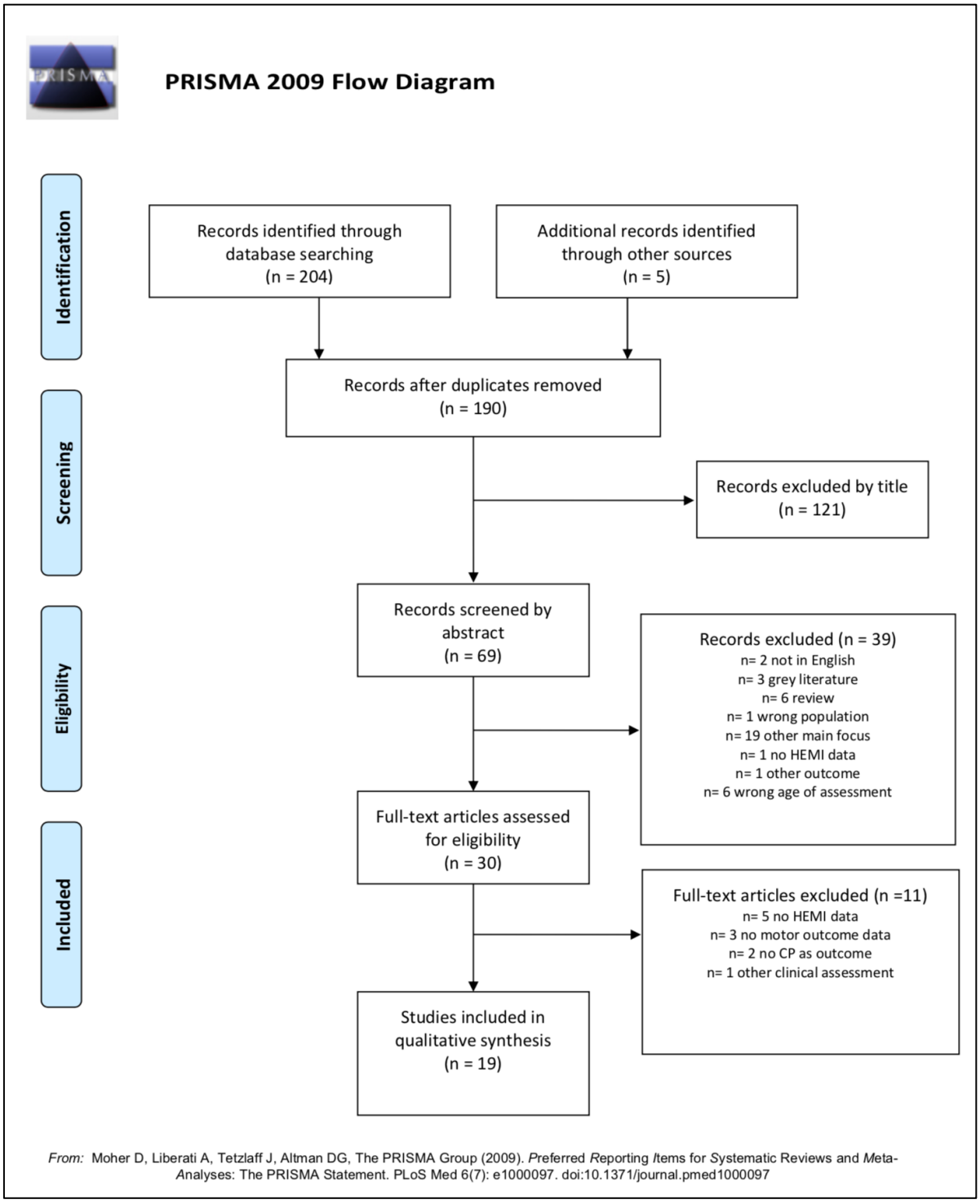
Studies characteristics

Nineteen studies were selected, 9 from Europe (11,14–21), 3 from China (22–24), 2 from United States (25,26), 2 from UK (27,28), 1 from UK and Sweden (29), 1 from Europe and Australia (30) and 1 study including 24 sites worldwide (10). The year of publication ranged from 1990 to 2021. Moreover, most of the studies were observational and perspective, aimed to explore the diagnostic validity of clinical assessments in population at risk of CP, while only one was retrospective. Most of the studies were cohort studies, while 6 had a case-control design.

Sample characteristics

As for the sample characteristics, subjects considered at risk of developing any kind of cerebral palsy were included in 6 studies (10,11,16,18,20,22,24,26,29), 9 studies were specifically focused on a population at risk of developing UCP (15,17,19,21,23,25,27,28,30), while 1 study took in consideration a sample of general population in order to assess the predictive value of abnormal general movements for severe neurodevelopmental disorders (14).

As for the characterization of brain lesion in extension and topography, in 14 out of 19 selected papers, MRI data were available, although with different degree of details (14,15,17,19,21–23,25–30). Differences concerning the time of execution, type of sequences acquired, and classification were identified. In most of the studies infants underwent the MRI scan around the term age, and only in three studies (16,21,25) neuroimaging was performed later. In 9 out of 14 studies (14,17,19,21,23,25,27,28,30) the sample was mainly represented by arterial infarction (ischemic/hemorrhagic), while in 5 studies, periventricular hemorrhage or asymmetrical cystic leukomalacia pictures were detected (15,16,22,26,29). Basal ganglia and thalamus involvement were not systematically reported by the authors in brain lesion description.



As for the clinical evaluation, General Movement Assessment (GMA) was performed in 15 studies (10,11,14–16,18–24,26,29,31), 4 of which also applied the Motor Optimality Score (10,22–24). The Hammersmith Infant Neurological Examination (HINE) was delivered (also in the Dubowitz version) in 5 studies (11,15,16,27,28). In 2 papers the Hand Assessment for Infants (HAI) was administered (19,30), while in 3 studies neurological examination with a specific focus on asymmetries in movements, postures and muscular tone was carried out (18,22,27). The Alberta Infant Motor Scale was applied in 1 protocol (29); detailed analyses of hand movements were performed by Guzzetta and colleagues (2003)(21), while Van der Heide and colleagues specifically evaluated kicking movements (20). Finally, a kinematic analysis of hand movements was proposed in 1 study by Mazzarella et al. (2020)(25). In 13 studies the authors proposed a combination of at least two

different assessments (10,11,15–20,22–24,28,29), while in 6 only one evaluation method was applied (14,25–28,30). Relevant details of the included studies are summarized in Table 1.

Specific results on the sensibility, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the different early (within 6 months of age, chronological or corrected) clinical assessments in infants at risk of UCP will be reported, when available. Then, we will retrospectively assess the sensibility of the clinical assessments in a population of infants with diagnosis of UCP.

2.3.1 Diagnostic accuracy of the clinical assessments in infants "at risk of UCP"

To realize a perspective analysis, the 19 selected studies were further sorted. The criteria for this further selection were: articles clearly focused on infants defined "at risk of UCP" or articles in which it was possible to deduce a precise number of infants at specific risk of UCP with clear information about the outcome. The risk was defined on the basis of neuroimaging data as the presence of "perinatal arterial ischemic stroke", "hemorrhagic stroke", "periventricular hemorragic infarction", "asymmetrical intraventricular hemorrage" or "asymmetrical periventricular leukomalacia" and, when assessed with cranial ultrasound, "unilateral intraparenchymal echodensity (UIPE)" or "asymmetrical ventricular dilatation". Studies on both preterm and full-term infants were included. According to these criteria, 13 papers out of 19 were selected (10,11,14–19,21,22,24,26,29). Results of the perspective analysis are reported below.

General Movement Assessment: predictive validity

Among the 13 selected studies, General Movements Assessment was provided in 6 (15–17,21,23). Two out of 6 reported data only at fidgety age (17,19), 1 reported data only at writhing age (23), while 3 reported data both on fidgety and on writhing period (15,16,21). As the main goal was to define the diagnostic accuracy of GMA in detecting pathological condition specifically in a population with early asymmetrical brain lesion, both infants with BCP and UCP were considered in the same outcome group, namely UCP outcome group, at this stage of analysis.

The sum of subjects potentially at risk of UCP from all the studies selected was used to calculate the sensitivity, the specificity, the PPV and the NPV of general movements assessment in detecting a later diagnosis of UCP.

Authors Year	Study subject sample	Age of assessment	GMA	HINE	Other assessments	Main result in UCP population
Bouwstra et al.,2010 (14)	455 (general population)	- 3 months	-DA GMs: 17 -Non-DA GMs (NO, NSO, MA): 438	No data	No data	1 UCP: a) NSO GMs
Cioni G et al., 2000 (15)	16 at risk of UCP	- 38-42 weeks PMA - 49-56 weeks PMA	38-42 PMA weeks: CS: 7 PR: 9 49-56 PMA weeks: Fidgety +: 3 Fidgety -: 13	HPT: 3 UCP; 1 mild ret. HPO: 1 UCP; 1 BCP ASY: 7 UCP; 1 BCP; 1 mild ret.	Movement Asymmetry score: - 49-56 weeks: UIPE score>controls; UIPE had a better movement repertoire in the ipsilateral side of the lesion then in the contralateral one Head posture: - 49-56 weeks UCP infants were less able to maintain head in the midline compared to control group	12 UCP: 7 right UCP: a) 7 Fidgety -; b) 7 left preference of segmental movements; c) 3 CS; 4 PR 5 left UCP: a) Fidgety -; b) 5 right preference of segmental movements; c) 3 CS; 2 PR
Bouza et al.,1994 (27)	5 at risk of UCP	-3-24 months	No data	Clinical Neurological Examination with focus on asymmetries: -3 months: 1 asymmetrical popliteal angle; - 6 months: 1 asymmetry in popliteal angles, scarf sign, resistance to shoulder elevation; 1 asymmetry in scarf sign, popliteal angle	No data	3 UCP: a) asymmetries in tone, posture, movement, hand function
Einspieler et al.,2015 (22)	61 with CP	-9-16 weeks after birth	-Fidgety +: 1 -Abnormal Fidgety: 1 -Fidgety +/- : 8 -Fidgety -: 50	No data	<u>Motor Optimality Score:</u> -MR reduced: 5 -MR inadequate to age: 56 -MP normal: 5 -MP abnormal: 42 -Normal=abnormal MP: 14 -P normal: 9 -P abnormal 44 -Normal=abnormal P: 8	4 UCP: a)1 Fidgety +; 3 Absent Fidgety
Ferrari et al.,1990 (16)	29 at risk of CP	-3 to 22 weeks of corrected age (GMA) -6-9-12-18-24 months corrected age (Dubowitz examination)	-PR: 9 -CS: 20 -Fidgety +: 7 -Fidgety -: 21 -Missing: 1	Dubowitz examination (before 6 months): -Hypotonia: 1 -Poor head control: 4 -Postural asymmetries: 3 -Hypertonia: 12 -Stifness:1 -Tonus asymmetry: 1	No data	5 UCP: 2 monoplegia leg>arm: a)2 Fidgety+; b)2 CS; c)1 asymmetry in arm movements; 3 hemiplegia arm>leg: a)1 Fidgety +; 1 Fidgety-; b)3 CS;

						c)3 asymmetries in posture, arm movements and tone
Guzzetta et al.,2009 (17)	13 at risk of UCP	12 weeks after birth	-Fidgety + : 7 -Fidgety - : 6	No data	<u>Analysis of hand movements</u> 1) Asymmetry of movement pattern: -hemi vs healthy: negative asymmetry index* for wrist movement (higher frequency of wrist movement in the unaffected arm compared to the affected one); -hemi vs healthy: negative asymmetry index for digit movements (higher frequency of wrist movement in the unaffected arm compared to the affected one; -no differences in the degree of asymmetry of global hand movements between groups; 2) Frequency of movement patterns: - hemi vs healthy: frequency (frequency per minute) of wrist movement in the affected hand was significantly different; - hemi vs healthy: frequency of digit movement in the affected hand was significantly different -no differences in the frequency of global hand movements between groups Asymmetry Index: (vCM-vIM)/(vCM+vIM); IM=ipsilesional movement, CM=contralesional movement	8 UCP: 2 left hemi a)1 F+; 1 F-; 6 right hemi a)1 F+; 5 F-
Hamer et al.,2011 (18)	44 at risk of CP	-10.2 weeks (median)	-Fidgety +: 4 -Fidgety +/-: 31 - Fidgety -: 9	No data	<u>Movement characteristics:</u> 1) Complexity and variation: -Present to a very limited extent: 31 -Absent:13 2) CS: -Frequently/ continuously present: 1 -Occasionally present: 4 -Absent: 39 3) ATNR pattern: -Frequently or persistently: 11 -No or variable occurrence: 33 4) Stiff movements (SM): -Predominantly stiff: 21 -Predominantly not stiff: 23 5) Jerky movements (JM): -Predominantly jerky: 14 -Predominantly not jerky: 30 6) Movement fluency (MF): -Predominantly fluent: 13 - Predominantly jerky: 10	2 UCP (1: Fidgety +; CS -; ATNR -; SM -; JM +; 2: Fidgety +/-; CS -; ATNR -; SM +; JM -)

					- Predominantly jerky& stiff: 4 - Predominantly stiff: 17	
Pascal et al.,2020 (19)	45 at risk of UCP	-10-15 weeks (GMA) -3-5 months (HAI)	-Intermittent Fidgety: 18 -Fidgety-: 13 -Sporadic Fidgety: 12 -Abnormal Fidgety: 2	No data	Hand Assessment for Infants: -Asimmetry Index significantly higher in USP infants compared to infants without CP (>23) -Contralesional Each Hand Sum Score significantly lower in infants with UCP compared to infants without CP; -Both Hand Sum Score significantly lower in infants with UCP compared to infants without CP	13 UCP: a)7 Fidgety-; 4 sporadic Fidgety; 2 intermittent asymmetrical Fidgety
Romeo et al.,2007 (11)	903 at risk of CP	-12 weeks post term age	-Fidgety+: 799 -Abnormal Fidgety: 46 -Fidgety-: 55	Hine score (median): -normal outcome: 62 -mild disability: 58 - CP (all): 36 - Hemiplegic-CP: 55 - Diplegic-CP: 35 - Tetraplegic-CP: 30.5	No data	13 UCP: a)1 Fidgety +; 1 Abnormal Fidgety; 11 Fidgety-; b)10 Hine score >50; 1 Hine score <50)
Van der Heide et al.,1999 (20)	11 at risk of CP	-1 and 3 months post term age	-CS: 5 -PR: 6	No data	Kicking movements analysis: -No significant differences were found in kicking frequency, temporal organization of the kick cycle, coordination among different joints and intelimb coordination; mild differences in segmental movement of the foot in CP-infants	1 UCP a)1 PR
Guzzetta et al.,2003 (21)	11 at risk of UCP	-3-6 weeks -9-16 weeks	-3-6 weeks (7 cases): 2 normal GMs; 5 PR - 9-16 wks (11 cases): Fidgety +: 5; Abnormal Fidgety: 6	No data	Assessment of asymmetry in spontaneous movements: -3-6 weeks: 2 symmetrical segmental movements; 5 asymmetrical segmental movements -9-16 weeks: 6 symmetrical segmental movements; 5 asymmetrical segmental movements	6 UCP: a) 6 abnormal Fidgety; b) 4 asymmetrical segmental movements at 3-6 weeks; 5 asymmetrical segmental movements at 9-16 weeks; 1 no asymmetry
Yin et al.,2021 (23)	27 at risk of UCP	-2.4 weeks post term age (median)	-CS:1 -PR: 22 -Normal: 2 -Missing: 2	No data	Motor Optimality Score: -Significant difference in global score between CP-group and non-CP group -Significant difference in contralesional limb scores between CP-group (lower scores) and non-CP group (distal rotatory components of upper limb and tremolous movement of lower limb)	7 UCP: a)6 PR; 1 missing; b)rotatory movement of both contralesional upper and lower limbs score lower than non-CP group
Mercuri et al.,1999 (28)	24 at risk of UCP	-3-5 days after birth	No data	Dubowitz examination: -Hypotonia: 11 -Asymmetries: 6 -Normal: 7	No data	4 UCP: a)2 hypotonia; b)2 mild asymmetry; 1 asymmetry
Mazzarella et al.,2020 (25)	11 at risk of UCP	-8-12 weeks of age	No data	No data	Kinematic analysis:	4 UCP

					-Movement frequency: Hemi-CP vs Non-CP move less often their involved side, and slower bilaterally; -Movement length: significant differences between the involved and the uninvolved hand in infants with Hemiplegic- CP -Length of hand path: significant differences between the involved and the uninvolved hand in CP -Straightness ratio: CP have larger straightness ratios in their involved side compared to their uninvolved side (trend) -Movement speed: significant differences in the involved hand between non-CP and CP	
Ryll et al.,2021 (30)	203 at risk of UCP	-13-53 weeks	No data	No data	Hand Assessment for Infants: The accuracy in predicting UCP for the Asimmetry Index, for the contralesional Each Hand sum Score (EaHS) and Both Hand Sum score (BoHM) increase with the age of assessment. From 3.5 month to 4.5 months the accuracy for all the three scales is very good, with the highest level of accuracy for the contraleisonal EaHS; from 4.5 to 5.5 months the contralesional EaSH and the asymmetry index show excellent accuracy , and BoHM is very good; from 5.5 to 6.5 months excellent accuracy for all the tree scales was found.	103 UCP
Glass et al.,2021 (26)	58 at risk of CP	-15.4 weeks (median)	-Fidgety +: 5 -Fidgety -: 2	No data	No data	2 UCP a) 2 Fidgety+
SkiÖld et al.,2013 (29)	53 at risk of CP (esxtremely preterm)	-13 weeks (median)	-NO GMs:14 -NSO GMs: 22 -MA GMs: 11 -DA GMs: 6	No data	Alberta Infant Motor Scale: AIMS Score <5th centile: 13	2UCP a) 1 normal suboptimal; 1 definitely abnormal; b) 1 AIMS <5th centile; 1 AIMS 25th centile)

Yang et al.,2012 (24)	79 at risk of CP	-9-20 weeks post term	-Fidgety +: 1 -Fidgety -: 78	No data	Motor Optimality Score: Quality of MP: -Predominantly normal: 11 -Predominantly abnormal: 52 -Equal number of normal and abnormal movements: 16 P Patterns: -Predominantly normal: 15 -Predominantly abnormal: 53 -Equal number of normal and abnormal patterns: 11 Movement character: -Monotonous/stiff/jerky /tremulous: 58; -CS: 21	5UCP: a)1 Fidgety +; 4 Fidgety-; b)3 asymmetry in segmental movements in the arm and leg
Einspieler et al.,2019 (10)	468 at risk of CP	-13 weeks (median)	-Fidgety +: 18 -Abnormal Fidgety: 5 -Absent Fidgety: 445	No data	Motor Optimality Score Quality of MP: -Normal hand regard was associated to diagnosis of UCP -Asymmetry of segmental movements was associated to diagnosis of UCP P Patterns: -UCP had more normal postural patterns than infants with diagnosis of bilateral CP -UCP infants had fewer atypical posture than infants with diagnosis of bilateral CP	92 UCP a)11 Fidgety +; 1 Abnormal Fidgety; 80 Fidgety-; b)58 higher frequency of segmental movements in the unaffected arm; 5 of the 11 subjects Fidgety + had an asymmetry of segmental movements

Table 1 DA GMs: Definitely Abnormal General Movements; Non DA-GMs: non definitely abnormal General Movements; NO: normal optimal; NSO: normal suboptimal; MA: mildly abnormal; UCP: Unilateral cerebral palsy; PMA: post-menstrual age; CS: cramped-synchronized; PR: poor repertoire; MR: motor repertoire; MP: movement pattern; P: posture

Writhing scoring

At writhing age (see Table 2), "poor repertoire" was investigated in infants at risk of UCP described in the 4 papers previously cited. The "poor repertoire" (PR) scoring reached a sensitivity of 53.33% (95% CI; 34.33%-71.66%), a specificity of 17.39% (95% CI; 4.95%-38.78%), a PPV of 45.71% (95% CI; 34.46%-55.28%) and a NPV of 22.22% (95% CI; 9.78%-42.96%). The "cramped-synchronized" (CS) scoring reached a sensitivity of 46.67% (95% CI; 28.34%-65.67%), a specificity of 100% (95% CI; 75.29%-100%), a PPV of 100% and a NPV of 44.83% (95% CI; 36.76-53.17%).

Table 2 Writhing scoring in infants at-risk of UCP

		CP*				No CP			
Author	**Total Population**	**TOT**	**N**	**PR**	**CS**	**TOT**	**N**	**PR**	**CS**
Cioni et al.,2000	N=16	13	0	6	7	3	0	3	0
Ferrari et al.,1990	N=7	6	0	0	6	1	0	1	0
Guzzetta et al.,2003	N=7	4	0	4	0	3	2	1	0
Yin et al.,2021	N=23	7	0	6	1	16	2	14	0
Total	**N=53**	**30**	**0**	**16**	**14**	**23**	**4**	**19**	**0**

CS: cramped synchronized; N: normal; PR: poor repertoire.
*Of the 30 infants with CP, 3 developed a bilateral CP.

Fidgety scoring

As regard the fidgety scoring (see Table 3), the sensitivity of Absent Fidgety (F-) in detecting a later diagnosis of UCP was 70.83% (95% CI; 55.94-83.05 %), the specificity was 86.05% (95% CI; 72.07-94.70%), the PPV was 85.00% (95% CI; 72.52%-92.41%), the NPV 72.55% (95% CI; 62.59%-80.67%), while combined with other pathological scoring of Fidgety (abnormal Fidgety, sporadic Fidgety), the sensitivity increased to 79.17% (95% CI; 65.01%-89.53%), the specificity decreased to 62.79% (95%CI; 46.73%--77.02%), the PPV was 70.37% (95% CI; 61.08%-78.24%) and the NPV was 72.97% (95%CI; 59.77%-83.07%)

Table 3: Fidgety Scoring in infants at-risk of UCP

		CP*				No CP			
Author	**Total Population**	**TOT**	**F+**	**F-**	**AF**	**TOT**	**F+**	**F-**	**AF**
Cioni et al.,2000	N=16	13	0	13	0	3	3	0	0
Ferrari et al.,1990	N=7	6	3	3	0	1	1	0	0
Guzzetta et al.,2009	N=13	8	2	6	0	5	5	0	0
Pascal et al., 2020**	N=45	15	3	8+4	0	30	15	5+8	2
Guzzetta et al.,2003	N=11	6	0	6	0	5	5	0	0
Total	**N=92**	**48**	**8**	**40**	**0**	**44**	**29**	**13**	**2**

F+: Fidgety normal; F-: absent fidgety; AF: abnormal fidgety
*Of the 48 infants with CP, 5 developed a bilateral CP; **In Pascal et al. Absent fidgety/Sporadic Fidgety are reported under the F- category.

Hammersmith Infant Neurological Examination: predictive value of asymmetries

Hammersmith Infant Neurological Examination (HINE) was performed in 5 studies (11,15,16,27,28) (see Table 4). In 4 of them, data on asymmetries detection were provided, while Romeo et al.,2007 reported only the final score, without detailed information on each items (11). The "asymmetries" in tone, postures, or movement during the HINE administration, reached a sensitivity in detecting a later diagnosis of UCP of

62.96% (95% CI; 42.37%-80.60 %), a specificity of 88.00% (95% CI; 68.78%-97.45%), PPV of 85.00% (95% CI; 63.35%-94.45%), and NPV of 68.75% (95% CI; 56.85%-78.60%).

Table 4: Asymmetries detected with HINE in infants at-risk of UCP

		CP*		No CP	
Author	**Total Population**	**TOT**	**ASY tone/movement**	**TOT**	**ASY tone/movement**
Cioni et al.,2000	N=16	13	8	3	1
Ferrari et al.,1990	N=7	6	4	1	0
Mercuri et al.,1999	N=24	5	2	19	2
Bouza et al.,1994	N=5	3	3	2	0
Total	N=52	27	17	25	3

*Of the 30 infants with CP, 2 developed a bilateral CP.

Motor Optimality Score (MOS): predictive validity

The Motor Optimality Score (MOS), as an additional movement assessment correlated to GMA, was proposed only by Yin et al.,2021 (23) in a population of infants at risk of UCP at writhing age. While it is not possible to calculate sensibility, specificity, PPV and NPV in UCP detection for the MOS score due to the characteristics of the reported data, authors reported a significant difference in the global score between non-CP and CP groups, with the latter scoring lower than the former (p=0,02). Furthermore, the CP group had lower MOS scores for the contralesional limbs than the non-CP group ($p<0.05$).

Other Assessment: predictive validity

In 6 out of 10 papers, other early neurological assessments were proposed, with a specific focus on the detection of early asymmetries in infant's neuromotor development profile (15,17,19,21,25,30).

Cioni and colleagues (2000) and Guzzetta and colleagues (2003) both proposed an assessment of asymmetries in spontaneous movements at fidgety age, with a specific focus on asymmetries in segmental movements that, considering data from the two studies together, reached an overall sensitivity in the detection of later UCP diagnosis of 94.74% (95% CI; 73.97%-99.87%), a specificity of 75.00% (95% CI; 34.91%-96.81%), a PPV of 90.00% (95% CI; 72.95%-96.78%), and a NPV of 85.71% (95% CI; 46.07%-97.68%).

Pascal and colleagues (2020) and Ryll and colleagues (2021) both reported data on sensitivity and specificity of the Hand Assessment for Infants (HAI). The first reported a sensitivity of 100% (95% CI; 66.4%-100%) and specificity of 100% (95% CI; 87.7%-100%) of the HAI to detect UCP in a population of infants with perinatal stroke. The second, aiming to investigate sensitivity, specificity and determine cut-off values for UCP diagnosis in a population at risk, found an increasing sensitivity ranging from 63% to 93%, specificity from 62% to 91% and accuracy from 73% to 94% by the age, from 3 to 12 months, when appropriate HAI cut-off were applied.

Guzzetta and colleagues (2009) reported early (3 months of age) higher frequencies of wrist and digit movements in the unaffected arm compared to the affected one in subjects later diagnosed with UCP. Mazzarella and colleagues (2020) instead, found differences in movement length and speed between the affected and unaffected hand with kinematic analysis of infants' movements at around 2-3 months of age.

2.3.2 Early sensitivity of the clinical assessments in infants with UCP diagnosis

A retrospective analysis was conducted on the 19 papers originally selected, in order to characterize the early clinical signs of infants later diagnosed with UCP by means of a quantitative analysis of early assessments tools sensitivity.

General Movement Assessment:

General Movement Assessment was provided in 15 studies (10,11,14–22,24,26,29,32). Five studies reported data on writhing period (15,16,20,21,32), while 13 studies considered the fidgety scoring in the time window of 9-16 weeks post term (10,11,14,16–19,21,22,24,26,29)(see Table 8) and 3 included data both at writhing and fidgety ages (15,16,21). In 2 papers (14,29) a classification of general movements by Mijna Hadders-Algra was used (33), although the assessment was referred to the videorecording of general movements during the fidgety period. As for defining the value of fidgety movements in predicting UCP outcome, we included also 3 subjects detected in these latter 2 studies considering the "normal suboptimal GMA" as intermittent normal fidgety and "definitely abnormal GMA" as absent fidgety.

Writhing scoring

At writhing age, 5 papers (15,16,20,21,32) reported data on infants with UCP diagnosis. The "poor repertoire" sensitivity was of 54,83%; the "cramped-synchronized" (CS) scoring reached a sensitivity of 45.16%. Data about writhing scores on BCP were more sporadic in these 5 papers and therefore are not reported.

Fidgety scoring

At Fidgety age, 13 papers reported data on infants with UCP diagnosis (10,11,14–19,21,22,24,26,29) . The sensibility of pathological Fidgety (absent, abnormal or sporadic) scored between 9 weeks and 5 months of age was 84,24%

Table 8: Fidgety Scoring in UCP population

Author	UCP detected	F+	F-	AF	+/-	Intermittent
Cioni et al.,2000	12	0	12	0	0	0
Einspieler et al., 2015	4	1	3	0	0	0
Ferrari et al.,1990	5	3	2	0	0	0
Guzzetta et al.,2009	8	2	6	0	0	0
Hamer et al.,2011	2	0	0	0	1	1
Pascal et al., 2020	13	0	7	0	4	2
Romeo et al.,2007	13	1	11	1	0	0
Guzzetta et al.,2003	6	0	0	6	0	0
Glass et al.,2021	2	2	0	0	0	0
Yang et al.,2012	5	1	4	0	0	0
Einspieler et al.,2019	92	11	80	1	0	0
Bouwstra et al.,2010	1	0	0	0	0	1
SkiÖld et al.,2013	2	0	1	0	0	1
Total	165	21	126	8	5	5

UCP= Unilateral Cerebral Palsy; F+: Fidgety normal; F-: absent fidgety; AF: abnormal fidgety; +/- Sporadic

Hammersmith Infant Neurological Examination

The retrospective analyses did not involve the Hammersmith Infant Neurological Examination because the papers and accordingly the cohort of probands remained the same as the perspective analysis.

Motor Optimality Score (MOS)

The Motor Optimality Score (MOS) was proposed in 3 studies (10,24,32) but, while Yang and colleagues (2012) and Einspieler and colleagues (2019) reported data at fidgety age, Yin and colleagues (2021), referred to the writhing period, with a scoring at term age, so the data of the three studies can't be put together for an overall analysis. In spite of the paucity of samples, results reported by the authors about data from 3 to 5 months post term were quite consistent as regard the sensitivity of MOS in UCP population. Yang and colleagues revealed that asymmetries in segmental movements had a sensitivity of 60.00% in infants with diagnosis UCP, while in a population later diagnosed with BCP, the sensitivity was 2.74%. Einspieler and colleagues (2019) found quite similar results since the asymmetry in segmental movement had a sensitivity of 63.04% in infants with UCP, while in a population with BCP, the sensitivity was 1.60%.

2.4 Discussion

The most recent international guidelines on early detection recommend a combination of neuroimaging, Hammersmith Infant Neurological Examination and General Movement Assessment as early as 3 months of age in order to achieve a diagnosis of Cerebral Palsy as soon as possible and to refer the infant to habilitative care (34). As for BCP, the absence of fidgety, combined with neuroradiological lesion patterns has been demonstrated to be highly sensitive and specific in detecting a motor impairment (10).

As for asymmetrical brain lesions or perinatal stroke, early UCP diagnosis and functional prognosis are still challenging if done only on the basis of neuroimaging, which still require a significant clinical contribution for the decisional algorithm to refer infant to early habilitative programs or avoid medical overtreatment in cases of low risk of UCP. The first achievement of the current review was the evidence that, despite the guidelines' advices, a consistent and standardized clinical assessment in infants at risk of UCP is still lacking. Literature selected reported, in fact, quite heterogeneous clinical evaluation methods to identify early signs of hemiplegia, mainly focusing on asymmetries that have been extensively hypothesized as potential biomarkers, but practically detected according to different criteria over time. Selected papers covered a period of almost three decades and, over years, some empirical observations on the characteristics of infant's movements, posture and muscular tone, before collected as discrete elements to enrich the standard observations, have been later implemented, better characterized and included in standardized scales (e.g. the items of the Motor Optimality Score, the items of the HAI) only clinically recently applied. More recently, data acquired by means of these standardized assessments however resulted not completely comparable from a quantitative point of view with those previously recorded, although they apparently refer to the same items (e.g. segmental movements asymmetries detection). As consequence, a quantitative analysis of results was possible only for some clinical tools, while for others, only a qualitative report was allowed given the paucity and the heterogeneity of the data.

In the perspective analyses, only studies including populations of infants at risk of unilateral cerebral palsy were considered eligible in order to give a general overview on the available clinical assessments and their reliability for a diagnostic purpose.

Considering the results on GMA, at writhing age, "cramped-synchronized" scoring has been found to have a very high specificity, while the sensitivity was moderate. This should be interpretated not as a specific marker of UCP, but rather of cerebral palsy in general. The "poor repertoire" had on the contrary very low specificity and moderate sensitivity.

The fidgety, considered the most predictive biomarker for CP (33,35,36), has been here investigated in respect of congenital hemiplegia. In the most recent studies, we found a more detailed scoring for the pathological expressions of this specific category of movements, while in older studies only the presence or the absence of fidgety was reported. In order to have homogeneous data we first hypothesized the worst condition of absent Fidgety (F-) to be the most predictive one for the diagnosis of UCP and we found that the sensitivity of (F-) in subjects at risk of UCP was quite high and the specificity even higher. Moreover, both the possibility that the absence of (F-) was associated to the absence of UCP (NPV) and the probability that an (F-) scoring was associated to the presence of the disease (PPV) were high. Considering the predictive value

of the pathological fidgety in general (absent, abnormal, sporadic) we found some increase in sensibility, while the specificity decreased and the positive and the negative predictive values remained almost the same.

Taken together, these data suggest that in a population at risk the writhing scoring demonstrate a high specificity only for the cramped-synchronized pattern, while the poor repertoire is less significant. The absence of fidgety had a good sensitivity and a quite high specificity in the detection of UCP in a population at risk, even if lower than the very high rate of sensitivity and specificity reported in the literature for the detection of CP in general. Considering, when possible, the characteristics of the at-risk samples, no significant differences were found in the fidgety scoring between preterm and full-term infants, as well as for the lesion patterns detected by neuroimaging (neonatal MRI or ultrasound). No significant correlation, in fact, were found in the fidgety scoring between the lesion patterns typical of the full-term infant (perinatal arterial/hemorragic ischemic stroke and hypoxic-ischemic encephalopathy) and those typical of the preterm infant (intraventricular hemorrage, periventricular leukomalacia).

The Hammersmith Infant Neurological Examination (HINE) was administered to infants at risk in 5 studies (11,15,16,27,37) but in none of them asymmetry score was reported. Nevertheless, in 4 studies "asymmetries" in tone, postures, or movement during the HINE administration were reported, with a moderate sensitivity and a quite high specificity in detecting a later diagnosis of UCP(15,16,27,37). In these studies, the older version of the neurological examination, lacking the asymmetry scoring, was applied. In the most recent paper by Romeo and colleagues (11), the focus was instead on the correlation between the global score and the motor outcome and no details on asymmetries were reported. Recently, Hay and colleagues (12) have supported the hypothesis that a combination of total HINE score with the introduction of an asymmetry score (which allow to quantify the number of items on the neurological exam that are different on right and left sides) can help clinicians to differentiate hemiplegic subjects from typically developing infants, but the data available are currently referred to infants older than 6 months of age.

Motor Optimality score has been introduced to integrate spontaneous motricity evaluation with GMA and it has been reported only in 1 study with a population at risk of UCP, even if in other study protocols some items lately included in the MOS assessment were already investigated as potential early markers, such as analysis of "movement characteristics" by Hamer, assessment of asymmetries in segmental movements by Guzzetta et al.,2003. The results of the study highlighted both the usefulness of the global score in discriminating, in a group at risk, the subjects with CP from the healthy ones and the possibility to detect asymmetries between the ipsilesional and contralesional limbs in the CP group.

The Hand Assessment for Infant (HAI) has been developed to early detect hands asymmetries and assess each hand motor development over the first months of life until 12 months in a population at risk of UCP. The HAI asymmetry score, calculated in 2 out of 19 studies offered an increasing accuracy by the age in predicting UCP diagnosis, with the score of the three scales (asymmetry index score, each hand sum score and both hands sum score) being significantly different in UCP population (12,30). Although in the literature, data on the application of this new instrument in at risk of UCP infants are still limited, data collected by now seems to suggest that HAI is a highly suitable, non-invasive and reliable tool to be used in early infancy.

The second part of the results has been conducted in an enlarged pool of infants with diagnosis of UCP and was dedicated to retrospectively investigate the characteristics of infants' neuromotor development and/or early neurological signs eventually identified with available clinical assessment tools.

On the basis of the available data, we can estimate a quite high sensitivity for the Absent fidgety scoring as well as for the detection of pathological fidgety in general. Asymmetry in segmental movements has been found in about the 60% of infants with UCP. Data on Hammersmith Neurological examination, instead, are still scanty. These data, although less informative, from a clinical point of view, than those described in the first part of the results report, represent however a contribute to confirm that fidgety (both absent or pathological in general), as well as segmental movements asymmetries and mainly at the upper limb could represent reliable early biomarkers for UCP detection.

Findings from the current review suggest that, to date, GMA, and notably, the fidgety scoring has demonstrated high sensitivity and specificity in the detection of UCP in a population at risk, as well as the

identification of asymmetries in tone, postures, and, above all in the segmental movements. The methodologies to detect asymmetries seem to remain quite heterogenic and mostly, qualitative. Data collected from the clinical assessments available, others than GMA, although scarce, seem encouraging in contributing to identify unilateral impairments. Of particular interest was the investigation of whether the combination of two different approaches would increase sensitivity, specificity, positive and negative predictive values of clinical neurological examination in predicting UCP outcome. Among the 13 studies which reported at least two clinical assessments on the same at-risk population, 7 commented just on data concerning every single assessment available (10,15,17,19,21,23,24), 6 qualitatively addressed this topic in the discussion (15,18,19,23,24,29) and only 2 studies (11,22) provided quantitative data. As for the qualitative data, authors commented underlying the relevance of carrying out a detailed infant neurological examination with a specific focus on asymmetries of segmental movements. Most of the studies emphasize the opportunity to assess movements of upper limb with a specific attention to contralesional wrist movements and distal rotatory components that were found to have high sensitivity and specificity in early detection of UCP, albeit so far, in small samples. As for studies reporting quantitative data, Romeo and colleagues reported that a combination of absent fidgety and HINE score above 50 points results in a CP outcome in 96% of the cases, mostly hemiplegic CP, while a combination of absent fidgety and HINE score under 50 points was highly correlated with bilateral CP (11). Einspieler and colleagues instead reported within the subgroup of infants with UCP and normal fidgety a significant asymmetry rate of segmental movements between the contralesional and ipsilateral side to the brain lesion, suggesting that in case of normal fidgety, the association of a detailed assessment of motor repertoire could be of help in supporting the hypothesis of a possible unilateral motor involvement (10).

Although the efforts to optimize the efficacy of the clinical resources in early detection of UCP, another issue related to the reliability of fidgety scoring is the interpretation of the significant rate of false positive and false negative in infants with unilateral brain lesions that affect the positive and negative predictive values. In order to give an explanation, we first evaluated the severity of the diagnosed CP in the selected studies on the basis of the Gross Motor Function Classification Scale (GMFCS). Only 7 out of 13 of the studies (10,18,19,22,24,26,29)proposing General Movement Assessment, reported data on the severity of motor impairment and there was a clear prevalence of low grade GMFCS (I-II) suggesting a prevalence of mild form of unilateral motor impairment which could contribute to clarify false negative results. No significant correlations were found between the different kind of pathological fidgety or the number of asymmetries in segmental movements and the severity of the outcome.

Furthermore, another significant aspect that we must consider before generalizing the results of this review, is related to the demographical characteristics of the population on which the GMA has been applied. In 14 studies the GMA has been conducted on samples considered at risk of CP development, with only 5 papers specifically addressed to infants at risk of UCP (15,17,19,21,23), and 9 papers considering subjects at general risk of developing any kind of CP (10,11,18,20,22,24,26,29,38), while only Bowstra and colleagues analysed the predictive value of abnormal general movements in the general population. Bowstra and colleagues demonstrated a lower PPV of GMA for CP in general population than in high-risk population, shedding light on the importance of prevalence which is heterogenic in the studies selected (14).

In conclusion, the current review of the literature suggests that, as for the unilateral cerebral palsy, the recommended diagnostic algorithm for early diagnosis of UCP could be less effective and reliable than what is generally prospected in the CP literature. This could be related to the still limited employment of a standardized asymmetries detection in neurological examination and in fidgety scoring itself. Neuroimaging, whilst highly predictive for CP, have still some limitations in the early UCP diagnosis and in the severity prognosis. In fact, even if the involvement of specific brain structures is, by now, considered highly predictive of motor impairment, it is still under debate whether the detection of their impairment at the neonatal age by mean of MRI could be sufficient in underpinning an early diagnosis of congenital hemiplegia. For this reason, the recommendation of keeping integrate instrumental data with clinical assessments at early stage has to be confirmed also for UCP subtype of CP. The presence in the general movement scoring of quite high rate of false positive and false negative in infants considered at risk of UCP shouldn't discourage the application of this valid technique, but rather support the need for a more extensive use of standardized

validated tools aimed to detect early segmental asymmetries, mainly in upper limbs, to enrich the information collected from the observation of infants spontaneous motricity, emphasizing the most ecological and less intrusive in order to better understand the neuromotor organization trajectory of the infant.

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Chapter 3

Early motor signs of Unilateral Cerebral Palsy (UCP): insights on the underlying physio-pathological mechanisms

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3.1 Background

Perinatal stroke prevalence has been estimated to be approximately 1 in 1,100 births (1), with infants' lifelong morbidity affecting motor, language, cognitive and sensory functions, being the main cause of Unilateral Cerebral Palsy (UCP)(2–4). Despite the achievements in terms of early diagnosis and intervention(5,6), this pathological condition keeps threatening infants' and families' quality of life and represents a significant socio-economic burden for the community (7). Many efforts have been made over the recent past years to maximize the diagnostic and prognostic power of the available instrumental and clinical tools to achieve an earlier identification of infants at greatest risk for developing a UCP. Early diagnosis is in fact intended to be crucial to immediately refer infants at high-risk to appropriate habilitative interventions, but also to avoid over-treatment of infants with stroke that could be however considered at low-risk for developing a UCP(5,8). The prognosis on the severity of the motor impairment is still challenging in infants at high-risk of UCP during the first months of life even if neuroimaging integrated with the clinical examination can guide the clinicians to formulate hypotheses (9–11).

Available protocols of early intervention in CP have been recently reviewed and a significant heterogeneity in response efficacy was found(6), supporting the need for a "stratified therapy approach", namely providing personalized interventions based on clinical biomarkers of sensorimotor function combined with information on structural and functional integrity of the corticospinal system(12). Indeed, the identification of specific patterns of sensorimotor re-wiring following the lesion could be responsible of the upper limb functional outcome as well as of the different responses to treatment. Jaspers and colleagues (12,13) have suggested some indirect strategies to investigate the corticospinal tract reorganization in the different patterns of disruption associated with UCP by means of behavioral measure as well as non-invasive brain stimulation techniques, the application of which is still limited in the very first months of life. Early finding of these biomarkers in infants with perinatal stroke could be very challenging due to the heterogeneity of the lesions, but also to the time-dependent maturational changes and the higher neuroplasticity opportunities that the young brain could exploit.

A detailed characterization of motor development from the first months of life and a systematic correlation with MRI structural imaging and tractography is required to better understand if any categorization is possible in a population of infants at risk for UCP in order to refer them to appropriate intervention. Recent guidelines for early detection of CP (5,6) recommend, in the first months of life, the systematic use of the General Movements Assessment (GMA) according to Prechtl's method to systematically evaluate infants at neurological risk and characterize the infant's motor profile in the very first months of life. *Fidgety movements* typically expressed between 9- and 20-weeks post-term age seem to be very useful in the early identification of congenital hemiplegia in infants with early unilateral cerebral lesions, although their diagnostic and prognostic accuracy remain still limited as to topography and severity of the impairment (14). More specifically, the absence of typical bilateral fidgety movements was shown to strongly correlate with the development of CP, although an heterogeneous expression of the fidgety pattern was reported. In children with UCP, patterns of asymmetrical representation of fidgety movements, bilateral absence of fidgety movements or sporadic but bilaterally present fidgety movements were all reported, suggesting a large variability in the expression of early motor repertoire in UCP. Understanding the neural basis of this variability in a population sharing a similar neurological risk (i.e. the development of UCP) can contribute to clarifying potential mechanisms of plasticity after perinatal stroke as well as predicting the motor outcomes and maximize the functional recovery by means of personalized early interventions.

Aim of the present study was to collect preliminary data on the characteristics of the early motor organization in a sample of infants with predominantly unilateral brain lesion at neonatal neuroimaging, thus considered at high-risk for developing a unilateral cerebral palsy. For this purpose, a videorecording of the spontaneous motricity and the Hammersmith Infant Neurological Examination with a focus on detection of asymmetries

were performed at 3 months of age (chronological or corrected) and early MRI data were collected and analysed.

We focused on three main hypotheses:

H1: Infants who will develop UCP have higher rates of pathological fidgety scorings (absent, sporadic, asymmetrical);

H2: Infants who will develop UCP have higher rates of asymmetries at the HINE;

H3: Brain lesion patterns can explain, at least partly, the heterogeneity of the GMA and HINE pictures in infants who will develop UCP.

3.2 Material and methods

Participants

A total of 12 infants (7 males and 5 females) with congenital unilateral brain lesion considered at high-risk for developing a hemiplegic cerebral palsy were enrolled in this observational longitudinal study. Each infant underwent a first clinical examinations at 3 months of age (chronological or corrected) and was evaluated with standard neurological examination and classified with Mini-MACS at 12 months of age to confirm or not the diagnosis of "Unilateral Cerebral Palsy". The study was conducted from April 2019 to June 2022 at the Infant Neurology Section of IRCCS Stella Maris Foundation. The subjects enrolled and the clinical data were collected from the database of an ongoing monocentric feasibility study, the E-TIPS-Early Therapy in Perinatal Stroke" study that involves IRCCS Stella Maris Foundation and approved by the Pediatric Ethic Committee of the Meyer Pediatric Hospital (registry number 142/2017).

Enrolment procedure

Enrolment of patients took place at the Infant Neurology Section of IRCCS Stella Maris Foundation when infants from the high-risk infant follow-up ambulatory service of the Neonatology Unit of Santa Chiara Hospital in Pisa or from other Italian hospitals were referred there as outpatients or inpatients for diagnostic or rehabilitative purposes.

Inclusion criteria were the following

1. Early diagnosis (before 3 months of life) of perinatal predominantly unilateral brain injury (ischemic stroke, haemorrhagic stroke, periventricular venous infarction, Asymmetrical PVL or IVH), documented with neuroimaging (Brain MRI).

2. Full acceptation of the informed consent by parents or legal guardians of the infants.

Exclusion criteria were the following

1. Extremely preterm infants (born before 26 weeks of g.a.)

2. Other medical conditions associated to brain vascular lesions (neurodegenerative disorders: genetic disease, metabolic disease, epileptic encephalopathy)

3. Predominantly bilateral brain lesions

4. Perinatal stroke with main involvement of the occipital, pre-frontal or temporal cortex (no clear correlation with motor impairment outcome)

All the subjects were regularly monitored through the clinical follow-up at IRCCS Stella Maris Foundation, independently from the participation to the study. The proposal to participate was formulated with a direct conversation with parents of eligible infants during clinical visits or during the period of hospitalization. When the parents expressed their will to participate, all the information about the study protocol and the detailed contact of the experimenters were provided to them.

Clinical Assessment

The study consisted of a longitudinal clinical follow-up of a sample of infants considered at high-risk for developing a UCP based on early neuroimaging. Specifically, at 3 months of age (chronological or corrected) the Hammersmith Infant Neurological Examination (HINE) and the General Movements Assessment (GMA) were performed. At 12 months all the subjects underwent a standard neurological examination and their hand functional ability characterized with the Mini-Manual Ability Classification System (Mini-MACS) in order to confirm or not the diagnosis of UCP.

General Movements Assessment (GMA)

The General Movements Assessment was first developed by Professor Heinz Prechtl in the 1990's as a new approach to neurologic examination of newborns and young infants, based on the qualitative observation of their spontaneous movement patterns. Two periods are described, characterized by two different patterns of movements: the "writhing period", from preterm age until 6-9 weeks post-term age and the "fidgety period" from 9 to 20 weeks post term age. General movements with "writhing" quality are described as gross movements involving the whole body with a variable speed, characterized by a writhing appearance due to the co-contraction of antagonist muscles; the body segments are involved in the movement in an indeterminate sequence. General movements with a "fidgety" quality are defined as restless, but smoothly rounded movements involving the whole body, characterized by small amplitude and moderate speed (15). An extensive literature has demonstrated a high correlation of this assessment with the neurological outcome. Currently, GMA is considered a standardized prognostic tool for early detection of Cerebral Palsy in high-risk infants with a reported sensitivity of 98% (95% confidence interval (CI): 86.79–99.58) and specificity of 94% (95% CI: 88.69–97.16) (16). A prognostic value is also reported for abnormal neurodevelopmental disorders different from cerebral palsy with a sensitivity of 54% and specificity of 97% (16). Fidgety movements, rather than writhing movements are considered to have the highest predictive power for the diagnosis of CP. In our study, GMA were conducted by a trained and certified researcher. GMs were obtained by a videorecording lasting at least 3 minutes of spontaneous motricity, when the infant was in an active state or wakefulness, without being touched or stimulated, lying supine, naked or in a nappy. The recording cameras were positioned 1 m above the infant. The classifications of "normal-continuous", "normal-intermittent", "sporadic", "asymmetrical", "absent" of fidgety were determined to define the risk of motor outcomes in each infant. Video scoring were performed off-line according to the Prechtl'method by two trained coders that worked independently, unaware about infants 'clinical history.

Hammersmith Infant Neurological Examination (HINE)

HINE is a neurological assessment tool for infants of 2-24 months of age. It consists of 26 items that assess cranial nerves, posture, movements, tone and reflexes; each item is scored on a scale of 0-3 and the total of all items provides a global score with a range of 0-78 (17,18). Global scores equal or above 73 at 9 to 12 months or equal or above 70 and 67 at 6 months and 3 months respectively are considered optimal, corresponding to the highest motor function (19). The HINE is considered to be predictive of Cerebral Palsy and useful for identification of the type and the severity with a high sensitivity (90-100%) and specificity (85-

100%). Romeo and colleagues (20), in a recent review of the literature have identified cut-off scores for defining the risk of cerebral palsy development. In particular, for preterm infants and infants at term, a global score equal or below 56 at three months and equal or below 65 at 12 months is considered highly sensitive and specific in prediction of cerebral palsy development while scores below 40 are associated with severe form of cerebral palsy (20). It takes approximately 5-10 minutes to be administered. In the present study HINE was administered at 3 months of age (chronological or corrected).

Mini-MACS

The Mini-MACS was first developed by Eliasson and colleagues in 2016 (21) as an adaptation of MACS (Manual Ability Classification System) for children aged 1-4 years. The aim of the test was to classify the child 'ability to handle objects in daily life. Since it was conceived to be addressed to young children, some changes were mandatory so the need for assistance in handling objects, as well as the description of specific "actions" rather than "activities" or "task performance" were judged to be more appropriate to describe the daily life of younger infants. The classification is still organized in five levels, which represent a spectrum of functional levels, ranging from level I describing only slight limitation in performing actions that require precision and coordination between hands and level V describing a complete absence of functional handling of the objects and the need of a constant assistance from the adult. Eliasson and colleagues (21) found a good interrater reliability of Mini-MACS administration between parents and therapists as well as between the therapists. Authors recommend considering the possibility to reclassify over time the manual ability since the level of the classification in younger children could be less stable than in the older.

Magnetic Resonance Imaging Classification:

For each subject available MRI were scored according to Mercuri et al.,1999 (22), using the following criteria: a) arterial distribution of the lesion; b) degree of involvement of cortical and subcortical structures.

Clinical data Collection

For each subject the following data were collected: gestational age, auxological parameters at birth, Apgar Index, perinatal anamnestic information, and neuroimaging results (Brain Magnetic Resonance). Acquired demographical and clinical data, as well as the scores of the HINE and the GMA were then gathered in a database. For privacy purpose, an ID code was assigned to each subject.

Statistical Analysis

Given the small sample size, analysis was primarily descriptive and qualitative, while no formal statistical analyses were performed.

3.3 Results

Sample characteristics

The sample included 12 infants, 7 full-term and 5 infants born preterm (2 late preterm and 3 very preterm). Among infants born at term, 3 were small for gestational age (SGA, E03; E06; E09), the others had an appropriate weight for their gestational age (AGA). All the preterm infants at birth needed respiratory assistance or resuscitation treatment, while only 1 infant born at term required ventilatory assistance. Seven infants (E01, E03, E04, E05, E07, E08, E09) showed focal clonic seizures and underwent EEG monitoring which revealed the presence of epileptic activity, and a pharmacological treatment was introduced. Brain ultrasounds performed during the perinatal period documented an altered signal in all infants, which was

also confirmed by later-performed sleep Magnetic Resonance. Detailed clinical characteristics of the sample are presented in Table 1.

Table 1: Study Subjects demographic and clinical characteristics.

ID	GA	SIBLING	BIRTH WEIGHT	APGAR INDEX	VENTILATORY ASSISTANCE	LESION TYPE	LESION SIDE	EPILEPSY	MOTOR OUTCOME	MINI-MACS LEVEL
E01	40+5	yes	3530 g	10 (V)	no	Stroke MCA	sx	no	R UCP	Level I
E02	29+6	yes	1520 g	6 (V)	yes	HI damage	sx	no	R UCP	Level I
E03	40+2	yes	2590 g	9 (V)	no	Head trauma	dx	no	Typical	na
E04	40+2	no	3372 g	9 (V)	no	Stroke MCA	dx	no	Typical	na
E05	41+5	no	4010 g	8 (V)	yes	Stroke MCA	sx	no	Typical	na
E06	40+2	no	2876 g	9 (V)	no	Malformation	sx	no	R UCP	Level II
E07	35+2	yes	2270 g	8 (V)	yes	Stroke MCA	sx	yes	R UCP	Level V
E08	41+1	no	*na*	7 (V)	no	Stroke MCA	sx	no	R UCP	Level II
E09	41+1	no	2870 g	8 (V)	no	Stroke MCA	dx	no	L UCP	Level II
E10	34+1	no	2300 g	*na*	yes	Stroke MCA	sx	no	R UCP	Level III
E11	32+4	yes	1605 g	8 (V)	yes	Stroke MCA	sx	no	R UCP	Level III
E12	30+4	no	1174 g	6 (V)	yes	PVL + IVH	sx	no	R UCP	Level I

GA=gestational age; MCA=middle cerebral artery; PVL=periventricular leukomalacia; IVH=intraventricular hemorrhage; HI=hypoxic-ischaemic; UCP=unilateral cerebral palsy; *na*=not available

Clinical Assessments

General movement assessment

Video-recordings of spontaneous movements were performed between 12 and 15 weeks of infants age (corrected for term age) to evaluate the fidgety movements with Prechtl's method of GMs assessment. Three infants (E02, E10, E11) were recorded at 12 weeks post-term, 3 (E03, E05, E08) at 13 weeks post-term, 4 (E01, E04, E09, E12) at 14 weeks post-term, and the last 2 (E06; E07) at 15 weeks post-term. Each infant fidgety movements were classified as "normal-continuous", "normal-intermittent", "sporadic", "asymmetrical", "absent". Findings on the classification of fidgety in each subject are shown in Table 2.

Hammersmith Infant Neurological Examination (HINE)

All the infants recruited underwent a neurological examination at 3 months of age (corrected for term age) according to the HINE procedure, and a total score was calculated (Table 2). The presence of asymmetries in any of the subareas (nerve function, posture, movements, tone, reflexes, and reactions) was marked on the score sheet. Nine infants received a total score above 50, while 3 received a total score below 50. Asymmetries were mainly detected in the domains of "Posture" and "Reflexes and reactions".

At least one asymmetry was detected in all the subjects of the sample, even in those eventually showing typical development. in infants receiving a diagnosis of hemiplegia, the asymmetries were generally, but not always, coherent with the side of the brain lesion; in two cases (E08; E09) part of the asymmetries detected were not coherent to the side of the motor impairment.

Table 2: GMA and HINE scores with type and side of the asymmetries

ID	GMA	HINE TOTAL	CRANIAL NERVES	POSTURE	MOVS.	TONE	REFLEXES AND REACTIONS	N° ASY
E01	NF, int	57	/	AAR: L>R	/	Pron/sup: L>R	/	2 L>R
E02	Asym F	45	/	Hands: L>R	/	/	TR: L>R	2 L>R
E03	NF, cont	57.5	/	/	/	/	TR: R>L	1 R>L
E04	NF, cont	58	/	AAR: R>L	/	/	/	1 R>L
E05	NF, cont	54	/	Hands: L>R	/	/	/	1 L>R
E06	Sporad F	45.5	/	AAR: L>R; Hands: L>R	/	/	LT: L>R	3 L>R
E07	Asym F	47	FCA: L>R	AAR: L>R; Hands: L>R	/	/	TR: L>R	4 L>R
E08	Asym F	52.5	/	Hands: R>L	/	PSE: L>R	LT: R>L	2 R>L; 1 L>R
E09	Asym F	52.5	/	Hands: R>L	/	PSE: L>R	LT: R>L; TR: R>L	3 R>L; 1 L>R
E10	Asym F	51.5	/	/	/	/	LT: L>R	1 L>R
E11	Asym F	54.5	/	Hands: L>R	Quant.: L>R	/	/	2 L>R

E12	NF, int	51	/	/	Quant.: L>R	/	LT: L>R; TR: L>R	3 L>R

AAR=arms at rest; F=fidgety; FCA=Following, complete arc; L=left; LT=lateral tilting; NF=normal fidgety; PSE=passive shoulder elevation; R=right; TR=tendon reflexes.

Brain MRI

All infants performed an early brain MRI at an age ranging from term age to 28 days post-term (see Table 3). Ischemic stroke in the territory of the middle cerebral artery (MCA) was detected in 8 infants. E02 presented with a bilateral, asymmetrical brain damage of ischaemic nature, with cortical-subcortical involvement (left post-central gyrus and concomitant reduction of the volume of the left frontal-parietal white matter and semioval centre white matter), while E12 presented with a hypoxic-ischemic damage correlated to premature birth, with a damage of periventricular white matter in the temporo-parietal regions. E03 reported a cortical damage in the frontal-parietal-temporal regions secondary to head accidental trauma occurred at five days of life; E06 showed a picture of brain malformation, possibly due to an early vascular damage (most likely a precocious venous infarction), which resulted in global hypotrophy of the left hemisphere and a selective damage of left basal ganglia and thalamus (see Table 3).

Twelve months neurological outcome

At 12 months infants were clinically evaluated with standard neurological examination and the diagnosis of Unilateral Cerebral Palsy was made in 9 infants, while 3 infants showed a typical neurodevelopment. The manual functional ability of each subject who developed a UCP was classified according to the Mini-MACS and is reported in Table 1.

Table 3: Type of lesion and involved structures

ID	LESION	LOBES	BG	THALAMUS	IC	BS	CEREB
E01	Stroke MCA, L	FPTO (L)	no	no	yes (L)	Asym (L)	no
E02	HI damage, L>R	FP (L)	no	no	yes (L)	no	no
E03	Head trauma, R>L	FTP (R)	no	no	no	no	no
E04	Stroke MCA, L	no	Anterior Striatum (L)	no	no	no	no
E05	Stroke MCA, L	FP (L)	no	no	no	no	no
E06	Malformation, L/R	no	BG (L)	yes (L)	yes (L)	no	no
E07	Stroke MCA, L	FTP (L)	Caudate/Putamen (L)	yes (L)	yes (L)	Mesnc (L)	no
E08	Stroke MCA, L	FPTO (L)	BG (L)	yes (L)	yes (L)	no	no
E09	Stroke MCA, R	FT (R)	Striatum (R)	yes (R)	yes (L)	no	no
E10	Stroke MCA, L	F (L)	BG (L)	no	yes (L)	no	no
E11	Stroke MCA; L	FTP (L)	VLN (L)	yes (L)	yes (L)	no	no
E12	PVL + IVH, L>R	no	no	no	yes (L)	no	no

BG=basal ganglia; BS=brainstem; F=frontal; IC=internal capsule; IVH=intraventricular haemorrhage; MCA=middle cerebral artery; Mesenc.=mesencephalon; O=occipital; P=parietal; PVL=periventricuar leukomalacia; T=temporal; VL=ventro-lateral nucleus.

Correlations with neuroimaging and clinical outcome

Correlation of GMA and HINE with clinical outcome

Correlations between GMA and clinical outcome are shown in table 4a. All infants with a normal outcome were sored as normal, continual Fidgety, while infants with Unilateral cerebral palsy, were scored as "sporadic Fidgety" (n=1), "intermittent Fidgety" (n=2), or "asymmetrical Fidgety" (n=6). While infants with intermittent fidgety showed a mild impairment, infants with asymmetrical fidgety showed various levels of severity at the MINI-MACS, ranging from level 1 to level 5.

Table 4a: Correlation between GMA and outcome

	TD	Hemiplegia				
		I	II	III	IV	V
NF, cont	○○○					
NF, int		○○				
Sporad F			○			
Asym F		○	○○	○○		○

Correlations between HINE and clinical outcome are shown in table 4b. All infants with normal outcome had a HINE score >50, while of the remaining nine, 6 scored above and 3 scored below 50. All infants showed at least one asymmetry at the HINE. Infants with normal development only showed 1 asymmetry, while those who developed UCP showed more than 1 asymmetry in all cases but one. The infant with the most severe level of impairment showed both a score under 50 and the highest number of asymmetries (i.e. 4).

Table 4b: Correlation between HINE and outcome

HINE asymmetries	TD	Hemiplegia				
		I	II	III	IV	V
n=1	○ ○○			○		
n=2		○●	○	○		
n=3		○	○●			
n=4						●

○=HINE>50; ●=HINE<50

Correlation of GMA and HINE with MRI lesion patterns

Correlations between GMA and MRI lesion patterns are shown in table 5a. All three infants with continual fidgety and normal outcome showed no asymmetries of the internal capsule, nor damage to the thalamus or the basal ganglia, except for E04 who showed a doubtful signal abnormality of the left anterior striatum. The two infants with intermittent fidgety and hemiplegia (false negatives) showed no damage to basal ganglia or thalamus but had asymmetrical PLICs. All the remaining 7 infants who developed hemiplegia showed abnormal movements, which consisted in either asymmetrical fidgety (n=6) or sporadic fidgety (n=1). Asymmetrical fidgety was always associated with abnormal signal of the internal capsule contralateral to the side of the body with no or minimal fidgety movements. In 6/7 subjects the basal ganglia and/or the thalamus were also involved on the same side. The only infant with sporadic fidgety showed a bilateral, asymmetrical brain malformation on MRI, also involving subcortical grey nuclei. Overall, "asymmetrical fidgety" was more likely in infants with lesions involving PLIC, basal ganglia and/or thalamus, while "normal fidgety", either continuous or intermittent, was more likely to be detected in infants without thalamus or basal ganglia involvement. The only subject with sporadic/absent fidgety had a very early onset brain damage (malformation), with involvement of both cortical and subcortical structures.

Table 5a: Correlation between GMA and MRI

	Nor PLIC	Nor BG and Th Abn PLIC	Abn BG and/or Th Abn PLIC
NF, cont	○○○		
NF, int		○○	
Sporad F			○
Asym F		○	○○○○○

Correlations between HINE and MRI lesion patterns are shown in table 5b. All infants without an involvement of the PLIC showed scores above 50 at the HINE and the lowest level of asymmetry (i.e. n=1). All other infants showed asymmetries ranging from 1 to 4, but no correlation was observed between number of asymmetries and lesion patterns. Most of the infants with an involvement of the PLIC, who later developed a UCP, showed more than 1 asymmetry.

Table 5b: Correlation between HINE and MRI

HINE asymmetries	Nor PLIC	Nor BG and Th Abn PLIC	Abn BG and/or Th Abn PLIC
n=1	○○○		○
n=2		○●	○○
n=3		○	○●
n=4			●

○=HINE>50; ●=HINE<50

3.4 Discussion

The results of the present study, albeit preliminary, given the small number of subjects, confirm that early clinical assessment can reliably lead to early diagnosis (before 5 months) of UCP in infants with clinical history of perinatal stroke. An extensive literature supports the use of GMA at 3 months of age combined with early MRI in the early diagnosis of CP (14,23–26), but as for the UCP detection, the diagnosis remains challenging (27,28). The rate of false positives and negatives in GMA at fidgety age is higher in UCP compared to the bilateral forms of CP, and early MRI as well as all the advanced diffusion techniques with high sensitivity in predicting motor outcome are not always available everywhere or for infants with delayed presentation of hemiplegia. At the same time, early structural neuroimaging is not always informative about the prognosis of severity of the motor impairment (29–34).

Over the years, different authors investigated the opportunity to assess asymmetries in movements, postures, tone, reflexes in order to qualitatively add information to standard examination. Guzzetta and colleagues (35) reported that asymmetry of wrist segmental movements and the absolute frequency of independent digit movements were significantly different between infants with or without hemiplegia at 3 months of age and previously, Cioni and colleagues (36) reported a high rate of asymmetries in segmental movements at fidgety age in a population of infants at high-risk for developing UCP compared to controls. In the last years there was an increasing attention to include the asymmetries in clinical examination not only from a qualitative point of view, but also by means of quantitative measures. Asymmetries detection was in fact included in the Motor Optimality Score (an integrative assessment of motor repertoire associated to general movements assessment) as a specific item to be scored (37,38) and an asymmetry index was recommended also in the HINE administration in order to discriminate infants at risk for congenital hemiplegia from the healthy ones (39). In the fidgety qualitative assessment, the category of “asymmetrical fidgety”, defined as the predominantly expression of fidgety movements in the ipsilesional side of the body, has become increasingly used (27).

Results in our sample confirm literature findings since infants at low-risk of developing UCP according to their MRI brain lesion pattern (i.e. absence of involvement of the PLIC, extension to only one structure among the cortex, basal ganglia and the thalamus) reported a normal fidgety. As for the infants with UCP outcome,

according to the most recent literature, different expressions of fidgety were expected (27,28,40–42). Asymmetrical fidgety was reported in 6 out of 9 infants later diagnosed with UCP, while 2 infants reported a normal intermittent fidgety and 1 reported a sporadic fidgety. A preliminary evaluation of these data suggested that asymmetrical fidgety reported a high sensitivity and specificity in detecting UCP, although, as expected, in infants at risk of UCP different kind of fidgety, other than "normal" were noticed.

Since the observation that an "asymmetrical fidgety" seems to be significant in UCP detection, understanding the neuroanatomical and/or the neurophysiological factors underlying its expression could be of interest, as well as clarify whether specific clinical and lesion characteristics could be related on the contrary, to intermittent or sporadic fidgety in infants at risk of UCP. Speculating on this specific point, we first looked at the neuroimaging lesion patterns in our population. We found that "asymmetrical fidgety" was more likely to be detected both in full-term infants and preterm infants with middle cerebral artery stroke or venous infarction with an involvement of the cortex, the thalamus and/or the basal ganglia. Otherwise, a pattern of "symmetrical fidgety" both intermittent or sporadic was detected in a full-term infant with a lesion involving only the cortex, in a very preterm infant with lesion mainly involving the periventricular white matter and in a full-term infant with very early (II trimester) venous infarction with a main involvement of subcortical structures; the last 3 infants, with typical outcome were full-term with small lesions limited to cortex or basal ganglia. These data are consistent with the extensive literature available, mainly on arterial ischemic stroke and venous infarction, which support that the involvement of the main branch of middle cerebral artery and the posterior limb of internal capsule with the coexistence of hemispheric cortical injury with basal ganglia and/or thalamus involvement are more likely to be correlated with a unilateral motor impairment, while injuries involving just one or two of these structures usually predict a better or normal motor outcome (22,24,29,43).

In several studies, authors focused on the specific role of the basal ganglia and/or the thalamus in affecting hand function both prospectively, specifically addressing the possible correlations between lesion patterns and hand motor function and retrospectively, addressing to neuroimaging to investigate whether after perinatal stroke neuroplasticity mechanisms exist which could affect the motor outcome. In the first case, authors (9–11,44) consistently found a significant correlation between poorer hand function sometimes associated also with sensory deficits and the involvement of basal ganglia and/or thalamus in the lesion. The motor outcome was in general assessed at pre-school or school age with AHA or other standardized tests specifically focusing on hand functional ability. Their data corroborate the hypothesis already supported by previous literature that basal ganglia and the thalamus have a key role in the sensorimotor integration; specifically, the thalamus is considered a critical structure given its role as relay station projecting to cortex and receiving afferences from periphery and from the basal ganglia themselves. Furthermore, the basal ganglia have been investigated as being crucial structure in motor learning processing during neuromotor development, thus having a specific role in shaping the motor outcome on the basis of sensory experiences. In the second case, since neuroanatomical studies suggested a more severe motor outcome in infants with thalamic or deep grey matter involvement, some authors (34,45–47) investigated the potential contribution of subcortical structures in driving plasticity after perinatal stroke occurrence. Connectivity and volumetric studies on the thalamus and the basal ganglia revealed that, after the acute phase, all these structures reported a certain degree of changes in terms of volume extension and networking modulation which seems to be potentially involved in defining the motor outcome.

In the present study we focus on UCP clinical signs detection in the very first months of age when only spontaneous motricity can be assessed beyond neurological examination of postures, tone, reflexes and no structured data on specific hand function outcome is available. Looking for a possible correlation between fidgety subtypes and MRI brain lesion patterns we could speculate that asymmetrical fidgety seems to be more likely to be expressed when the lesion is extended to hemispheres, thalamus and/or basal ganglia. In the immature brain, early transient thalamocortical and intracortical networks are established autonomously and generate patterns of spontaneous activity responsible for the functional template that gradually shape the more mature cortical architecture. The cortical subplate is the transient structure, that before the cortical plate, contribute to establish these connections and foster the transition from a spontaneous activity to a

sensory driven activity that is responsible for the creation of more stable and long-lasting connections with a progressive enrichment of cortical-peripheric fashions increasingly based on genetic and environmental interaction (48). Considering this general framework of neural development, as for the movement appearance, it was hypothesized that the activity generated by the central pattern generator network in the spinal cord and brainstem is modulated by the projections of the cortical subplate. Authors theorised that the emergence of the fidgety movements may be induced by the developmental changes from transient networks to permanent circuitry shaped by the increasing afferent inputs that allow for more specific goal-directed movements (49). The fidgety general movement period seems then to reflect this phase of gradual shifting from subplate activity to cortical plate activity with consequently developmental changes in the permanent networks in the primary sensorimotor cortices (50). It is then hypothesized that fidgety phase should be characterized by activity-dependent reorganization of functional connections between corticospinal tract fibres and spinal motoneurons and thalamic nuclei are highly involved in this process as relay structures (49).

We can speculate that an early vascular accident affecting cortical and most of all, subcortical structures so deeply implicated in this delicate interplay between central sources and peripheral activity should break down the typical maturation of the sensorimotor network thus affecting the fidgety as an opportunity of postnatal calibration of the motor system on the basis of the proprioceptive experiences. The asymmetrical pattern of the spontaneous motricity could then be hypothesized to be the expression of a miswiring of the sensorimotor system development in a specific time window in which peripherical afferences and central projections are expected to shape one each other in an activity-dependent manner in order to pave the way for more advanced goal-directed movements. Considering our sample, the hypothesis of asymmetrical fidgety as a “miswiring biomarker” could find correspondence in the MRI pattern of our subjects since this pattern was found, independently from the gestational age, when a cortical-subcortical mismatch was likely to happen, while intermittent fidgety was found when only one structure was affected given the opportunity of a more extensive connective plastic reorganization and finally, the sporadic fidgety was noticed when a very early disruption of thalamus/basal ganglia occurred thus a precocious disruption of sensorimotor development could have affected the whole networking process.

Even if the paucity of sample doesn’t allow any conclusive consideration on the origin of asymmetrical fidgety, we can although observe that the detection of this kind of spontaneous motor pattern could be of help in early detection of UCP, but most of all, in suggesting new potential target for very early models of intervention which could be tailored to the sensorimotor network early restoring and connectivity rewiring taking advantage on the principles of environmental enrichment and motor-learning.

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Chapter 4

Asymmetry in sleep spindles and motor outcome in infants with unilateral brain injury

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4.1 Abstract

Aim: To determine whether interhemispheric difference in sleep spindles in infants with perinatal unilateral brain injury could link to a pathological network reorganization that underpins the development of unilateral cerebral palsy (CP).

Method: This was a multicentre retrospective study of 40 infants (19 females, 21 males) with unilateral brain injury. Sleep spindles were detected and quantified with an automated algorithm from electroencephalograph records performed at 2 months to 5 months of age. The clinical outcomes after 18 months were compared to spindle power asymmetry (SPA) between hemispheres in different brain regions.

Results: We found a significantly increased SPA in infants who later developed unilateral CP (n=13, with the most robust interhemispheric difference seen in the central spindles. The best individual-level prediction of unilateral CP was seen in the centro-occipital spindles with an overall accuracy of 93%. An empiric cut-off level for SPA at 0.65 gave a positive predictive value of 100% and a negative predictive value of 93% for later development of unilateral CP.

Interpretation: Our data suggest that automated analysis of interhemispheric SPA provides a potential biomarker of unilateral CP at a very early age. This holds promise for guiding the early diagnostic process in infants with a perinatally identified brain injury.

A common neuroanatomical substrate for the development of cerebral palsy (CP) is early structural brain damage, which compromises the growth and organization of neuronal networks during early infancy, leading to the wide range of clinical CP phenotypes.1 Neonatal stroke is a well-known cause of adverse neurodevelopmental outcomes, including unilateral CP. The global prevalence of neonatal stroke is around 1 in 3000 live births, and over half of the affected children develop long-term disabilities, including unilateral CP.2,3 Growing evidence supports the importance of early identification of infants at high risk for CP, as early detection might allow for timely therapeutic interventions aiming to exploit the neuroplastic potential of the developing brain.(4,5)

Sleep spindles are hallmarks of non-rapid eye movement sleep, representing a stereotypical, widely coordinated neuronal activity in the thalamo-cortical and cortico-cortical networks.6–8 The relative ease of sleep spindle detection with electroencephalography (EEG) makes it an attractive candidate for an early functional biomarker of neurodevelopmental network diseases such as CP. Indeed, studies on adults after hemispheric stroke suggest that functional asymmetry between hemispheres, and in particular a reduction in sleep spindles over the lesioned hemisphere, is correlated with stroke severity and outcome.9–14 These effects, however, have not been studied in infants where rapid functional and structural development of brain networks adds another dimension to be inspected: the sleep spindles themselves emerge around the second month of life, which is long after the occurrence of the structural brain lesion.(7,8)

In the present work, we set out to study whether spatial reorganization of sleep spindle activity is present after unilateral neonatal brain injury. We hypothesize that a marked interhemispheric asymmetry of sleep spindles' amplitude would be specific for a pathological network reorganization after unilateral brain injury that leads to unilateral CP

4.2 Method

Study design and participants

A multicenter cohort (n = 40; 19 females, 21 males) was retrospectively collected from three hospitals in Italy (Azienda Ospedaliera-Universitaria di Pisa [n = 8), Azienda Ospedaliera-Universitaria Meyer [n = 7], and Azienda Ospedaliera-Universitaria Careggi [n = 4]) and the Children's Hospital of the Helsinki University

Hospital in Finland (n = 21). Patients were identified from a 9-year period (1st January 2011 to 31st December 2019) from each local EEG archive. Details of the study population are reported in Table 1. The median gestational age at birth was 40.1 weeks (interquartile range [IQR] 1.18; range 36.6–42.3). The median age at recording was 3.0 months post-term (IQR 1.5; range 1.5–5.0). The median age at the last follow-up was 24 months post-term (IQR 18; range 18–117).

Inclusion criteria were: (1) a diagnosis of unilateral brain injury such as focal ischemic or hemorrhagic injury confirmed on neonatal magnetic imaging resonance (MRI); (2) at least one sleep-EEG recording between weeks 8 and 20 after term age, with at least 5 minutes of clear N2-NREM sleep; and (3) clinical follow-up of at least 18 months.

Exclusion criteria were: (1) preterm birth (before 36 weeks gestational age); (2) bilateral brain injury; (3) a known other brain malformation; (4) isolated hypoxic-ischemic brain injury; (5) epidural or subdural hematoma; (6) intraventricular hemorrhage without signs of parenchymal venous infarction.

Standard protocol approvals, registrations, and patient consents

For the Italian cohort, the relevant local institutional research review board approved the study protocol and ethics approval was obtained by the Tuscany Pediatrics Ethics Committee (SPACE2020; nr.187/2020) and for the Finnish cohort, the study protocol was approved by the Institutional Research Review Board at Helsinki University Hospital Medical Imaging Centre, Helsinki University Hospital. In both countries, a waiver of consent was granted because of the retrospective and observational nature of the study.

MRI acquisition and classification

All infants underwent standard diagnostic brain MRI during the first 2 weeks of life using a 1.5T scanner (Helsinki: Philips Intera Achieva, Philips Medical Systems, Best, the Netherlands; Santa Chiara Hospital and Meyer Children Hospital: GE, Signa Horizon, Milwaukee, WI, USA) or a 3T scanner (Helsinki: Siemens Magnetom Skyra, Siemens Healthcare GmbH, Erlangen, Germany). Acquisition protocols slightly differed among the clinical centres, but all included at least T1-, T2-, and diffusion-weighted images. For the Italian cohort, retrospective classification of brain lesions was performed by authors (VM, RR) based on the available MRI scans. For the Finnish cohort, as 11 infants were the same as in Nevalainen et al.,15 we used the scores assigned in that study; for the rest of the infants, MRI data were classified from the radiology reports based on the identification of the different vascular territories as previously reported by Kirton et al. and Goveart et al. and recently adapted by Nevalainen et al.15–17 Classification of stroke subtypes included the following: (1) proximal middle cerebral artery (MCA) territory including lateral lenticulostriate arteries, leading to infarction of basal ganglia and distal MCA territory; (2) distal MCA territory, involving the M1 segment, sparing basal ganglia, and lateral lenticulostriate arteries; (3) posterior trunk territory including parietal and posterior temporal lobes; (4) anterior trunk territory including frontal lobes and anterior temporal lobes; (5) other territory of the distal segments of the MCA not classifiable as posterior trunk or anterior trunk; (6) other arterial territories not classifiable as MCA territory; (7) territory of the lateral lenticulostriate arteries (mainly putamen, caudate body, and posterior limb of internal capsule); (8) medullary venous territory infarction extending into the periventricular white matter with a relative spare of basal ganglia and cortex; (9) periventricular venous infarction secondary to intraventricular haemorrhage; and (10) intracerebral lobar haemorrhage.

EEG acquisition

EEG recordings of the identified patients were retrospectively collected from the hospital archives. All the patients had undergone a full video-EEG recording of at least 40 minutes, including sleep and wake phases, with at least 5 minutes of clear N2-NREM sleep. Three different EEG systems were used in these hospitals (Meyer Children Hospital and Santa Chiara Hospital: Brain Quick, Micromed, Italy; Careggi General Hospital: Galileo, EBNeuro, Italy; Helsinki University Hospital: NicoletOne, Natus, Middleton, USA). Recordings were

sampled at 256Hz, and the EEG signals were acquired using paediatric EEG caps with at least 10 Ag/AgCl electrodes located at Fp1, Fp2, F3, F4, C3, C4, T3, T4, O1, O2 according to the international 10–20 system. All visual and computational analyses were performed on a standard bipolar montage: Fp1-C3, C3-O1, Fp1-T3, T3-O1, Fp2-C4, C4-O2, Fp2-T4, T4-O2. A low-pass filter with a cut-off of 70Hz and a high-pass filter with a cut-off of 0.3Hz was applied.

Sleep epochs were visually identified (by VM, PN, RR, FM), and the N2-NREM sleep epochs extracted from every EEG recording. The periods with obvious artefacts were removed by visual inspection and the channels with poor signal were excluded from the analysis. EEG data epochs were then exported into .edf format and imported into MATLAB (2018B, MathWorks, Natick, MA, USA) using customized routines.

Automated spindle detection and spindle power analysis

Spindle events were selected using an automatic spindle detector employing a customized algorithm in MATLAB (written by author VM) based on previously published procedures.6,10,18,19 For each event on every channel, we then calculated the spindle oscillation frequency and the mean spindle power. The algorithms can be found in an open GitHub repository (https://github.com/vivi-mar/EEGspindles_SPA). According to the maturational and spatial patterns,18 we then categorized the spindles depending on their respective frequency ranges as slow (11–13Hz), fast (13–15Hz), or full-band (11–15Hz). The mean spindle power was then computed for each of these frequencies and for every bipolar channel: fronto-central, fronto-temporal, centro-temporal, centro-occipital, and temporo-occipital.

Asymmetry in spindle power was regionally calculated as the ratio between the spindle spectral power of the lesioned and non-lesioned hemispheres. As a result, 15 separate spindle power asymmetry (SPA) indexes were obtained depending on the region and the frequency assessed: fronto-central-full-SPA, fronto-temporal-full-SPA, centro-temporal-full-SPA, centro-occipital-full-SPA, temporo-occipital-full-SPA, fronto-central-slow-SPA, fronto-temporal-slow-SPA, centro-temporal-slow-SPA, centro-occipital-slow-SPA, temporo-occipital-slow-SPA, fronto-central-fast-SPA, fronto-temporal-fast-SPA, centro-temporal-fast-SPA, centro-occipital-fast-SPA, temporo-occipital-fast-SPA.

Details of the spindle selection procedure and steps of power analysis and interhemispheric asymmetry are reported in Figure S1.

Motor outcome

As part of the standard care for infants with brain injury, all patients were included in the neurodevelopmental follow-up programme performed by the paediatric neurologists of the four tertiary centres. Motor outcome (classified as typical motor outcome and unilateral CP) was collected after retrospective review of the medical record (authors VM, PN, CA, RR) of the last follow-up visit (range 1 year 6 months–9 years 10 months; minimum 18 months).

Statistical analysis

Statistical analysis was performed with IBM SPSS statistics software v.23.0 (IBM SPSS Statistics, Version 23.0. Armonk, NY, RRID: SCR_002865). Demographic characteristics were compared for infants with and without unilateral CP using the $\chi 2$ test for binary variables. For comparisons of continuous variables between the outcome groups, we used the Mann–Whitney U test given that the data did not present a normal distribution (Shapiro–Wilk test, as the sample was below the 50 participants). We chose a p-value lower than 0.05 as the level of statistical significance in a two-tailed test.

SPA indexes which differed significantly between the two groups ($p < 0.05$) were then entered in a binary logistic regression model to predict the outcome. The forward conditional method was run by setting the

criterion value to enter the model in the forward selection as 0.05, and the criterion value to leave the model in the backward elimination as 0.1. By default, the starting model was the constant model.

Finally, we calculated the sensitivity, specificity, positive and negative predictive values, and area under receiver operating characteristic curve for the SPA index that was most accurate for predicting CP based on the logistic regression model. At the end, we applied the Grubb's test to check for the presence of any significant outliers and rebooted the analysis after their removal.

Data availability

All relevant data are within the paper and Appendix S1. Raw EEG data supporting the findings of this study are available from the authors in an anonymized format, upon reasonable request from a qualified investigator and approval by the ethics boards of the corresponding institutions for purposes of replicating procedures and results. The algorithms used for the spindle analysis are available from an open GitHub repository (https://github.com/vivi-mar/EEGspindles_SPA).

4.3 Results

In total, 13 out of 40 neonates developed unilateral CP. There were no significant differences in the demographic variables between infants with versus without unilateral CP (Table 1).

Neonatal MRIs were classified according to the stroke patterns. Most of the lesions (n = 27) occurred in the territories of the MCA: 10 infants had lesions involving the proximal tract of the MCA, five were in the distal branches, three in the anterior trunk, seven in the posterior trunk, two in the lateral lenticulostriate arteries territory. In two infants, lesions occurred in other arterial territories than MCA. Of the remaining cases, six presented periventricular venous infarction, three intracerebral haemorrhage, and two periventricular haemorrhagic infarction. In our sample, all infants who developed unilateral CP had unilateral brain injury involving the MCA territory, while those having a positive motor outcome were distributed across all MRI classification groups.

SPA and outcome

We first examined whether early SPA significantly differed between the clinical outcome groups. The infants with eventual unilateral CP had a greater SPA in their wideband spindles (11–15Hz) in the centro-temporal and centro-occipital regions (centro-temporal-full-SPA $p < 0.001$; centro-occipital-full-SPA $p < 0.001$). Frequency-wise analysis showed the group difference to be frequency specific: the slow spindles (11–13Hz) differed between clinical outcome groups in the same centro-temporal and centro-occipital derivations (centro-temporal-slow-SPA $p < 0.001$; centro-occipital-slow-SPA $p < 0.001$), while the fast spindles (13–15Hz) showed a group difference also in the fronto-central derivations (fronto-central-fast-SPA $p = 0.014$; centro-temporal-fast-SPA $p < 0.001$; centro-occipital-fast-SPA $p < 0.001$). Table 2 and Figure 1 summarize results from all comparisons. There was, however, no significant association between SPA and the patterns of MRI-detected structural brain injury.

We then tested the ability of SPA to predict unilateral CP at the individual level by entering all the seven SPA indexes that significantly differed between the centro-occipital outcome groups in a binary logistic regression model. An asymmetry between fast spindles over centro-occipital regions (centro-occipital-fast SPA) was found to provide the best prediction (centro-occipital-fast SPA Wald = 08.61, $p = 0.003$), explaining 44.6% (Nagelkerke R2) of the variance in outcome and correctly classifying 95.0% of cases, if considered alone in the regression model. The area under the receiver operating characteristic curve for centro-occipital-fast SPA was 0.93 (standard error: 0.07, asymptotic significance: 0.00, asymptotic 95% confidence interval: 0.79–1.00; see Figure 2). An empiric cut-off level for SPA at 0.65 gave a positive predictive value of 100% (sensitivity 100.0%) and a negative predictive value of 93.3% (specificity 92.3%) for later development of unilateral CP.

We finally checked the presence of outliers by applying the Grubb's test (two-sided p = 0.05) to the whole population and no outlier was selected (mean = 0.834, SD = 0.332). When we applied the Grubb's test by splitting the population in the two different outcome groups, it showed the presence of significant outliers in the unilateral CP group: one outlier was identified when tested for temporo-occipital and for centro-occipital SPA indexes. No outlier was detected in the group with typical motor outcome. After removing the outliers, we confirmed our results. For centro-occipital-SPA, the statistical difference between the outcome groups was even stronger (centro-occipital-full-SPA, $p < 0.000$; centro-occipital-slow-SPA $p < 0.000$; centro-occipital-fast-SPA $p < 0.000$); for temporo-occipital-SPA, the removal of outliers confirmed the non-different distribution of SPA indexes between the two outcome groups. The subsequent steps of the analytical process confirmed the selection of centro-occipital-fast SPA as the best predictor.

4.4 Discussion

Our results show that unilateral brain injury in newborn infants links to interhemispheric asymmetry of sleep spindles in those infants that later will develop unilateral CP. Our work extends prior research by showing that sleep spindles, a functional bedside measure of thalamo-cortical networks, may be a clinically useful biomarker in neurophysiological assessments at an early age. These findings corroborate recent studies on adults suggesting that sleep spindles may provide an endogenous functional biomarker of neuromotor outcomes after vascular brain injury.10,13

Sleep spindles emerge during the second month after term birth and are clearly seen over the central areas which involve the crucial networks implicated in the neuromotor functions affected in CP.7,18 The brain networks sustaining sleep spindles show rapid activity-dependent growth and organization during the early development of brain circuitry.20,21 In our study, asymmetry of the fast spindles over the centro-occipital regions proved to be the best predictor of later motor impairment. This is not surprising as during the first year of life the fast spindles (13–15Hz) peak over the central regions, while the slow spindles (11–13Hz) become more dominant over the frontal regions after the second year of life.18 Overall these findings confirm that the integrity of motor-sensory feedback over the somatosensory cortex could play a critical role in later motor development.

Sleep spindles are sustained by and are hence considered to reflect the integrity of thalamo-cortical circuitry.7,21 Sleep spindle activity is thought to be involved in sleep-dependent brain plasticity, memory consolidation, and learning,7,20–22 but also to be particularly sensitive after pathological events, such as focal brain injuries. Studies in adult patients showed that the occurrence and synchrony of spindles are greatly reduced after an injury affecting the sensory-motor cortex,9,11,14 the thalamic nuclei, or the thalamocortical projections.6,13,23 Moreover, after hemispheric stroke, amplitude asymmetry of spindles significantly correlates with long-term motor outcome of adult patients,9,14 indicating that poststroke sleep changes could be used as early biomarkers of atypical motor development.10,13 Our study extends these findings to neonatal brain injuries, a time window when spindles are yet to emerge, suggesting that the spindle asymmetry reflects an underlying disruption in thalamocortical networks, leading to the long-term neurodevelopmental consequence of unilateral CP. Recent studies on structural imaging are in line with the key role of thalamocortical pathways in the emergence of both unilateral and bilateral types of CP after perinatal stroke.24–26 Moreover, abnormalities in the thalamo-cortical projections are even a stronger predictor of motor outcome compared to findings of the corticospinal tract.24

Early prediction of unilateral CP is crucial for identifying infants to early intervention programmes, as well as to adequately support and counsel caregivers. Current clinical recommendations support the combination of structural brain MRI with the General Movements'Assessment to estimate the risk of unilateral CP before the 5th month of age.4 The sleep spindles assessment could provide an independent predictive measure to this age range. Previously, clinical qualitative interpretations of neurophysiological recordings were used to predict outcome after perinatal brain injury.15,27–29 In contrast, the hereby introduced SPA indexes offer

an objective and fully automated measure to be extracted from the clinical EEG recordings, which are often routinely performed during epileptological follow-up of perinatal stroke. As such, SPA indexes may support clinicians in the selection of high-risk infants after unilateral brain injury, providing a quantitative measure of risk of unilateral CP. Further studies are needed to determine whether SPA indexes provide information over and above the current diagnostic standards (neonatal brain MRI and neurological assessment). Nevertheless, SPA indexes might still offer an opportunity for early assessment of unilateral CP risk in areas where MRI is not easily available, like low- and middle-income countries.

Our study has some limitations that may affect the practical conclusions. First, this work is based on retrospective data collection from specialized medical centres, which might lead to selection bias towards higher risk populations; however, the incidence of unilateral CP in our cohort was comparable to the existing literature.30 Second, the retrospective study design does not allow a formal assessment of predictive performance or a standardized clinical protocol in the diagnostic thresholds. For instance, early motor assessments (i.e. General Movements Assessment)4 or identical neuroradiological protocols were not available for all individuals which limits a thorough comparison to alternative risk stratifications that are currently used in the clinical workups. Third, the size of the patient cohort is limited, and much larger cohorts are needed to establish definitive diagnostic thresholds, prediction performance, and generalization across data sets. Finally, the spindle detector algorithm was customized for this study as a standard part of the methodological development. While it cannot explain the key findings (the SPA group differences), it should be acknowledged that more detailed neurophysiological interpretations of the present results would benefit from a detailed technical validation of the spindle detector in larger cohorts. While any of these issues are unlikely to challenge the overall conclusions in our work, future prospective studies with multicentre settings are needed to accurately define the diagnostic added value of SPA in the clinical context.

In conclusion, the automatically computed measure of interhemispheric sleep SPA holds promise as an early, easy to obtain, and totally automated biomarker for predicting unilateral CP. Should these results be confirmed, SPA could be a valuable tool for the follow-up of infants at neurodevelopmental risk.

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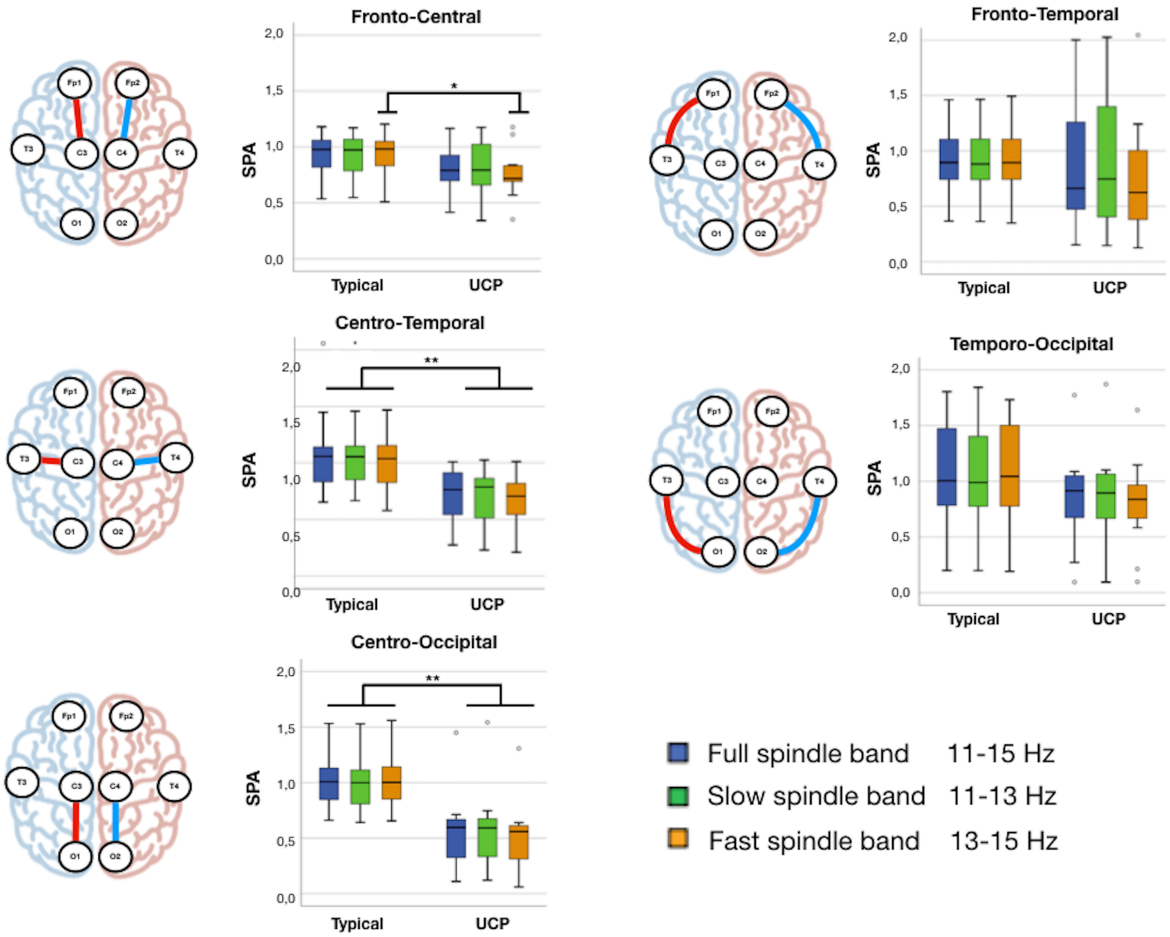

Figure 1: Graphical representation of regional spindle power asymmetry (SPA) indexes. Colour boxplots are grouped based on different outcomes (UCP: unilateral cerebral palsy; typical: typical outcome). The boxplots represent median and interquartile range; whiskers represent 95% confidence intervals; outliers are depicted as circles. For each region a schematic drawing of the bipolar electrode placement is reported.

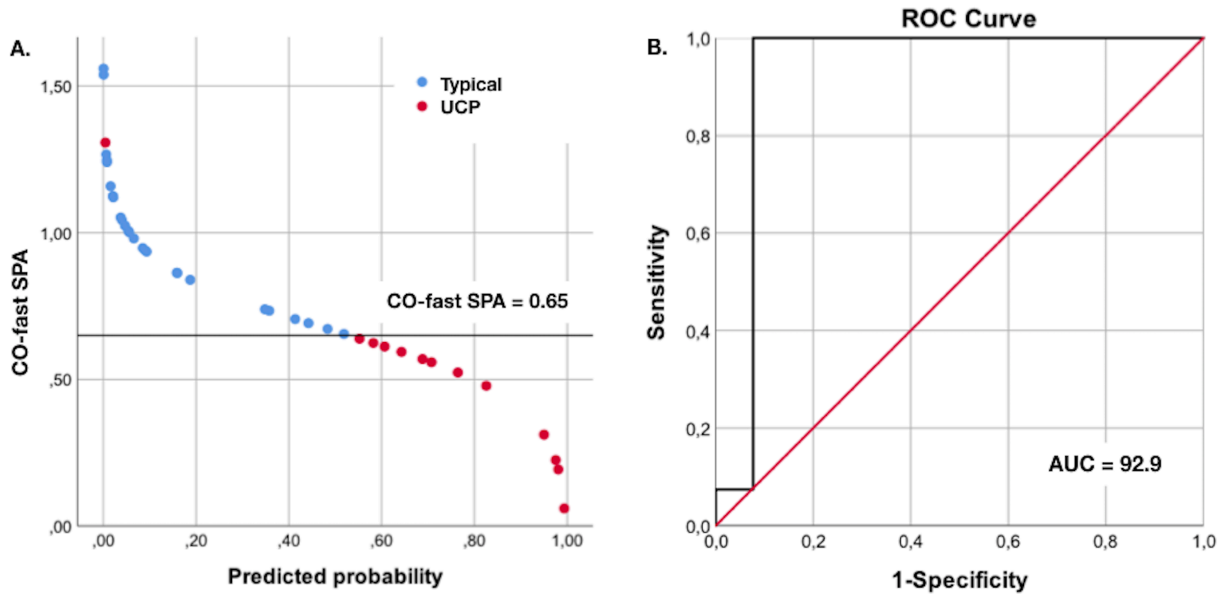

Figure 2: Unilateral cerebral palsy (UCP) prediction by centro-occipital fast sleep spindle power asymmetry (CO-fast-SPA). (a) Distribution of CO-fast-SPA values according to the corresponding predicted probability of developing UCP. Each circle represents one participant; colors represent different outcomes: red for UCP and blue for typical motor outcome. The selected cut-off for CO-fast-SPA is represented by the horizontal black line. (b) Receiver operating characteristic (ROC) curve and relative area under receiver operating characteristic (AUC) curve of the CO-fast SPA for UCP prediction.

	All groups, *n* = 40	**Unilateral CP, *n* = 13**	**Typical outcome, *n* = 27**
Females (%)	19 (48)	6 (46)	13 (48)
Lesion side, left (%)	22 (55)	8 (61)	14 (52)
Median (IQR) age at EEG (months)	3.0 (1.5–5.0)	3.0 (1.5–5.0)	3.0 (2.0–5.0)
Median (IQR) age at follow-up (months)	24.0 (18.0–117.0)	35.9 (19.0–117.0)	22.5 (18.0–84.0)
Median (IQR) gestational age (weeks)	40.1 (36.6–42.3)	40.4 (37.6–41.9)	40.0 (36.6–423)

Table 1: Demographics. Abbreviations: CP,cerebral palsy; IQR, interquartile range; EEG, electroencephalogram.

Asymmetry		Median	IQR	Mann–Whitney *U*	*p*
FC-full-SPA	Typical	0.98	0.28	111.00	0.064
	UCP	0.79	0.26		
FT-full-SPA	Typical	0.89	0.38	151.00	0.493
	UCP	0.66	0.90		
CT-full-SPA	**Typical**	**1.06**	**0.31**	**61.00**	**<0.001**
	UCP	**0.76**	**0.45**		
CO-full-SPA	**Typical**	**1.01**	**0.31**	**33.00**	**<0.001**
	UCP	**0.60**	**0.39**		
TO-full-SPA	Typical	1.00	0.73	135.00	0.252
	UCP	0.92	0.43		
FC-slow-SPA	Typical	1.97	0.32	125.00	0.151
	UCP	0.79	0.40		
FT-slow-SPA	Typical	0.88	0.37	157.00	0.608
	UCP	0.75	1.06		
CT-slow-SPA	**Typical**	**1.05**	**0.32**	**62.00**	**<0.001**
	UCP	**0.79**	**0.44**		
CO-slow-SPA	**Typical**	**1.00**	**0.33**	**39.00**	**<0.001**
	UCP	**0.59**	**0.42**		
TO-slow-SPA	Typical	1.00	0.66	137.00	0.276
	UCP	0.90	0.43		
FC-fast-SPA	**Typical**	**0.98**	**0.26**	**91.00**	**0.014**
	UCP	**0.72**	**0.18**		
FT-fast-SPA	Typical	0.89	0.39	123.00	0.135
	UCP	0.62	0.73		
CT-fast-SPA	**Typical**	**1.04**	**0.35**	**46.00**	**<0.001**
	UCP	**0.71**	**0.36**		
CO-fast-SPA	**Typical**	**1.00**	**0.32**	**25.00**	**<0.001**
	UCP	**0.56**	**0.35**		
TO-fast-SPA	Typical	1.04	0.73	132.00	0.217
	UCP	0,84	0.43		

Table 2: Regional spindles power asymmetry indices. Abbreviations: IQR, interquartile range; FC, fronto-central; SPA, spindle power asymmetry; UCP, unilateral cerebral palsy; FT, fronto-temporal; CT, centro-temporal; CO, centro-occipital; TO, temporo-occipital. Note: *p*-values less than 0.05 are statistically significant and marked in bold.

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SECTION 2

Building new models of early intervention in infants at high-risk of CP: family-centered care framework and its neurobiological foundation

Chapter 5

Parent-infant interaction during the first year of life in infants at high-risk for cerebral palsy: a systematic review of the literature

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5.1 Abstract

Perinatal adverse events put neonates at high risk for short and long-term disabilities, including cerebral palsy (CP). The most recent guidelines about early intervention in infants with brain damage have emphasized the importance of family involvement from the very first phases of development. Early parent-infant interactions are pivotal in promoting infant cognitive and social developmental trajectories. However, little is known about the extent to which severe adverse perinatal events can affect the quality of early parent-infant interactions. Patients and Methods. We systematically searched five databases (PubMed, PsycINFO, EMBASE, CINAHL, and Cochrane Library) for the publications assessing parent-infant interactions in infants at high neurological risk within 1 year of age. Articles were selected if they involved direct comparison between high-risk populations and healthy controls or low-risk populations, and if quantitative or semiquantitative tools were used to assess the parent-infant interaction. Measures of parent-infant interaction included infant interactive behaviors, parental interactive behaviors, and dyadic interactive patterns. Results. The search yielded 18 publications that met the inclusion criteria. The articles represent a high level of heterogeneity in terms of infant neurological risk, infant age, and tools assessing interactive behaviors. Both infant and maternal behaviors within the investigated interactive exchanges were reported to be compromised, leading to subsequent overall impairment of the dyadic patterns. Conclusion. While the studies reviewed here provide general and important information, the review did not yield a clear picture of early dyadic interactions in high-risk infant populations. Further observational studies are warranted in order to provide a more accurate knowledge of the early dyadic exchanges between infants at high neurological risk and their parents, as they might provide a critical opportunity for early family centered habilitative interventions.

5.2 Introduction

The role of parent-infant interaction during early development has been studied extensively in the past decades. Newborn's brain is known to be prone to interactive exchanges at birth or even before [1, 2]. Neonatal imitative processes, occurring from the very first hours of life [2], represent the first signs of reciprocation between parents and infants that, within the first months of life, evolve towards actual *protoconversations,* characterized by reciprocal multimodal exchanges and rhythmic vocal, facial and gesture imitations [3–5]. Murray and colleagues have recently suggested the existence of a functional architecture of mother-infant engagements, active from the very first weeks of life and apt to support the development of infant intersubjective skill [6]. Authors reported that the occurrence of mirroring or marking maternal responses to infant social expressions predicts the increase of such infant behaviors over time. More importantly, they stressed the importance of contingency more than frequency of maternal responses, thus suggesting that infants are able to capitalize on relatively limited exposure to specific parental behaviors, already at very early developmental stages.

Primary dyadic interactions support infant's cognitive, motor and social skills maturation[7, 8]. Studies in typically developing infants have widely demonstrated that the quality of early interactions can strongly influence later infant's developmental outcomes [9–12]. For instance, Feldman and Greenbaum [10] reported that maternal affective attunement and dyadic synchrony within the interaction of 3-month-old infants and their mothers were predictive of infants'quality of play, verbal IQ, and regulation capacity at 2 years of age. The contingency within interactive exchanges at 3 months of age has been reported to be the precursor of infants 'attachment style at 1 year [13], while maternal sensitivity to infants distress has been described as predictor of secure attachment [14]. Accordingly, studies focusing on clinical populations showed that the occurrence of either parent adverse conditions (e.g. maternal depression, anxiety or early traumatic experiences, poor socioeconomic family) or infant pathological conditions (e.g. preterm birth, autism, cleft lip palate) are associated with poor outcomes, likely due, at least partly, to a disruption of the quality of early dyadic interactions [7, 15–17]. So far, little attention has been given to the quality of early

dyadic interaction in infants at high-risk of neuromotor disabilities, and more specifically of cerebral palsy (CP), as studies on term infants with brain damage are very scanty, while the majority of the studies on preterm infants have focused on low-risk prematurity[18, 19].

CP is the most common physical disability in childhood [20]. Despite the progressive improvements in perinatal and neonatal care, extreme prematurity as well as perinatal insults are still associated with major neonatal morbidities with long-term sequelae such as neurodevelopmental delay, neurosensory disorders and cerebral palsy (CP) [21–25]. More specifically, in very or extremely premature infants or in full-term infants with a history of perinatal asphyxia, CP has still a prevalence rate consistently above 10% in high-income countries [26–30]. Scientific evidence is rapidly growing in support of the importance of an early diagnosis of CP for the improvement of long-term outcome[31]. This is essential for a prompt referral to early intervention programs aimed at promoting and maximizing neuroplasticity and minimizing further medical complications [31], and provide emotional support for parents [32–34].

The most recent guidelines about early intervention in infants with brain damage have greatly emphasized the importance of family involvement from the very first phases of development [35]. Indeed, review studies in infants at very high-risk of CP indicate that early interventions focusing on parents' empowerment and supporting early parent–infant relationships, may have greater impact on later cognitive and neuromotor outcomes compared to those with an exclusive focus on infant functional impairment [36,37]. A deep knowledge of general and detailed aspects of parent-infant interaction in populations at high-risk for CP would be therefore essential to inform new strategies for early clinical support in both infant and parents. Unfortunately, very little is known about how the parent-infant dyad is affected by the occurrence of severe perinatal events in the infant. In order to contribute bridging this knowledge gap, we systematically reviewed the existing literature on early dyadic interactions between parents and infants at high-risk for neurological impairments. The main objective of this paper was to review current knowledge on the influence of severe adverse perinatal events on the quality of early parent-infant interaction, focusing on infant behavior, parental behavior, and dyadic interactive patterns. We specifically focused on papers evaluating dyadic interactions occurring within the first year of life, as the optimal time window of the emergence and early development of infant and parental interactive patterns.

5.3 Methods

Literature Search and selection of studies

A systematic literature search was performed in February 2018, through the following electronic databases: PubMed/MEDLINE, PsycINFO, EMBASE (OVID), CINAHL and Cochrane Library. No publication date limits were applied to the searches.

The following search strategy, including both MeSH headings and keywords, was used: (Parent-child relations (Mesh) OR Mother-Child OR Father-Child OR Parent-Child OR Mother-Infant OR Father-infant OR Parent-infant) AND (Interaction* OR Relation* OR Attachment* OR Bond* OR Intersubjectiv* OR Transact*) AND (Brain injuries (Mesh) OR Brain damage* OR Brain Injur* OR Brain lesion* OR Brain malformation OR Asphyxia OR Hypoxia OR Ischemia OR Encephalopathy OR Hypoxic Ischemic Encephalopathy OR Cerebral stroke OR Leukomalacia OR Hemorrhage OR Haemorrhage OR High-risk) AND (Infant (Mesh) OR Infant OR Newborn* OR Neonate* OR baby OR Preterm OR Premature).

The list of records was first checked for duplicates using EndNote (EndNote X8.2, bld 13302). Subsequently, two authors (FF and CA) independently reviewed the remaining records for suitability by title and abstract. Finally, full-text of articles addressing the topic of interest were screened in order to exclude those not meeting inclusion criteria. Secondary searches involved checking of publication reference lists and manual searches of relevant journals.

Agreement for articles inclusion was reached upon discussion between authors (FF, CA, AG).

Inclusion criteria

Article selection was restricted to peer-reviewed research articles published in English and to human studies. Articles were selected if they met all the following criteria: 1) the study involved direct comparison between at least one population of infants at high-risk for neurological impairment and either healthy controls or low-risk populations; 2) mother-infant and/or father-infant and/or both parents-infant interactions were assessed; 3) quantitative or semi-quantitative tools were used to assess interaction; 4) the study included assessments within the first year of the infant's life.

High-risk for neurological impairment was defined by one or more of the following conditions: gestational age at birth under 30 weeks; birth-weight below 1500 g; perinatal asphyxia or hypoxic ischemic encephalopathy; cerebral stroke; periventricular leukomalacia; severe intraventricular hemorrhage (grades III or IV) or any type of documented brain damage occurring within the first month of life. Populations were defined as at high-risk for neurological impairment if at least 50% of their participants met the above criteria.

No limitations for article inclusion were applied to parent-infant interaction assessment methods, which could include feeding sessions, face to face interactions and free or structured play session, either toy-centered or non-toy-centered. Similarly, early interaction scoring modalities including scoring scales, manuals or checklists were all included, provided that a clear description of the analyzed parental and/or infant interactive dimensions (e.g. maternal intrusiveness, infant engagement, dyadic synchrony) were reported in the main text.

Data extraction

Descriptive information of all included articles was systematically extracted and gathered in an electronic database. These included authors, year, study design, sample size, inclusion and exclusion criteria for clinical and/or control samples, age of infants at the time/s of the parent-infant interaction assessment, assessment methods (e.g., place, duration), scoring modalities (e.g., behavioral annotation), and main findings relative to the early parent-infant interaction. Additional parental, neonatal, or developmental outcome measures and any other results relevant to the current report were scored and gathered, if reported. The quality of the included studies was assessed by using the National Heart, Lung, and Blood Institute (NHLBI) Quality Assessment Tool for Case-Control Studies [38], which was chosen based on the study design of the included articles. Two authors (OC and FF) independently evaluated the items of the tool as "yes," "no," "not applicable," "cannot determine," or "not reported." The comprehensive evaluation of all items was then used to rate the global quality of each study as "good," "fair," or "poor."

5.4 Results

The flow chart in Figure 1 summarizes the whole selection process and exclusion criteria at each selection step. Database and secondary searches yielded 2910 articles, of which 2673 were excluded after screening for titles and abstracts. Eighty-two full-text articles were scored, of which 18 met all predetermined inclusion criteria and were therefore included in the present review. Relevant details of the included studies are summarized in Table 1.

Table 3: Characteristics of the included studies.

Study	At high-risk sample	Control Sample	Exclusion criteria	Timing of assessment	Method	Main results
Greene et al. (1983)[39]	16 PT, with RDS (S-PT) 16 FT, with birth asphyxia (S-FT)	14PT, Healthy (H-PT) 16 FT, Healthy (H-FT)	Not defined	At 3 months (CA)	-Free play interaction -Video recording-15 min -At the Laboratory -Checklist by Lewis et al., (1974)[40]	- Infant-Look/gaze at mother: H-PT, H-FT > S-PT, S-FT - Mothers- vocal responsivity: H-PT, S-PT > H-FT, S-FT - Mothers-proximal and kinesthetic stimulation: S-FT> S-PT, H-FT, H-PT -Mothers-affective and distal stimulation: S-FT< S-PT, H-FT, H-PT <u>Other measures</u>: S-FT, S-PT lower scores in orientation cluster at NBAS
Lasky et al. (1984)[41][41]	40 PT, BW: <1500 g and/or requiring mechanical ventilation (PT)	25 FT, Healthy (H-FT)	Not defined	At 12 months(CA)	-Free interaction in 5 different situations: waiting, physical exam with a nurse present, physical exam with nurse absent, the nurse return, and blood drawing -Live observation- 5, 2, 2, 2, 4 min respectively -At the Hospital -Checklist by Lasky et al.,1984 [41]	-Mothers-restrain infant, positioning near infant, looking at the infant, not smiling at infant during blood draw: H-FT> PT <u>Other measures:</u> Bayley Mental Developmental Index (MDI) and Psychomotor Developmental Index (PDI): PT< H-FT
Minde et al., (1985) [42]	20 PT, BW <1500g (PT)	20 FT, Healthy (H-FT)	No Physical malformation	At 1, 2 and 3 months (CA)	-Visit 1 and 2: Routine feeding, Visit 3: face to face play sequence. -Live observation, visits 1-2: duration	-Feeding 4 weeks: Infant-alert/focused: H-FT>PT Mother-look en face: PT>H-FT Mother-vocalize to others: PT>H-FT Mother-touch: PT< H-FT Mother-smile: PT< H-FT

					not reported, visit 3: 10 min -At Home - Scoring of infant and maternal behaviors (as in Minde et al., 1980) [43]	-Feeding 8 weeks: Infant-leg movements: H-FT> PT Mother-vocalize to others: PT>H-FT Mother-vocalize to baby: PT> H-FT Mother-touch: PT< H-FT Mother-smile: PT< H-FT -Play 12 weeks: Infant-head/mouth movements: PT> H-FT Mother-look: PT>H-FT Mother-vocalize to baby: PT>H-FT Mother-smile: PT>H-FT <u>Other measure:</u> "Sicker infants" defined according to a morbidity scale by Minde (Minde et al., 1982)[44] display more behavioral disorganization during feeding, cry more and
Landry et al., (1986)[45]	20 High Risk (HR) PT with IVH III-IV, RDS or BPD (HR-PT)	20 Low Risk (LR) PT with transient to moderate RDS and/or IVH I-II (LR-PT) 20 FT: Healthy Full term (H-FT)	Moderate to severe Cerebral Palsy, sensory handicaps, non-IVH related hydrocephalus	12 months (CA)	-Free play interaction -Video recording-10 min -At the Laboratory -Coding of mother's attention-directing and infant's exploratory strategies [45]	-Mother-attention directing behaviors: HR-PT> LR-PT; -Mother-questions: H-FT> LR-PT; <u>Other measures</u>: Mothers of HR-PT used questions more often with infants with higher MDI, while use attention-directing gestures with infants with lower MDI

Farel et al., (1991) [46]	37 High Risk (HR) infants with intracranial Hemorrhage and/or perinatal asphyxia and/or seizures and/or meningitis and/or BW<1500 (HR)	37 Healthy infants matched for age, sex and race (H-FT)	Not defined	At 8 months (CA)	-Interactions during a feeding episode, a session in which the mother is asked to teach the child an age-appropriate task and a free play session. -Live observation-120 min in total. -At Home -NCAFS, NCATS, HOME scale (including an interview to the mother)[47, 48]	-Dyads-Scores at NCAFS and NCATS: HR<H-FT <u>Other measures</u>: a strong association between NCAFS and risk-status was reported.
Landry et al., (1993)[49]	11 PT with IVH III or IV (HR-PT)	16 PT with RDS or IVH I and II (LR-PT) 12 FT: Healthy (H-FT)	Other medical complications	At 12 months (CA)	-Toy-centered play interaction -Video recording-10 min -At the Laboratory -Coding of mother's attention-directing and infant's exploratory strategies[45]	-Infant-exploratory play in response to mother's structured strategy: LR-PT>H-FT -Infant-exploratory play: HR-PT< H-FT, LR-PT -Infant-exploratory play in response to unstructured versus structured strategies: H-FT>LR-PT, HR-PT - Infant-exploratory play in response to structured versus unstructured strategies: LR-PT> H-FT, HR-PT

Smith et al., (1996) [50]	89 PT with BPD, IVH III or IV and/or PVL (HR-PT)	123 PT with IVH I or II, transient RDS (LR-PT) 128 FT: Healthy (H-FT)	Sensory impairments, meningitis, encephalitis, congenital abnormality of the brain	At 6 and 12 months (CA)	-Toy play session and naturalistic observation of daily activity -Live observation-10 min and 60 min -At Home -Mixed Rating scale and Microanalytic coding system [45, 51]	- Daily Activities and toy play at 6 and 12 months: Mother-interactive behaviors: HR-PT=LR-PT-H-FT <u>Other measures:</u> 6 months -MDI, daily living, receptive language: FT, LR-PT>HR-PT -Expressive language: HR-PT<H-FT -Positive correlation between mother-attention maintaining behavior and infant mental age and receptive language score (during daily activity and toy play): HR-PT, LR-PT> H-FT -Positive correlation between mother-attention maintaining behavior and infant expressive language score (during daily living activity): HR-PT, LR-PT> H-FT -Positive correlation between mother-attention maintaining behavior and infant expressive language score (during toy play):HR-PT>LR-PT, H-FT
Schermann-Eizirik et al.,(1997)[52]	67 PT, GA <32 ws required IC* (VPTIC) 75 PT, GA <36 ws required IC* (PTIC) 66 FT, required IC* (FTIC) *IC, intensive care for CPAP or parenteral nutrition o severe asphyxia	70 FT, Healthy (H-FT)	Chromosomal abnormalities and severe cerebral malformations.	At 2, 4 and 6 months (CA)	-Undressing of the infant and face to-face interaction. -Video recording-variable time, 3 min -At the Laboratory -Mother-infant interaction coding (Bohlin et al., 1989)[53]	-2,4,6 months: Mother-interactive behaviors: VPTIC, PTIC=H-FT Infant-interactive behaviors: VPTIC, PTIC=H-FT Dyad-positive interaction:VPTIC, PTIC=H-FT -2 months: Mother-interactive behaviors:FTIC=FT Infant-interactive behaviors:FTIC=FT -4 months: Mother-sensitivity/involvement:FTIC<FT Infant-interactive behaviors: FTIC<FT Dyad-positive interaction: FTIC<FT -6 months: Mother-sensitivity/involvement: FTIC<FT Infant-interactive behaviors: FTIC=FT

Davis et al., (2003)[54]	50 PT, <1500 g, with neurobiological risk (defined by NBRS) (HR-PT)	Normative data	No congenital anomalies	Within the 6th month	-Interaction during feeding -Video recording-20 min -At Home -NCAFS[47]	-Total feeding score: HR-PT< Normative data -Mother-interactive behaviors: HR-PT=Normative data -Infant-responsivity to caregiver: HR-PT<Normative data Other measures: According to The Coping Health Inventory for Parents[55], mothers, mothers who coped better, had more responsive children at three months after discharge.
Muller-nix et al., (2004)[56]	28PT, HR: defined by PERI [57](HR-PT)	19 PT, LR defined by PERI (LR-PT) 25 FT: Healthy (H-FT)	Infant malformation, chromosomic abnormalities, foethopaty	At 6 months (CA)	-Mother-child toy-play interaction -Video recording-10 minutes -Context not specified -CARE index[58]	-Mother-sensitivity: HR<LR<FT -Infant-interactive behaviors: HR-PT=LR-PT-H-FT Other measures: Mother-Posttraumatic Stress Symptoms :HR-PT>FT More stressed mothers were less sensitive and more controlling in dyadic play.
Schmücker et al., (2005)[59]	n=79 PT, BW: < 1500 g, and/or with IVH, PVL, SGA or required more than 28 days on mechanical ventilation. (HR-PT)	35 FT, Healthy (H-FT)	Not defined	At 3 months (CA)	-Interaction during diaper change and free play -Video recording-10 minutes -At the Laboratory -Microanalytic coding system to rate early mother–child interaction[60]	-Infant-vocalize: HR-PT>H-FT -Infant-vocally responsive: HR-PT> H-FT -Infant-facially responsive: HR-PT<H-FT-Mother-facially responsive: HR-PT<H-FT Other measures: The higher the neurobiological risk of the infant, the more mothers were judged to lack sensitivity.

Feldman (2006)[61]	17 PT, BW: < 1000 g, <30 ws (HR-PT) 29 FT: >36 ws, >2500 g (H-FT)	25 PT BW 1700-1850 g, 34-35 ws (LR-PT)	IVH III e IV, asphyxia, metabolic and genetic diseases.	At 3 months (CA)	-Face-to-face interaction -Video recording-5 min -At Home -Scoring with Monadic Phase Manual.[62]	-Mother-infant synchrony: H-FT>HR-PT, LR-PT, HR-PT=LR-PT -Mother-infant degree of synchrony (coherence): FT>LR-PT>HR-PT -Infant-negative emotionality: HR-PT, LR-PT>H-FT <u>Other measures:</u> Biological rhythms analysis revealed that sleep–wake cyclicity, vagal tone, orientation, and arousal modulation are each uniquely predictive of mother–infant synchrony at 3 months.
Feldman and Eidelman (2006)[63]	18 PT, SGA, BW: <1000g (SGA<1000) 28 PT, AGA, BW: <1000g (AGA<1000)	22 PT, SGA, BW: >1000g (SGA>1000) 52 PT, AGA, BW: >1000g (AGA>1000)	IVH IV, asphyxia, metabolic or genetic or syndromic disease, SNC Infection	At 3 months (CA)	-Mother-infant interaction -Video recording-10 min -At Home -CIB [64]	Mother-intrusiveness: (SGA<1000)>(SGA>1000)>(AGA<1000), (AGA>1000) Infant-Negative engagement: (SGA<1000)=(SGA>1000)=(AGA<1000)=(AGA>1000) <u>Other measures:</u> At 12 months, SGA<1000 showed poorer cognitive development at MDI.SGA<1000 scored significantly lower on orientation and motor maturity compared with other groups.
Feldman (2007)[65]	34 PT, BW: < 1500 g;(HR-PT) 21 PT, IUGR, <1500g (HR-PT)	38 FT: Healthy (H-FT)	Maternal and/or paternal depression and anxiety	At 4 months (CA)	-Interaction mother-infant father-infant, triadic interaction -Video recording 5 min each -At Home -Coding Interactive Behavior Manual (CIB)[64]	-Mother-Intrusiveness: HR-PT>H-FT -Mother-Sensitivity: HR-PT<H-FT -Infant-Negative Emotionality: HR-PT>H-FT -Dyad-reciprocity: HR-PT<H-FT <u>Other measures:</u> -Family cohesion: HR-PT<FT -Family rigidity: HR-PT>FT Mother of IUGR infants showed the highest Intrusiveness scores and IUGR infants showed the highest negative emotionality. Family also showed the highest rigidity.

Korja et al., (2008)[66]	30 PT, BW: <1500 g, GA: <32 (PT)	36 FT: Healthy (H-FT)	major congenital anomalies	At 6 and 12 months (CA)	-Free play mother-infant interaction (toy optional) -Video recording-5 min -At the laboratory -PC-ERA[67]	-6 months: Infant-interactive behaviors: PT=H-FT Mother-interactive behaviors: PT=H-FT -12 months: Infant-quality of play and attention: PT<H-FT Infant-sober and withdrawn: PT<H-FT Mother-interactive behaviors: PT=H-FT <u>Other measures:</u> Duration of holding at 5 months (CA) was positively associated with the good quality of mother–infant interaction at 6 and 12 months in PTPTs cried (combined fussing and crying) more often and were held more than H-FT
Agostini et al., (2014)[68]	29 PT, BW: <1000 g (HR-PT) 40 PT, BW: <1500 g (PT)	80 FT: Healthy (H-FT)	infant chromosomal abnormalities, CP, malformations and foetopathy.	At 3 months (CA)	-Face to face interaction -Video recording- 5 min -At the Laboratory -Global rating scale (GRS)[69]	-Mother-sensitivity: HR-PT=PT=H-FT -Mother-intrusiveness:HR-PT>H-FT -Mother-Remoteness: PT, HR-PT<H-FT -Infant-Interactive behaviors: HR-PT=PT=H-FT <u>Other measures:</u> In H-FT mothers, higher degree of remoteness was associated to the presence of depressive symptoms.
Neri et al., (2015)[70]	32 PT, BW: <1000 g (HR-PT) 45 PT, BW:<1500 g (PT)	20 FT, Healthy (H-FT)	Infant chromosomal abnormalities, cerebral palsy, malformations and foetopathy	At 3 months (CA)	-Face to face interaction -Video recording- 5 min -At the Laboratory -Global rating scale (GRS)[69]	-Mother-Sensitivity: PT>H-FT -Mother-Intrusiveness: HR-PT>H-FT -Mother-Remoteness: HR-PT<H-FT -Infant-Communicative dimension: PT> FT <u>Other measures:</u> Mother-Signs of depression: HR-PT, PT>H-FT

Sansavini et. al., 2015[71]	20 PT: GA<28 ws (HR-PT)	20 FT: > 37 ws (H-FT)	major cerebral damage,PVL, IVH > II grade,hydrocephalus.	At 12 months (CA)	-Mother-child toy-play interaction -Video recording-10 minutes -At the laboratory -R-RCS for dyadic co-regulation and Lunkenheimer's coding system for coding affective intensity[72, 73]	-Dyad-frequency of symmetric co-regulation patterns: HR-PT< H-FT -Dyad-frequency of unilateral co-regulation patterns: HR-PT> H-FT -Infant-Emotional Involvement:HR-PT< H-FT -Mother-frequency of high positive affective intensity: HR-PT< H-FT Infant-frequency of neutral affective intensity:HR-PT> H-FT Infant-frequency of high positive affective intensity: HR-PT< H-FT Infant-frequency of low positive affective intensity: HR-PT> H-FT Infant-duration of neutral affective intensity: HR-PT> H-FT Infant-duration of high positive affective intensity: HR-PT< H-FT Infant-duration of low positive affective intensity: HR-PT< H-FT Infant-duration of neutral affective intensity: HR-PT> H-FT Infant-duration of low negative affective intensity: HR-PT> H-FT

PT=Preterm infant; **FT**=Full Term Infant; **S-PT**= Sick Preterm; **S-FT**= Sick Full Term; **H-PT**=Healthy Preterm; **H-FT**=Healthy Full Term; **HR-PT**=High risk Preterm; **LR-PT**=Low risk Preterm; **VPTIC**=Very Preterm who required Intensive Care; **PTIC**=Preterm who required Intensive Care; **SGA**=Small for Gestational Age; **AGA**=Appropriate for Gestational Age; **BW**=Birth weight; **GA**: Gestational Age; **CA**=Corrected Age; **RDS**=Respiratory Distress Syndrome; **BPD**=Bronchopulmonary dysplasia; **PVL**= Periventricular Leukomalacia; **IVH**: Intraventricular Hemorrhage; **IUGR**=Intrauterine growth retardation; **NBAS**=Neonatal Behavioral Assessment Scale; **MDI**=Bayley Mental Developmental Index; **PDI**=Bayley Psychomotor Developmental Index; **NCAFS**=Nursing Child Assessment Feeding Scale; **NCATS**= Nursing Child Assessment Teaching Scale; **HOME**=Home Observation Measurement of the Environment; **NBRS**= Neurobiological Risk Score; **CIB**=Coding Interactive Behavior; **PC-ERA**= Parent-Child Early Relational Assessment.

Table 2: Assessment and Scoring Scales used in the studies.

Assessment And Scoring Scales	Description	Reference	Studies
Global interaction scales			
Global rating scales (GRS)	Assessment of the quality of mother-infant interaction. Maternal behavior is rated on 4 dimensions: sensitivity, intrusiveness, remoteness, and signs of depression. Infant behavior is rated on 3 dimensions: communicative, inert, distressed. One dimension assesses the quality of the overall interaction between mother and infant. A 5-points Likert-type scale is used to rate each dimension, with 1 being the poorest and 5 being the optimal rating.	Gunning et al., 1999 Murray et al., 1996a,b [69, 74]	Agostini et al., 2014; Neri et al., 2015 [68, 70]
NCAST Feeding (NCAFS) and Teaching (NCAST) PCI Scales	The NCAST-PCI evaluates 149 items related to maternal and infant behaviors. It comprises two scales: NCAFS and NCATS. infant and parent items are coded as Yes or No, items are then added to provide a total score. Each scale includes 4 subscales, measuring maternal behaviors and 2 subscales, measuring child's behaviors. Maternal subscales are: Sensitivity to cues, responsivity to child's distress, social-emotional growth fostering and cognitive growth fostering. Infant subscales are: clarity of cues, responsiveness to parent.	Barnard et al.,1987[47]	Davis et al, 2003[54] Farel et al., 1991[46]
Coding Interactive Behavior (CIB)	Global rating system of parent-child interaction in different play or interaction situations, including 42 codes: 21 for parents, 16 for infants, and 5 for dyads. Each score is rated with a Likert-type scale, where 1 corresponds to the poorest and 5 to the optimal rating. Five composite scales are included: maternal sensitivity, maternal intrusiveness, child's social involvement, dyadic reciprocity.	Feldman, 1998[64]	Feldman and Eidelman, 2006; Feldman, 2007[63, 65]
Parent-Child Early Relational Assessment (PC-ERA)	Semi-structured assessment to evaluate affective and behavioral quality of parent–infant interaction during 4 situations: feeding, administration of a structured task, free play and a separation-reunion task. Three parental subscales (29 items) are coded: positive affective involvement and verbalization; negative affect	Clark (1985)[67]	Korja et al., (2008)[66]

	and behaviors; intrusiveness, insensitivity and inconsistency. Three Infant subscales (28 items) are coded: positive affect, social and communicative competence; quality of play, interest and attentional skills; Dysregulation and irritability. Two Dyadic subscales (8 items) are coded: mutual enjoyment and reciprocity, tension and disorganization. A 5-points Likert-type scale is used to rate each item.		
CARE-Index	Assessment of the quality of adult-infant interaction. Three adult behaviors are scored: sensitivity, control, and unresponsiveness. Four infant behaviors are scored: cooperativeness, compulsiveness, difficultness, and passivity. The scores range from 0 to 14, with 0 score being the worst score.	www.patcrittenden.com, Crittenden 1979-2004 [58]	Muller-nix et al., 2004[56]
HOME	Inventory designed to identify the presence of risk for developmental delay due to lacking of appropriate quantity and quality of stimulation from home environment. Forty-five binary items, organized in six subscales, are scored using a combination of semi-structured mother interview relative to children routine activities, observation of mother-infant interaction during play and interview and assessment of kinds of play materials available to the child. Six subscales are coded: emotional and verbal maternal responsivity, maternal avoidance of restriction and punishment, maternal involvement with the child, organization of the environment, provision of appropriate play materials, variety in daily stimulation.	Bradley&Cadwell 1979 [48]	Farel et al., 1991[46]
Micro-analytic			
Coding system of Minde (1980)	Micro-analytic system recording the occurrence of the 10 maternal and 11 infant behaviors. Infant behaviors: arm, head, leg, hand and to mouth; eyes open; scan; grimace; cry; vocalize; smile; yawn. Maternal behaviors: look; look *en face*; verbalize to baby and to others; instrumental and non-instrumental touch; hold; feed; smile; standing further than 1 meter away from the baby. Behaviors are recorded by a digital simultaneous event recorder (procedure described in Celhoffer et al., 1977).	Minde et al. 1980 [43]	Minde et al., 1985 [42]

Microanalytic coding system to rate early mother–child Interaction by Jo¨rg (1994)	Mycro-analytic system which rates interactive behaviors at fixed time intervals of 1, 15 and 30 seconds. In particular, maternal behaviors rated per second are: direction of gaze, vocalization, facial expression, content of interaction, proximity; infant behaviors rated per second are: direction of gaze, vocalization, facial expression; joint mother-child behaviors rated every 15-30 seconds are: appropriateness of stimulation, maternal responsiveness, child responsiveness.	Jo¨rg et al., (1994) [60]	Schmùcker et al.,2005 [59]
Coding system of Landry (1986)	This coding system is based on the recording of the occurrence of mothers' attention-directing strategies and infant's responses. The variables scored are: maternal attention-directing attempt; verbal technique-question; verbal technique-Imperative; verbal technique-attention verbs; non-verbal techniques attention directing-gesture; non-verbal techniques attention directing-demonstrate; non-verbal techniques attention directing-give; Initial focus of attention-maintain; Initial focus of attention-introducing; initial focus of attention-redirecting; Infant response-no response; infant response-look; Infant response-manipulate.	Landry (1986)[45]	Landry (1986); Landry (1993)[45, 49]
Monadic Phase Manual	Coding system in which the stream of affective behavior of eah partner is coded using 6 expressive modalities for the parent, which are vocalization, direction of gaze, head orientation, facial expression, body position, specific handling of the infant and 5 for the infant which are vocalization, direction of gaze, head orientation, facial Expression. Combination of expressive modalities, checked second by second, are transformed in one of the following seven Adult Monadic Phases: Avoid, Avert, Monitor, Elicit, Set, Play, Talk. Six Infant Monadic Phases are also coded: Avoid Avert, Monitor, Set, Play, Talk.	Tronick et al. 1980 Feldman 2003 [62, 75]	Feldman 2006 [61]
Revised relational coding system (R-RCS)	This coding scale assesses Dyadic co-regulation based on 5 patterns: symmetrical, asymmetrical,unilateral, disruptive, unengagged. One additional pattern, no code, can be used for missing information.	Fogel et al., 2003 [72]	Sansavini 2015 [71]

Lunknenheimer's coding stystem	This scale codes parent and infant positive and negative affective intensity in 30-s intervals. An ordinal 3-point scale (non, low, high) is used to code affective behaviors based on a combination of voice tone, facial expression, eye contact, and body language.	Lunknenheimer et al., 2011 [73]	Sansavini 2015[71]
Checklist			
Checklist by Lewis	Checklist sheet for recoding 13 infant and 12 maternal behaviors. Behavior are coded within 10-seconds periods:occurrence, initiation or response. Two principal types of summary variables are computed from discrete infant and maternal behaviors: general behavior and responsivity. Maternal general behaviors: frequency of general stimulation; frequency of proximal stimulation, frequency of distal stimulation, frequency of kinesthetic stimulation, frequency of positive affect expression, frequency of vocal stimulation. Infant general behaviors: Frequency of fret/cry; frequency of vocalization; frequency of look/gaze at mother. Maternal responsivity: proportion of general responsivity; proportion of proximal responsivity; proportion of distal responsivity, proportion of vocal responsivity. Infant responsivity: proportion of general responsivity.	Lewis et al., 1974[40]	Greene et al. (1983) [39]
Checklist by Lasky 1984	Observational method based on rating the presence of maternal and infant behaviors in 5 different situations. 10 behaviors initiated by the infant and 12 behaviors initiated by the mother are checked.	Lasky et al.,1984 [41]	Lasky et al.,1984 [41]
Checklist by Bohlin, 1989	Observational method based on on 5-point scale (higher score indicating higher frequency or better performance) rating of maternal, infant and dyadic items. Maternal items are grouped into three variables: sensitivity, intrusiveness and involvement. Infant items are grouped into two variables: infant interactive behaviors. The Dyadic variable corresponds to a global evaluation of quality of positive interaction.	Bohlin et al., 1989[53]	Schermann-Eizirik et al., (1997)[52]

Other			
Mixed Rating scale and Microanalytic coding system	Five points rating scale to code a composite measure labeled "warm sensitivity" which comprises three maternal behaviors: positive affect, warm concern/acceptance and flexibility/responsiveness combined to a micro-analytic coding scheme developed to quantify maternal attention-directing events defined as verbal and non-verbal behaviors (frequency of events is considered for analysis).	Landry et al.,1986, Smith et al.,1996 [45, 51]	Smith et al., 1996[50]

Level of neurological risk of the study populations

All studies included preterm populations, while few of them involved mixed populations of preterm and full-term infants at high-risk for neurological impairments. The severity of infant risk-status was quite variable among the selected articles. In eight articles, the high-risk population presented intraventricular haemorrhage (grades III– IV), periventricular leukomalacia, severe perinatal asphyxia, seizures, meningitis or other severe medical conditions [39, 45, 46, 49, 51, 52, 56, 59], while in the remaining studies, the high-risk population included infants on the basis of intrauterine growth retardation (IUGR), birth weight or prematurity with or without mild or moderate medical complications [41, 42, 54, 63, 65, 66, 68, 70, 71].

Seven of the 18 studies explicitly excluded infants with severe brain lesions or perinatal asphyxia from their high-risk samples, however the resulting populations still met at least one of our inclusion criteria for high neurological risk (i.e. BW or GA) and were therefore retained in the current review.

Parent-infant interaction assessment modalities

The time-duration for observation of early parent-infant interaction was quite heterogeneous, varying from few minutes long video-recorded sessions (from 3 to 20 minutes) [39, 49, 52, 54, 59, 63, 65, 68, 70] to much longer (up to 120 minutes) live observations [41, 42, 46, 51]. A wide variety of parent-infant interaction coding modalities was used, including micro-analytic coding systems [42, 45, 49, 59, 63, 71], rating scales [46, 54, 56, 63, 65, 66, 68, 70] and scoring checklists [39, 41]. Only one study used a mixed coding system which included both micro-analytic and global rating scores [50]. Assessment details are summarized in Table 2.

The timing at which parent-infant interaction assessment were performed was equally distributed over the first year of life, with about half of the included studies collecting data within the first semester of life [39, 42, 52, 54, 59, 63, 65, 68, 70] and half focusing on older infant's ages, from 6- to 12-month-old infants [41, 45, 46, 49, 51, 56, 66, 71]. Most of the studies involved a cross-sectional design [39, 41, 45, 46, 54, 59], while few of them implemented a longitudinal-design, with data collection at multiple time-points[42, 50]. Three studies [49, 56, 63] evaluated mother-infant interaction over a longer temporal span, which goes beyond the first year of life. For these studies, however, only assessments falling within the first year of life were considered in the present review.

Finally, all studies focused on mother-infant interaction. Feldman [65] was the only study that, in addition to the mother-infant interaction, also included father-infant and triadic early interactions. The authors found no differences between father-infant and mother-infant interactions therefore, we abstain from discussing this topic further in this review.

Comparison of interactive patterns between high-risk and non-high-risk dyads prior to 6 months

Overall, all of the included studies described compromised dyadic interactive patterns between mothers and infants who experienced adverse perinatal events, compared to those occurring within control populations. Importantly, while most of the studies focused on interactive behaviors considering mother and infant as discrete units which interact to each other, few studies analyzed the quality of early interactive exchanges in a dyadic perspective.

Infant interactive behavior

Within the first semester of life (or corrected age for prematurity), infants at high-risk were mostly described as **less active**, **less engaged in the interaction** and **more fretful** than controls. Minde and colleagues [42] showed that preterm infants were less alert and focused, as revealed by the fact that they spent less time with their eyes open during feeding sessions, at one month of age; although they became more physically active by 3 months of age. Davis et al. [54], described preterm infants at risk as less responsive to their caregivers compared to typically developing infants, during feeding. Schmücher et al, 2005 [59] and Feldman 2006 [65], instead, found that high-risk preterm infants were less optimally engaged in dyadic interaction

with their mothers, as they showed less facial expressions and more negative engagement cues, respectively, compared to controls. Interestingly, however, Schmücher and colleagues (2005) [59] also reported that preterm infants were more vocally active and responsive than full-term infants, thus indicating that the extent of responsiveness to the caregiver can be different depending on the communicative channel.

Two studies explored populations of full-term infants at high neurological risk, reporting abnormal infant behavior within the parent-infant interaction [39, 52]. Specifically, Greene and colleagues [39] found that, healthy infants looked significantly more at their mothers than sick infants, with sick full-term infants, corresponding to the group at highest neurological risk, having the lowest scores compared to healthy controls. Similarly, results by Schermann-Eizirik and colleagues (1997) [52], revealed that, unlike preterm born infants, full-term infants who required intensive care at birth, thus supposedly corresponding to the most impaired infants,differed from healthy full-term controls in their interactive patterns, with the first group showing significantly less interactive behaviors than the second one. Finally, more recent studies investigating early mother-infant interactions within the first trimester of life showed no significant differences in terms of negative engagement or interactive patterns between preterm infants and full-term controls [63, 68].Only one study [70] reported more communicative behaviors in VLBW infants compared to full-terms ones,.

Parent (mother) interactive behavior

Consistent with results on infant behaviors, during the first months of infants' life mothers of high-risk infants were described as **less sensitive**, **more vigilant or intrusive** and **less emotionally involved** than mothers of healthy or low-risk infants.

At 3 months of age, Greene and colleagues [39]reported that high risk infants, and specifically high-risk full-term infants, during free play interactions received more proximal and kinesthetic stimulation but less distal and affective behaviors from their mothers, compared to healthy infants. At the same infant's age, other studies [63, 65, 68] reported that mothers of high-risk infants had more intrusive and less remote behaviors during face to face interaction than mothers of controls. Similarly, Minde and colleagues [42], reported that during both early feeding sessions (1 and 2 months) and later play interactions (3 months), mothers of premature infants at higher risk, provided more compensatory care (e. g. vocalization and en face look), but less affect (e.g. smiling,) to their infants compared to full-term mothers.

More inconsistent results were found about maternal sensitivity. In fact, while some studies [54, 59, 68] failed to find differences between study and control mothers' groups in the sensitivity dimension, others showed that mothers of high-risk infants are less sensitive than mothers of controls [52, 61]. In particular, Schermann-Eizirik and colleagues [52], overall, did not find differences in interactive behaviors between mothers of preterm infants, which either require or not intensive care, and mothers of healthy full-term infants; while they observed significant less sensitivity and less interactive involvement in mothers of high-risk full-term infants compared to healthy full-term, when infants were 4- and 6-month-old. Only one study reported enhanced sensitivity in mothers of high-risk infants, which was however associated to a higher level of intrusiveness [70]. Taken together, all these studies support the idea that mothers of high-risk infants are particularly focused on close monitoring and stimulating their infants rather than affectively or socially interact with them.

Dyadic interaction

Among the studies included in the current review, some approached a dyadic perspective, besides analyzing discrete maternal and infant dimensions, and focused on compromised patterns of synchrony [61], reciprocity [65] or positive exchanges within the dyad [52]. Two studies focused on the dyadic synchrony and reciprocity in the first semester of life and revealed that dyads at risk were less synchronized than control dyads, showing less reciprocal rhythmic and fluent exchanges [61, 65]. The study by Schermann-Eizirik and colleagues [52] revealed that high-risk dyads showed less positive exchanges compared to control dyads.

Comparison of interactive patterns between high-risk and non-high-risk dyads from 6 to 12 months

Studies focusing on older infants evaluated more heterogeneous and difficult to compare interactive parameters, such as play, social and communication skills. Consequently, also results were more heterogeneous than those observed during the first semester of life.

Infant interactive behavior

During toy-centered play sessions, both Muller-nix et al. 2003 [56] and Landry et al.,1986 [45], found no significant differences between the study and the control group in infant play-interactive patterns, at 6 and 12 months respectively. Conversely, significant differences in play-strategies between 12 month-old high-risk and control infants were found by Landry et al. 1993 and by Korja et al. 2008 [49, 66]. Results by Landry and colleagues 1993 [49] revealed that high-risk infants showed, in general, less exploratory capacities compared to low-risk and full-term infants and, more specifically, that high-risk infants were more dependent on mothers' structuring strategies than controls. Similarly, Korja et al.(2008) [66] described 12-month-old preterm infants as less skilled in play, less attentive and more apathetic, passive and avoiding than controls, during free play interactions.

A different approach was used by Farel and colleagues [46] who investigated interactive behaviors during feeding at 8 months of age and found that high-risk infants showed less clarity of cues and less responsiveness to their mothers than controls. Finally, Smith and colleagues [50] found that high-risk infants had significantly lower expressive language abilities than controls during daily activity.

Parent (mother) interactive behavior

As far as maternal behaviors are concerned, results of studies focusing on older infants highlighted two main altered dimensions. Specifically, mothers of high-risk infants seemed to be more stimulating and less sensitive toward their infants, as already revealed by studies focused on younger infants. For example, Landry et al. (1986)[45] found more attention-directing behaviors in high-risk mothers than mothers of controls; while Farel and colleagues [46] found that mothers of high-risk infants reached lower scores in fostering cognitive growth behaviors than control mothers.

Less affective behaviors were found in mothers of high-risk infants by Lasky et al. [41], Muller-nix et al. [56], and Sansavini et al. [71]. Lasky and colleagues (1984)[41] reported that mothers of preterm restrain less their infants during a stressful clinical examination, maybe because they were more used to this kind of procedure. Muller-nix et al. (2005) [56]found a negative gradient of maternal sensitivity, with mother of HR infants being less sensitive than mothers of LR infant than, in turn, were less sensitive than mothers of full-term infant. The study by Sansavini and colleagues (2015)[71], instead, revealed that mothers of extremely small for gestational age infants showed lower positive affect compared to full-term infants.

Finally, two studies [51, 66] found no differences in maternal interactive behaviors between high-risk and control infants. However, both studies, differently from the others analyzing multiple discrete maternal dimensions, only reported a global score of maternal behaviors, thus leaving open the question of whether significant differences would have been found, should single discrete maternal behaviors be analyzed.

Dyadic Interactive Pattern

Among the studies focusing on dyadic interactive patterns at later infants' ages, Farel and colleagues (1991) [46] showed that high-risk dyads reached significantly worse total interactive scores, during feeding at 8 months, compared to control dyads. Korja and colleagues [66] did not find any differences in dyadic mutuality, flatness and disorganization and tension between 12-month-old high-risk and control infants. Finally, the study by Sansavini and colleagues (2015) [71] reported that ELGA dyads showed less frequent

symmetrical co-regulation and more frequent unilateral co-regulation, specifically meaning that mother observes, initiates or demands doing something, while infants do not respond to her.

Coding Systems Used in Multiple Studies

In order to evaluate if similar patterns of mother-infant interaction could be inferred, we compared the quantitative results of those stud- ies using the same coding systems. Only four scales were applied in more than one study (see Table 2). The scale by Gunning et al. [69] and Murray et al. [15, 73] was used in two studies, in populations with the same characteristics and at the same age of assessment [67, 69]. Consistent results were reported in "intrusiveness" and "remoteness" dimensions, describing mothers of high-risk infants as more intrusive and less remote than mothers of controls, while inconsistent results were reported for the "sensitivity" dimension. The NCAST scale was also used in two studies [46, 53]. The authors reported similar results in the feeding subscale, with a higher score in populations considered at high risk compared to control groups or normative data. However, investigated populations differed in clinical risk and age at the time of the mother-infant interaction assessment. The CIB was used in other two studies [62, 64]. They both found significant differences in the "intrusiveness" dimension, with mothers of the risk group reported to be more intrusive than mothers of the control group. However, the population characteristics of the two studies and the age at the time of the mother-infant interaction assessment differed between the two studies. Finally, two studies by Landry et al. [45, 50] used the same coding system which was developed by the authors. Comparison of the data was however not feasible, as the results in one paper [45] were only related to mother behaviors, while the results of the other [49] were only related to infant behaviors. No further quantitative comparisons, nor meta-analysis, were feasible with the selected articles, due to the heterogeneity of the publications and because the assessment tools and the dimensions analyzed were not consistent across the reports.

5.5 Discussion

In the last thirty years, extensive research has provided evidence that early interactive exchanges are fundamental for fostering later social and cognitive development, as they steadily drive, throughout early infancy and toddlerhood, emerging infant social abilities toward intentional and more complex relational capacities [3–6, 9, 10, 73]. The occurrence of adverse perinatal events, however, negatively impacts the overall infant neurodevelopment with consequent detrimental effects also on infant social and relational dimensions [7, 8, 11, 15, 17]. The extent to which severe neonatal complications, such as the extremely preterm birth or low birth weight at birth or the occurrence of neonatal brain insults, might affect early interactive exchanges between infants and their primary caregivers is, however, mostly uncharted. Our main objective was, therefore, to review the studies which focused, even partially, on the emerging behavioral interactive patterns of parent-infant dyads in infant population at high neurological risk and compared them to those of control populations. We specifically focused on parent-infant interactions occurring over the first year of life, as it represents one of the most critical stages in infant's neurodevelopment and therefore is considered an optimal timeframe for early intervention on infants experiencing brain damage or developmental delays.

Most of the eighteen studies resulting from our systematic search revealed that both infant and maternal behaviors within early interactions are compromised, and result, in turn, in a more general impairment of the dyadic patterns. During the first six month of life, infants at high neurological risk are described as less engaged and active than control infants, which makes them less responsive social partners, unable to provide cues sufficiently clear to their caregivers [46, 59, 61]. The most likely explanation of this finding is that these altered behaviors are mainly dependent on infant's neurophysiological immaturity and medical conditions, which necessarily affect their propensity to interact. This is consistent with the findings of Feldman, 2006 [61] who evaluated neonatal biological rhythms and their relationship with mother-infant synchrony at 3 months of age, finding that immature or dysregulated endogenous rhythms, due to perinatal insults, limit

the capacity of arousal modulation and negatively affect infant emotionality. During the same timeframe, i.e. the first semester, mothers of high-risk infants are more intrusive and overstimulating but, at the same time, less sensitive and affective [52, 61] . Authors have generally interpreted these behaviors as the result of major maternal concerns relative to the health-status of their infants. In this view, mothers tend to be more focused on infant's caretaking while lacking of emotional involvement [39, 59]. It is of interest that the abnormalities observed in infant behavior tend to persist beyond the first semester of life, with infants being less engaging in the interaction and less focused during play sessions, while the intrusive maternal behaviors observed in early interactions, evolve into more controlling and attention-directing behaviors in the second semester of life [45, 49]. This has been interpreted as a process by which mothers become more conscious of their infants' compromised motor, cognitive and communicative skills and move from early concerns to the adoption of more suitable strategies to optimize their interactions with infants, such as directing their attention. Taken together these results suggest that communicative patterns between mothers and infants at risk are less fluent and more disorganized compared to those typically observed in healthy populations, and that the resulting quality of interaction is significantly impaired on the short and long-term.

Findings from the current review do not provide an exhaustive characterization of early mother-infant relationships in population at very high-risk of developing CP. In fact many of the studies excluded infants with major neurological complications, thus leaving low-birth weight and extremely prematurity as their main selection criteria. While this selection approach has limited the heterogeneity of the samples, it clearly reduced the overall level of neurodevelopmental risk of the populations defined as high-risk. In spite of this important limitation, the available data support the concept that illness, rather than the degree of prematurity, gives the greater contribution to the disruption of early infant interactive behaviors and, in turn, of maternal responses. In particular, our findings show that infants with more severe illness, either premature or full-term, have the less optimal interactive approaches toward their mothers, as opposed to those with lower levels of neurodevelopmental risk [39, 41, 42, 45, 46, 51, 52, 59]. Similarly, maternal behaviors are strictly related to infants' medical status with greater levels of risk associated with greater alterations of maternal interactive behaviors [42, 51, 52, 59]. Mothers of infants who faced major neurological complications at birth were also more distressed and anxious, as revealed by postnatal interviews or questionnaires, and both these emotional states influenced mothers' interactive behaviors toward a less efficient perception of their infants' cues [56, 59, 68, 70]. Specifically, more negative emotional states were associated to poorer or more negatively affected maternal interactive patterns. Finally, few and discordant results were identified on the extent to which maternal emotional state compared to infants' risk can alter the interactive patterns [55, 58, 64, 69]. Further investigations are therefore needed to disambiguate such aspects and, more importantly, to provide deeper insights on the maternal emotional state following the occurrence of perinatal adverse events and on the extent to which they can influence maternal interactive patterns over time.

It is of interest that the studies included in this work cover a time span of over thirty years. However, only older studies focused on very–high neurological risk populations, while most recent ones mainly focused on prematurity. Two main considerations can be made. First, it is plausible that since first attempts in investigating early interactions in such complex populations have not been fully successful, subsequent attempts have mainly deviated toward more homogeneous populations including only premature infants. On one hand, this approach has led to more consistent and reliable knowledge on early interactive exchanges in premature populations. On the other hand, however, this has also left open many questions relative to the role played by neurological illness on early mother-infant interactions. Second, the survival rate of infant at high-risk for neurological impairments as indicated by current guidelines was significantly lower in the past decades, while it has significantly increased following advances in perinatal and neonatal care. The need of promptly refer high-risk infants to diagnostic-specific early intervention, promoting early social interactions besides purely medical conditions is currently critical more than even.

It is important to underline that findings were not always consistent across studies. Inconsistent results were found in relation to maternal dimensions, in particular maternal sensitivity [56, 65, 68, 70], as well as to infant dimensions, in particular communicative patterns [59, 63, 70]. A number of factors might support these

inconsistencies. Firstly, methods and scoring modalities used to investigate the interaction were very heterogeneous, varying from short video sessions to very long live observations and from micro-analytic to global rating scoring systems. Different observational approaches and analyzed dimensions may result in heterogeneous pictures of dyadic exchanges. Secondly, studies were conducted at different infants' ages, albeit within the first year of life. Consequently, observational analyses were quite different across studies and specifically aimed at capturing the most appropriate interactive behaviors at different developmental stages. The last and the most important factor is that all articles included in this review focused on infant populations at high-risk or neurologically impaired, but inclusion selection criteria were relatively variable, namely varying from prematurity only to severe brain lesions. Therefore, not unexpectedly the extent of the interaction impairment was proportional and strictly related to the severity of infant medical risk. Few studies have reported that early coping maternal behaviors influence later interaction maternal status [54]. Our results show that mothers of older infants demonstrate adaptive interaction strategies based on the impairment level of their infants. In particular, mothers of infants who clearly showed developmental delays as revealed by outcome measurements, chose alternative strategies to properly communicate with their infants (i.e. g attention-directing gestures vs questioning [49, 51]. These findings might indicate a natural maternal attitude to adapt their behaviors based on infant needs over time [45, 49, 54], therefore early interventions fostering such attitude as early as possible would be of crucial importance.

In conclusion, results from this work extend previous research which has mainly focused on preterm populations, providing more specific information relative to early interactions involving infant population with or at high risk for neurological impairments. In fact, while our findings confirm that premature infants displayed behaviors similar to those previously observed in healthy populations, extremely preterm infants and full-term infants with severe illness showed markedly more impaired interactive patterns. Similarly, when maternal behaviors were taken into account, results showed that mothers of high-risk infants were more likely to show altered interactive patterns. However, while the studies reviewed here provided important information, the review did not yield a clear picture of early dyadic interactions in high-risk infant populations. Therefore, further investigation focusing on less heterogeneous populations (e.g., targeting infants with severe perinatal insults only versus controls) and embracing a longitudinal and comprehensive perspective, including, for instance, the systematic evaluation of maternal mental states and their impact on the interaction, are necessary to better characterize the extent to which early parent-infant interactions are impaired following severe perinatal insults. This is an essential step in order to determine the specific impact of addressing the promotion of positive parent-infant interactions as part of early intervention in infants at high neurological risk.

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Chapter 6

Feasibility of early intervention through home-based and parent-delivered Infant Massage in infants at high-risk for cerebral palsy

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6.1 Abstract

Infant massage (IM) can be considered an early intervention program that leads to the environmental enrichment framework. The effectiveness of IM to promote neurodevelopment in preterm infants has been proved, but studies on infants with early brain damage are still lacking.

The main aim of this study was to assess the feasibility, acceptability and usability of IM, carried out by parents at home, on infants at high risk for Cerebral Palsy.

An IM daily diary and an ad hoc questionnaire, called Infant Massage Questionnaire Parent-Infant Experiences (IMQPE), were developed. IMQPE consisted of a total of 30 questions, divided into 5 areas. The parents were trained to carry out the IM with a home-based course, conducted by an expert therapist. The intensive IM program was set according to a defined daily length of at least 20 minutes, with a frequency of at least 5 days per week for a total of 8 weeks. Data collection consisted in the selection of the variables around the characteristics, both of the infants and the mothers, IM dosage and frequency, different body parts of the infants involved and IMQPE scores. Variable selection was carried out by minimizing the Bayesian Information Criteria (BIC) over all possible variable subsets. 19 high-risk infants, aged 4.83 ± 1.22 months, received IM at home for 8 weeks. The massage was given by the infants' mothers with a mean daily session dose of 27.79 ± 7.88 minutes and a total of 21.04 ± 8.49 hours. 89.74% and 100% of mothers performed the IM for the minimum daily dosage and the frequency recommended, respectively. All the families filled in the IMQPE, with a Total mean score of 79.59% and of 82.22% in General Information on IM, 76.30% in Infant's intervention-related changes, 76.85% in IM Suitability, 79.07% in Infant's acceptance and 83.52% in Time required for the training. Different best predictors in mothers and in infants have been found. These data provide evidence of the feasibility of performing IM at home on infants at high risk for CP. Study registration: www.clinicaltrial.com (NCT03211533 and NCT03234959).

6.2 Introduction

Infant massage (IM) is defined as any form of systematic tactile stimulation of the infant by human hands, often combined with other types of stimulation such as rocking, kinaesthetic stimulation, talking or eye contact (1). Nowadays this technique is widespread in Neonatal Intensive Care Units (NICU) (Bennett et al., 2013), since it is considered as a valid model of environmental enrichment (2) given its positive effects on the stress of newly-born infants and parent-infant bonding.

A large amount of literature has focused on the effects of IM in infants born preterm without brain lesions. A first meta-analysis and systematic review by Vickers and colleagues (1) analyzed studies that took into account populations composed of infants born preterm and/or low birthweight without any medical complications. The authors highlighted that IM seemed to improve daily weight gain in the treated group compared with controls; a trend in the reduction in terms of length of stay in hospital was also reported even if they argued that there was some methodological bias towards the studies supporting this last finding. However, a meta-analysis by Wang and colleagues (3) confirmed the increased daily weight gain in medically stable massaged preterm populations and supported the hypothesis that massage administration leads to a reduction in the length of stay in hospital. In addition, these authors reported that the possible correlations between IM and neurobehavioural development are still weakly supported in the studies selected due to a lack of consistency, not only in the design of the studies, but also to the lack of follow-up data, to the many differences in the characteristics of the patients and the disparity of treatment protocols.

Updated meta-analyses by Badr and colleagues (4) and Lu and colleagues (5) confirmed data on increased daily weight gain in massaged preterm infants medically stable compared with controls. Badr also added data on higher neurodevelopmental scores (assessed with structured developmental scales) in infants that received IM in the NICU compared with controls treated with standard care (6).

In two other systematic review of literature (6,7) the authors corroborated the hypothesis, with qualitative data, that administering IM to hospitalized preterm infants could have a potential benefit on their growth. In particular, the major findings by Juneau and colleagues in the preterm population treated with massage were a more significant weight gain, less response to pain in terms of less increase in heart rate caused by a painful procedure, more social engagement in parent-infant interaction and a greater score at the Bayley Scale administered at 12 months (6). Álvarez and colleagues (7) reported that the studies selected in their systematic review supported the benefits of the administration of IM in hospitalized preterm infants in terms of increased vagal activity, increased gastric activity, increased serum insulin; positive effects on the maturation of brain electrical activity and visual function were also reported (8,9).

Most of the studies carried out on infant massage, as also confirmed by the meta-analysis and systematic reviews available in literature, focused on populations of clinically stable newborns, while a paucity of studies is dedicated to infants with major medical complications. A study by Livingstone and colleagues (10) was developed with the aim to demonstrate the feasibility and safety of IM on infants with complex medical conditions, defined as "fragile infants" and to collect the level of satisfaction of parents reporting positive preliminary results. Significantly, most of the protocols of IM are meant to be applied in the Neonatal Intensive Care Unit (NICU) environment (9,11–20). The vast majority of them require a nurse or a therapist to massage infants, while in a minority of protocols the mothers were trained to massage their infants (10,21–27).

A study by Goldstein-Ferber (25) proposed comparing the effects of IM delivered by mothers and by professionals in different populations of preterm infants and found that the expected weight gain was achieved both in the group massaged by the mothers and in the group massaged by a therapist; in addition, a significant decrease in depression symptoms was seen in mothers of preterm infants. This result on the mothers' emotional status was also supported by other studies arguing that anxiety and depression symptoms assessed with self-report questionnaires by the mothers were significantly lower after one or more massage sessions with their infants. This finding was true both for mothers of preterm babies (28,29) and those of infants born at term (30–32).

All these data contribute to supporting the idea that IM can be proposed as an early intervention (EI) in order to promote physical maturation and neuropsychological development. The increasing number of papers in literature on the beneficial effects of massage on the neurodevelopmental outcome of infants, on the emotional status of mothers in the post-partum period and its positive influence on the quality of parent-infant interaction, as well as the extensive experience in the preterm population, has paved the way for the further application of massage. In particular, to our knowledge, no studies have focused on infants at high risk for Cerebral Palsy (CP). Recent literature focused on the sheer importance of early diagnosis and early intervention for this pathological condition that represents the most common physical disability in childhood with a prevalence of 2.1 cases per 1000 in high-income countries (33). As regards the intervention, it is recommended that it is carried out as early as possible to take advantage of the plasticity of the brain when it is at its maximum level. It should also be intensive, personalized, multi-axial, family-centred and affordable for families and the health service (34). In a recent systematic review of interventions for preventing and treating children with CP, the results of feasibility studies of some EI programs have been included (35). We hypothesized that an innovative application of IM as a home-based intervention administered by the parents, who had previously been adequately trained by a therapist, in the very first months of life after discharge from the NICU in a population of infants at high risk of CP could represent an active standard care of EI. It was included in a larger Randomized Clinical Trial (RCT) comparing the effects of a new technological system, called CareToy-Revised system to the IM (36).

Given the novelty of this hypothesis, feasibility studies of these new proposed approaches such as EI in preterm and at term infants with brain lesions and at risk for CP were required before assessing their effectiveness. CareToy-R Training feasibility had already been assessed by Beani and colleagues (37). The present study aimed to assess the feasibility, acceptability and usability of IM as a new home EI program.

6.3 Materials and methods

This feasibility study is part of a larger CareToy-R RCT study described in detail by Sgandurra and colleagues (36). The study was approved by the Paediatric Ethics Committee of Tuscany (84/2017) and registered (NCT03234959) on Clinical Trials.gov.

In a first stage of the project, families were asked to sign an agreement to participate in an observational phase (www.clinicaltrial.gov, NCT03211533) and it was only when the inclusion and exclusion criteria for the infants' enrolment were assessed that the parents were asked to sign and give consent to participate in the interventional trial.

The randomized, evaluator-blinded, multi-centre interventional study compared two home-based EIs with two investigative arms (CareToy-R training and Infant Massage) lasting 8 weeks. Eligible infants at high risk of developing CP were randomly assigned to one of these two investigative arms.

This feasibility study focuses on IM provided for an intensive and continuous period of time to infants at high risk of CP by their parents.

Participants

The participants of the CareToy-R study were recruited by a child neurologist in the NICUs or on the occasion of neurodevelopmental follow-up visits in 3 University Hospitals in Tuscany (Italy): the Meyer Children's Hospital and the Careggi General Hospital, in Florence, and the Santa Chiara Hospital in Pisa. The intervention study was managed by clinical and rehabilitation staff of Developmental Neuroscience, IRCCS Fondazione Stella Maris, Pisa.

The subjects deemed eligible for the CareToy-R study were both preterm or full-term infants with brain lesions as reported by Neonatal Brain Ultrasonography (US) or Magnetic Resonance Imaging (MRI). Infants with polymalformative syndromes, severe sensory impairments (retinopathy of prematurity grade > II, deafness or blindness) and cerebral malformations were excluded. The selection process included a clinical and neurological examination of infants at risk at 3 months corrected age, using the General Movements assessment (GMA) and the Hammersmith Infant Neurological Examination (HINE).

The subjects were selected when atypical patterns at the GMA and/or specific neurological signs at the HINE were observed.

When the infants selected achieved pre-established motor skills (starting from the initial head control) defined on the basis of the cut-off scores of the Ages & Stages Questionnaire, they were randomly allocated to one of the two investigative arms (CareToy-R Training or the IM intervention) of the RCT.

Recruitment for this preliminary study on feasibility started once the approval of the Ethics Committee was obtained. This feasibility study involved those infants randomly assigned to the IM intervention.

Study design and procedures

The minimum sample size for the IM group was set at 19 infants. Recruitment started in September 2017 and ended in June 2020.

During the intervention, infants continued to benefit from the standard care (SC) provided by the National Health System (NHS) and parents were asked to complete a diary to define and quantify the content of the SC. A child neurologist and a therapist evaluated infants at the following times:

i) T0 (baseline), the week before starting IM or CareToy-R Training interventions

ii) T1 (primary endpoint), a week after the end of the intervention

iii) T2, 8 weeks after the end of the intervention

iv) T3 (last follow-up), at 18 months corrected age of the infant

Standardized clinical tools and questionnaires were administered at all time point assessments. The primary outcome measure of the RCT study was the Infant Motor Profile (IMP) (38,39), a video-based assessment of motor behaviour in infancy that can also be used to assess infants at high risk of CP (40,41). Secondary measures included Peabody Developmental Motor Scales-Second Edition (PDMS-2) (42,43), Bayley Scales of Infant Development Cognitive subscale (BSID-III) (44), standardized video-recordings of parent-infant interaction (45,46), Teller Acuity Cards® (47) and Actigraphic analysis (Motionlogger Microwatch) (48). Moreover, parents were also asked to fill in the BSID-III Social-Emotional Scale (49) and the Parenting Stress Index questionnaire (PSI) (50).

After the intervention period, families were asked to fill in a questionnaire on the feasibility of their intervention: "CareToy-Revised Questionnaire Parent-Infant Experiences" (37) and "Infant Massage Questionnaire Parent-Infant Experiences" (see details below), respectively.

It should be noted that some post-intervention evaluations have been delayed due to the COVID-19 pandemic breakdown.

Intervention

Infant Massage intervention

We proposed massage as a home-based early intervention to be provided by parents who had been previously trained by a therapist. The intervention lasted 8 weeks and parents were asked to massage their infants at least 20 minutes a day (in one or more daily sessions) for a minimum of 5 days per week. They were also asked to write information in a daily diary about the duration of each IM session and the sequences of movements provided each time.

The IM course was provided at home and organized in 5 sessions of 1-hour each, scheduled every 7-10 days.

During the IM session the therapist first assisted the parents in creating the optimum setting so as to derive the greatest benefit from the interaction with their infant. The therapist then performed massage sequences on a doll while the parents imitated the sequences of massage on their infants.

The order of the IM sequences taught was not mandatory; the therapist explained and showed all the sequences in different orders depending on the tolerability of the infant and his/her response to the massage. Once all the sequences had been illustrated (sequences: legs and feet, stomach, chest, arms and hands, face and back), the parents were invited to personalize the order of the massage sequences according to the infants' preferences.

A team of clinical and rehabilitative professionals (mainly child neurologists and therapists) was available throughout the duration of the study to answer any requests from the families regarding the IM intervention. The therapist, in some cases, was available for assistance and, on occasion, if necessary, sent some explanatory videos or scheduled video calls with families to resolve doubts about the massage sequences.

Outcome measures

The feasibility of IM was evaluated according to three different thematic areas which focused on the intervention, on the study design and its procedures and on the acceptability and usability of the intervention from the parents' point of view.

For each area a general main question was formulated, and a multi-dimensional answer was elaborated on the basis of defined criteria that had to be fulfilled.

The feasibility criteria for this study were taken from recommendations that can be found in literature (51–54).

Feasibility of the intervention

The main question asked regarding this point, was "Is the intervention suitable and acceptable for the participants?". The answer was formulated on the basis of the parents' daily diaries on the intervention and these measures were taken into account:

- Intervention compliance and motivation: difference between IM (days and hours) requested and total IM administered (days and hours).
- Intervention adherence: total number of days in which at least 20 minutes of IM was performed.
- Intervention and participation in appointments: number of lost SC appointments during the IM intervention due to tiredness or physical discomfort of the infant.

Definitions and measurements for the feasibility criteria of this intervention can be found in Table 1.

Feasibility of the study design and its procedures

The main question asked regarding this point, was "Is the intervention suitable and acceptable for the participants?". To answer this question, data included in the RCT study database were used and the following measurements were analyzed:

- Participation willingness: percentage of families that agreed to participate in the study.
- Participation rate: percentage of dropouts (percentage of infants who abandoned the 8-week intervention).
- Data loss in the follow-up: percentage of data recorded on time at all timepoints.
- Assessment time scale: time required for collecting all the outcome measures at each timepoint.
- Assessment procedures: number of patients who failed to complete the outcome measures during follow-up.

Definitions and measurements for the feasibility criteria for this intervention can be found in Table 1.

Acceptability and usability of the intervention from the point of view of parents

As far as this point is concerned, the main question was "To what extent is the intervention acceptable and usable according to the participants?". To answer this question, an ad hoc questionnaire on the standard definition of acceptability (55,56) and usability (57–59) criteria was compiled. The pivotal role of parents in providing IM was taken into account to create the 'Infant Massage Questionnaire Parent-Infant Experiences (IMQPE)' as well as for the questionnaire created for the CareToy-R Training (37).

All families were asked to reply to the IMQPE in order to understand and collect their opinions on IM in the post-intervention period.

There are 30 questions in the IMQPE, which are divided into 5 areas (with 6 questions for each area with a maximum total score of 150 points): 1) General information on IM, 2) Infant's intervention-related changes, 3) IM suitability, 4) Infant's acceptance and 5) Time required for the training. Questions were measured with a 5-point Likert scale (families were instructed to choose the most appropriate response, ranging from "totally agree" (score 5) to "strongly disagree" (score 1) and with some open questions in which parents could express their thoughts or add qualitative comments.

Data collection and Statistical Analysis

Dedicated Case Report Forms (CRF) were developed in order to collect both the infant's and mother's demographic characteristics as well as the Parents Stress Index questionnaire scoring results.

For IM data, parents were asked to fill in a daily diary with a detailed description of the IM sessions in terms of body regions massaged and duration of the sequences provided.

At the end of each intervention, the information reported in the parents' diaries was digitalized in a spreadsheet by filling in the following items: number of times parents performed a sequence dedicated to a particular body region (legs and feet, arms and hands, stomach, chest, face and back) during the 8 weeks of intervention, number of times these sequences were performed considering only the period after training (in this case the variable was defined "Post Training" or PT), duration of the IM expressed in minutes per day and total amount of hours spent delivering IM during the 8-weeks period. For each item, the mean and the standard deviation were calculated.

Subsequently, the IMQPE questionnaire was administered by a psychologist via phone call to the parent responsible for the massage. This procedure made it easier for families to understand all the questions since they could ask the interviewer directly for clarification and they could feel free to express their own opinions.

The IMQPE questionnaire scores were also reported in a spreadsheet, as well as the relative percentages, calculated with respect to the total of the questions (Total score) and to each area.

For each item, a linear regression model was fitted after a variable selection step. The best model was found by minimizing the BIC (Bayesian Information Criteria) over all possible predictors subsets. The analysis was carried out with R - version 4.0.1 (2020-06-06). Significance level was set to 0.05.

6.4 Results

Participants

Nineteen infants were allocated to the IM intervention group and all the families completed all the assessments planned for the study and filled in the IMQPE questionnaire.

The study population was composed of 10 males, 9 females; 6 single-born subjects, 13 with siblings and 6 of the latter had a twin. Thirteen infants were preterm (2 late preterm, 6 very preterm and 5 extremely preterm) and 6 were born at term.

All the subjects had a brain injury on early neuroimaging: 4 of them were affected by an hypoxic-ischemic encephalopathy (HIE); 6 of them suffered an intraventricular hemorrhage from grade II to grade IV (IVH) (1 subject with grade II, 3 subjects with grade III, 2 subjects with grade IV); 7 of them reported a periventricular leukomalacia (PVL) and 2 subjects had an history of perinatal stroke.

The mean age of the infants at T0 assessment was 4.83± 1.22 months (range 3.00-6.74 months). In all the families, IM was administered by the mothers, whose mean age was 33.16±7.03 years (range 19 – 45 years).

68% of the mothers were Italian and 32% were of foreign origin (2 Moroccans, 1 Albanian, 1 Macedonian, 1 Russian and 1 Chinese). Families participating in the study lived in different Regions of Italy. The mean distance from IRCCS Fondazione Stella Maris was 167.64 ± 225.82 km, ranging from 12 km (Livorno, the nearest place) to 993 km (Santa Maria di Leuca in Puglia, the farthest).

The demographic characteristics of the mothers and infants can be found in Table 2.

Feasibility of the intervention

The feasibility criteria were met as follows:

- ✓ Intervention compliance and motivation: IM was performed in all cases above the minimum requested by the study. 89.47% of mothers performed IM for more than the minimum number of hours recommended (i.e. 13.33 hours) for the study with a total range of IM between 13.55 and 40.08 hours. Only in two cases was the total amount of IM lower, 8.63 and 11.42 hours. Infants received a mean total IM of 21.04 ± 8.49 hours.

- ✓ Intervention adherence: All mothers massaged their infants at least 5 days per week, but four of them in some days were not able to massage the infant for at least 20 minutes every day.

  The daily mean length of massage administration was 27.79 ± 7.88 minutes.

- ✓ Intervention and participation in appointments: mothers were able to organize the IM in their daily routine and integrate it with SC (visits, physiotherapy, follow-up).

  In particular, 84% of infants attended motor therapy sessions with the following frequency: 4 infants were monitored with one session every two weeks, 3 infants attended the rehabilitation treatment once a week, 6 infants twice a week and 4 infants three times a week.

  Moreover, all the infants had monthly follow-up visits, paediatric visits, and some of them received neurodevelopmental assessments in third level centres. Most of them are also subject to mandatory vaccinations according to the NHS.

Feasibility of the study and its procedures

The feasibility criteria of this study were fulfilled as follows:

- ✓ Participation willingness: all the families accepted the invitation to participate in the study when asked.

- ✓ Participation rates: all participants completed the intervention.

- ✓ Data loss in the follow-up: it was possible to record all the data of all outcome measures and there were no missing data

- ✓ Assessment time scale: follow-up measurements of 74% of participants were collected within 1 week after the end of the intervention period (range 0-7 days after the end of IM). 26% follow-up measurements were collected between 8 and 17 days after the training because of the COVID-19 pandemic breakdown, the distance from the centre and the holiday period (mainly Christmas and summer holidays). The follow-up at T1 was carried out after a mean of 6.72± 5.13 days from the end of the IM period.

- ✓ Assessment procedures: all participants completed the assessment at all the timepoints.

The IMQPE Questionnaire

All 19 families accepted to fill in the questionnaire and the semi-structured interview was carried out by a psychologist of the NICU of Santa Chiara University Hospital in Pisa.

All participants reported a total score above 102 points (68.00%) at the IMQPE, with a range of 102-137 points and a mean total score of 119.39± 9.27 points (79.59%).

Regarding the five sections scores, in "General information on IM" the range of the raw scores was between 20 and 30 points (mean of 83.52%); in "Infant's intervention-related changes" the range of the raw scores was between 16 and 30 points (mean of 76.30%); in "IM suitability" the raw score was between 19 and 26 (mean of 76.85%); in "Infant's acceptance" the raw score was between 15 and 30 points (with a mean of 79.07%) and in "Time required for the training" it was between 20 and 29 points (with a mean of 82.22%).

Median and 95% confidence interval of percentages scores in the questionnaire (both total and section scores) are shown in Figures 1 and 2.

Relationship between infant and mother characteristics

Considering the mothers' characteristics and the amount of IM administration (mean daily and total hours of IM and frequency of execution of each sequence during the entire intervention and post training) the best predictive significant models were found between nationality of the mother and the frequency of the provided Arms and Hands sequences, post training Arms and Hands sequences and post training Legs and Feet sequences. The results are shown in Table 3. The mother's nationality factor predicted three variables of model, as reported in Table 4.

Considering the characteristics of the infants and the amount of IM administration (mean daily and total hours of IM and frequency of execution of each sequence during the entire intervention and post training) the best predictive models are shown in Table 5.

The characteristics of being a twin predicted three variables of model, gestational age predicted two variables, being siblings and having a stroke lesion predicted one variable each, as reported in Table 6.

Relationship between IMQPE questionnaire and IM

The best significant predictor models considering the results of the IMQPE questionnaire and the amount of IM administration (mean daily and total hours of IM and frequency of execution of each sequence during the entire intervention and post training) are shown in Table 7.

The "Infant's acceptance" score predicted three variables of the model, the "Infant's intervention-related changes" score predicted one variable and "General information on IM" score predicted one variable, as reported in Table 8.

Relationship between IMQPE questionnaire and mothers' and infants' characteristics

Considering the PSI scores of the mothers and the IMQPE questionnaire scores, the best predictor factor was found between the Parental Distress subscale and the "Infant intervention-related changes" score, as shown in Table 9. The "Infant intervention-related changes" area predicted one variable of model, as reported in Table 10. The results of the IMQPE questionnaire and the infants' characteristics are reported in Table 11.

The characteristic of being a twin predicted one variable of the model, as well as brotherhood and IVH or PVL lesion. The results were reported in Table 12.

6.5 Discussion

To our knowledge, this is the first study in literature which assesses the feasibility, the acceptability and the usability of IM as an EI program dedicated to a population of infants at high risk for CP, delivered at home by their parents, who had been previously trained by an expert therapist.

The home-based nature of the IM early intervention, ongoing for 8 weeks, significantly differs from the other programs proposed in the vast majority of the studies in literature. These studies mainly investigate the short-term clinical benefits of a usually brief cycle of massage administration in the NICU before discharge from hospital (1,3,4,6,7).

This study population is also different from the previous studies as it involves both preterm and at term born infants with a brain injury and atypical patterns at standardized neurological examinations with a consequent high risk for developing a CP. Our protocol proposal combines most of the key factors required for an EI program according to the most recent literature on CP (34,60–62).

We expect a positive impact of this intervention on neurodevelopmental outcome of infants at high risk for CP. Previous studies have suggested the potential value of IM as an intervention in the framework of the environmental enrichment (63,64). Specifically, a sensitive parent-infant bonding and a stimulating home environment have been associated with an effective shaping of cortical plasticity and with a better neurodevelopmental outcome in preterm infants. In this framework, IM seems to be characterized by some of the key features of a successful early developmental intervention program for preterm babies since it is based on parents 'empowerment and can be performed in the NICU as well as at home, after the hospital discharge, even if its efficacy has to be proven also for a population at high risk for CP that may include pathological conditions other from prematurity alone (62).

We proposed an intensive family-centred approach, where the IM would be delivered by the parents at home, even by those living far from our clinical centre, for 8 weeks. The choice that the IM intervention could be delivered directly by the parents is consistent with literature that underlines the importance of taking into account the parents' emotional status and the beneficial effects of massage on depression and anxiety symptoms, mainly in mothers after giving birth (28,29,31,65). Moreover, the home-based nature of this intervention has allowed the families to personalize the administration of the massage, albeit in the context of the instructions provided by the therapist regarding the duration and the frequency of the sessions. Furthermore, the parents had the possibility to choose the best timing and the most suitable sequences of IM according to the infants' and parents' preferences. The feasibility analysis of this type of EI is very innovative. Most of the feasibility studies available in literature are mainly focused on the feasibility of home-rehabilitation with technologies (37,66) so in this study the feasibility evaluation criteria were customized to a home intervention conducted without the use of technological tools (51–54).

As regards the feasibility of the study and its procedures, the data collected supported the high rate of acceptance of the general RCT project since all the families who were asked to join agreed to participate. All the families completed the protocol of intervention participating in all the scheduled follow-up visits. No dropouts were reported. Even if the IM training and delivering was proposed both to mothers and fathers, only mothers conducted the intervention. This may be due to the higher availability of the mothers, who are usually at home on maternity leave. The mothers' intervention compliance and the motivation were high. All the mothers, indeed, administered a daily mean of IM (in minutes) above the minimum duration requested; most of them administered the IM more than 5 days per week which was the minimum weekly frequency required. We have also found some interesting best predictors of the mothers' characteristics. The foreign mother provided Legs and Feet and Arms and Hand sequences more frequently.

Moreover, the parents of twins provided less IM in terms of mean daily sessions and total hours of IM conducted; in particular the Arm and Hands sequence was the least performed by this group. However, they were related to higher values of IM suitability, and not to encountering difficulties in running sequences in

both infants. By contrast, the mothers with only one child performed more IM in terms of total hours than parents with more than one child. We can hypothesize that parents with only one child may find it easier, in terms of time, to include the massage among the daily activities of the family. Furthermore, the lower the value of gestational age, the higher the amount of IM carried out. It could be related to the mothers' greater interest in having tactile contact with their infants, as they usually stay until the term period in the NICU. Furthermore, thanks to the description reported in the parents' daily appointments diaries, we found that the mothers were able to combine the IM administration with SC (visits, physiotherapy, follow-up). The mothers did not observe their infants suffering from fatigue after the massage and they were therefore able to participate in the rehabilitative sessions provided by the NHS even on the same day as the IM.

As regards the type of lesion the infants presented, stroke had been identified as a predictive factor for receiving a greater mean daily amount of IM. The interpretation of these data is not simple on the basis of the available literature, given the lack of studies on the population at neurological risk. We can speculate that this population had fewer medical complications related to their neurological illness, so they were possibly willing to receive IM sequences. For the IVH or PVL lesion types, the mothers needed to spend a greater amount of time, even if they did not carry out a higher amount of IM. More sessions for a potential higher neurological impairment of these infants may be necessary, even if this hypothesis needs to be confirmed in the clinical RCT study.

As regards the acceptability ad usability investigated by means of the IMQPE, very interesting results have been obtained. In particular, in the "General information on IM" area the parents reported a higher score since they widely appreciated this kind of intervention from many points of view, considering it useful in enhancing and promoting interaction and attunement with their infants. The lowest score was reported in the "Infant intervention-related changes" area, although in the Likert scale, the score obtained highlighted how a certain degree of change was perceived by the parents due to the IM intervention. However, considering the PSI subscale scores, a higher score in the mothers Parental Distress subscale was predictive of a lower perception of changes in the infant which could be related to the emotional difficulties in perceiving such changes. High levels were found for the "IM suitability", as the mothers had not encountered any difficulties in carrying out the sequences and generally did not need any additional assistance from the therapists. High scores were achieved also in the areas of "Infant's acceptance" and "Time required for the training". Questions related to the infants' acceptability of IM and the role of the parent while performing the intervention were included in these areas. This could highlight that the infants had appreciated the IM and the mothers (who had conducted the IM) felt free and confident with this approach; moreover, most of them reported that the time required to dedicate to IM was adequate. It should also be pointed out that the higher levels of acceptance on the part of the infants were related to the massage of Arms and Hands (during the entire intervention and in the post training period) even if lower levels of changes in the infants were perceived. However, the results of the standardized clinical outcome measures in the RCT can provide evidence of the effectiveness of the IM. Finally, from the general analysis of the interviews conducted with the mothers, it emerged that a very good experience was had by all. The mothers recounted how they had felt a deep sense of involvement in IM practice and a sense of satisfaction in sharing IM sequences with their infants and found the approach calming, pleasant, beautiful, engaging, and relaxing. Furthermore, they reported that the IM experience was an occasion to get to know each other better.

In the present study, there are some limitations that need to be discussed. First of all, the answers given in the questionnaire could be overestimated because the mothers when interviewed were not blind to the intervention but were active actors and fully devoted to delivering it. In addition, the interviews done through phone calls could not allow an objective evaluation of the mothers. Furthermore, some results are difficult to interpret due to the lack of clinical measurements that may allow an objective evaluation of their changes and of IM effectiveness, for example those comparing the perception of changes with the real clinical changes, or the impact of IM on Parental Stress. Moreover, the heterogeneity of the brain injuries without a stratification and the small sample size represents an important limitation and requires caution in results interpretation and in generalizing the feasibility of this approach to the large CP population; for this reason, we suggest considering out study as a pilot study. Another limitation that we can point out is the lack of

information on the parental coping with respect to the NICU communication on the neurological risk of their infants. The literature supports the importance of early diagnosis and of effective communication strategies for diagnosis disclosure to the parents (67,68). This aspect could also be considered prognostic for family acceptance of an EI proposal, but, unfortunately, we did not collect systematically information concerning this specific issue and therefore correlation with the feasibility indices could not be analyzed.

Finally, a cost-effectiveness detailed analysis of the study was not carried out. This analysis is crucial to assess the real possibility of using IM to reduce the costs of health services and to offer a relatively inexpensive home-intervention. Besides these limitations, the current study, and the previous publication of Feasibility of the other arm of the study on CareToy-R training (37) lay the groundwork for the feasibility of two active EI home based programs in infants at high risk for CP. Moreover, the innovative use of standard criteria to assess the EI feasibility could be useful to encourage, and the compare future studies. The parent's participation and commitment and the feasibility of EI programs at home are absolutely crucial for home-based intervention.

Table 1: Feasibility, usability and acceptability criteria

	Main question	Areas	Definition	Feasibility question	Feasibility criterion for success	Measurement
Feasibility of intervention	*"Is the intervention suitable and acceptable for the participants?"*	**INTERVENTION COMPLIANCE AND MOTIVATION**	Parents motivation and compliance to perform infant massage	Are participants compliant and motivated to perform training intervention?	Difference between IM (days and hours) requested and total Infant Massage administered (days and hours)	Parents' daily massage diaries
		INTERVENTION ADHERENCE	The extent to which the families followed the instructions for administering the massage provided for in the study	Do participants perform at least 20 minutes of infant massage per day?	Total number of days in which a IM of at least 20 minutes was performed	Parents' daily massage diaries
		INTERVENTION AND PARTICIPATION IN APPOINTMENTS	To evaluate whether infant massage fitted in with the families' daily play and rehabilitation activities (Standard Care)	Does the infant massage fit in with the families' daily play and rehabilitation activities?	Number of missed SC appointments during the IM intervention due to tiredness or physical discomfort of the infant	Parents' daily appointments diaries
Feasibility of study and its procedure	*"Is the intervention suitable and acceptable for the participants?"*	**PARTICIPATION WILLINGNESS**	Percentage of families that accepted to participate in the study	What is the participation rate?	At least 80% of eligible participants agreed to join the project	Caretoy database
		PARTICIPATION RATES	Percentage of dropouts	Do all eligible participants agree to perform the infant massage intervention?	80% of participants who gave consensus participated in the study	Caretoy database
		DATA LOSS IN THE FOLLOW-UP	Percentage of data recorded on time at all timepoints	Can all data be collected without any problems?	90% of the outcome measures were collected	Caretoy database
		ASSESSMENT TIME SCALE	Time required for collecting all the outcome measures at each timepoint	Can follow-up data be collected within a week after the training period?	Time from end of training period to first follow-up data collection	Recorded data of the beginning and the end of the infant massage (daily massage diaries) and data of assessments

		ASSESSMENT PROCEDURES	Number of patients who failed to complete the outcome measures during follow-up.	Is the loss to follow-up acceptable? Is missing a follow-up appointment acceptable?	Less than 20% of participants failed to complete outcome measures on all follow-up assessments	Collection of data report by examiners
Acceptability and usability of the intervention from parents' point of view	*"To what extent is the intervention acceptable and usable according to the participants?"*	**AD HOC QUESTIONNAIRES**	An ad hoc questionnaire on the standard definition of acceptability and usability was compiled	Is the intervention acceptable and usable for participants?	At least 65% of total score in IMQPE questionnaire was achieved	results IMQPE questionnaire

Table 2: Sample characteristics. Abbreviations. PSI: Parent Stress Index, PD: Parental Distress; P-CDI: Parent-Child Dysfunctional Interaction, DC: Difficult Child, TS: TOTAL SCORE

Sample characteristics	
Infants' characteristics	
Infants' sex: n (%)	male: 10 (53%) female: 9 (47%)
Mean gestational age ± SD (range) (weeks)	31.84±5.90 (24^{+0}- 40^{+10})
Mean infant age ± SD (range) at T0 (months)	4.83±1.22 (3.00 – 6.74)
Brotherhood: n (%)	13 siblings (68%) 6 only-child (32%)
Twin: n (%)	6 twins (32%)
Type of lesion: n (%)	Hypoxic-ischemic encephalopathy: 4 (21%) Intraventricular Hemorrhage: 6 (32%) Periventricular Leukomalacia: 7 (37%) Stroke: 2 (10%)
Mothers' characteristics	
Mean mothers' age ± SD (range)	33.16± 7.03 (19.00-45.00)
Mothers'emotional status nationality: n (%)	Italian: 13 (68%) Foreign: 6 (32%)
Mother employment: n (%)	Employed: 12 (63%) Unemployed: 7 (37%)
PSI subscales score: mean ± DS	PSI PD: 30 ± 10.33 PSI-CDI: 21.31 ± 7.81 PSI-DC: 25.10 ±9.35 PSI-TS: 76.42 ± 22.70

Table 3: Mothers' characteristics and the amount of IM SE: Standard Error; PT: post training

	R^2	Estimate	SE	t-value	p
PT Legs and Feet					
Mother nationality (Italian)	47.50%	-0.67	0.29	-2.29	0.04
Arms and Hands					
Mother nationality (Italian)	28.80%	-9.53	3.63	-2.62	0.02
PT Arms and Hands					
Mother nationality (Italian)	33.10%	-9.80	3.38	-2.90	0.01

Table 4: Variables of model between mothers' characteristics and the amount of IM PT: post training

	N° of model	Variables
Mother nationality	3	PT Legs and Feet
		Arms and Hands
		PT Arms and Hands

Table 5: infants' characteristics and the amount of IM SE: Standard Error; Ab:IM, infant massage

	R^2	Estimate	SE	t-value	p
Mean daily IM					
Twins	60.40%	-11.66	3.79	-3.08	<0.01
Gestational age		-1.41	0.66	-2.15	0.05
Lesion stroke		11.53	5.22	2.21	0.05
Total Hours IM					
Brotherhood	45.30%	8.99	3.88	2.32	0.03
Gestational age		-0.71	0.34	-2.08	0.05
Twins		-14.83	4.37	-3.39	< 0.01
Arms and Hands					
Twins	30.50%	-10.48	4.17	-2.51	0.02

Table 6: Variables of model between infants' characteristics and the amount of IM; Ab: IM,infant massage

	N° of model	Variables
Twins	3	Mean daily IM
		Total Hours IM
		Arms and Hans
Gestational age	2	Mean daily IM
		Total Hours IM
Lesion stroke	1	Mean daily IM
Brotherhood	1	Total Hours IM

Table 7: IMQPE questionnaire and the amount of IM Ab:SE,Standard Error; IM,infant massage; PT,post training

	R^2	Estimate	SE	t-value	p
Mean daily IM					
Infant's acceptance	20.70%%	0.84	0.41	2.05	0.06
Arms and Hands					
Infant's acceptance	49.40%	1.91	0.54	3.54	< 0.01
Infant's intervention related changes		-1.35	0.53	-2.55	0.02
PT Arms and Hands					
Infant's acceptance	29.10%	1.25	0.052	2.40	<0.03

Table 8: Variables of model between IMQPE questionnaire and the amount of IM; Ab: IM,infant massage; PT,post training

	N° variables of model	Variables
Infant acceptance	3	Mean daily IM
		Arms and Hands
		PT Arms and Hans
Infant's intervention related changes	1	Arms and Hands
General information on IM	1	Arms and Hands

Table 9: IMQPE questionnaire and mothers' characteristics; Ab: SE,Standard Error; PSI,Parent Stress Index; PD,Parental Distress

	R^2	Estimate	SE	t-value	p
Infant intervention related changes					
PSI-PD	44.60%	-0.72	0.23	-3.05	<0.01

Table 10: variables of model between IMQPE questionnaire and mothers' characteristics; Ab:PSI,Parent Stress Index; PD,Parental Distress

	N° variables of model	Variables
Infant intervention related changes	1	PSI-PD

Table 11: IMQPE questionnaire and infants' characteristics; Ab:SE,Standard Error; IM,infant massage; IVH, intraventricular haemorrhage; PVL, periventricular leukomalacia

	R^2	Estimate	SE	t-value	p
IM suitability					
Twins	48.90%	2.46	1.01	2.44	0.03
Time required for the training					
Brotherhood	56.20%	-3.09	1.00	-3.08	<0.01
Lesion IVH or PVL		-6.58	2.54	-2.59	0.02

Table 12: Variables of model between IMQPE questionnaire and infants' characteristics; Ab: IM,infant massage; IVH,intraventricular haemorrhage; PVL, periventricular leukomalacia

	N° variables of model	Variables
Twins	1	IM suitability
Brotherhood	1	Time required to the training
Lesion IVH or PVL	1	Time required to the training

Figure 1: Answers of IMQPE sections

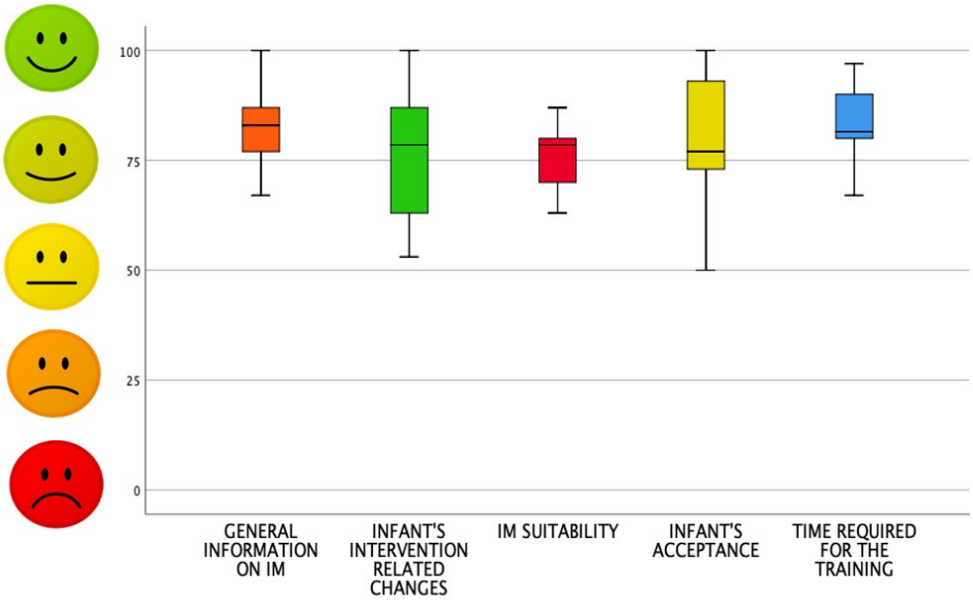
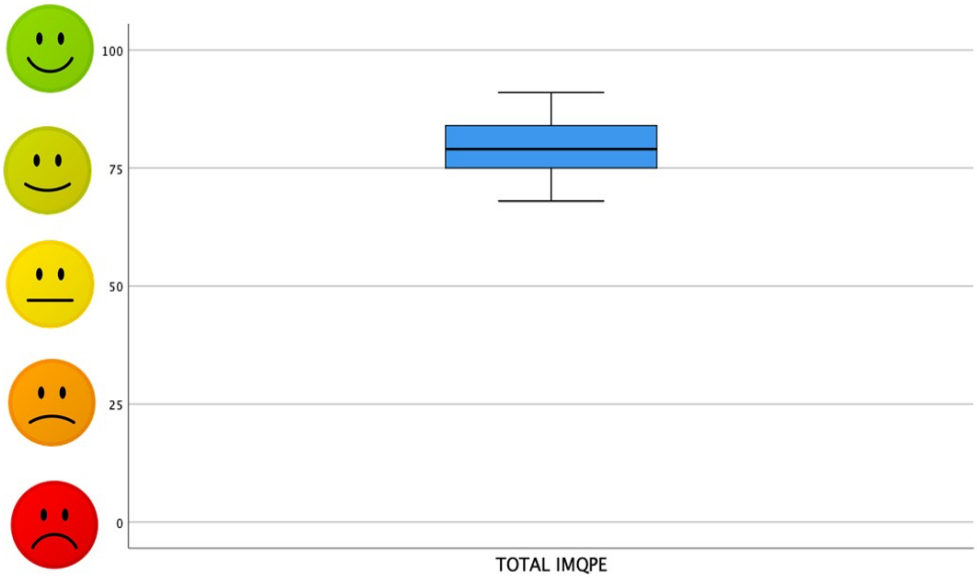


Figure 2: Total answer to the IMQPE



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Chapter 7

Conclusions

Final Considerations

Data collected in the context of my PhD project, both from the literature reviews and from the observational and feasibility studies underline the need, especially during the developmental age, for a continuous interplay between clinical experience and neurobiological principles both for early diagnosis and early intervention in CP.

The increasing demand of more effective therapeutic strategies to face a chronical condition highly impacting on patients' and families' quality of life, as well as on the national health service, encourages the definition of standardized diagnostic tools which could ensure earlier and more individualized habilitative approaches. In the last decades, the neuroscientific achievements in the field of neuroplasticity have provided a robust framework of evidence within which is mandatory to elaborate new diagnostic and intervention proposals.

In the first section of the PhD thesis, the review of the literature as well as the observational studies about the early diagnosis of UCP sustain that detecting precocious functional and/or electrophysiological markers represents a critical challenge to define the functional outcome relying both on the characteristics of the primary lesion and on the subsequent neuroplasticity mechanisms. We found that as early as 3 months of age (corrected for term) the detection of asymmetrical fidgety is more likely to be correlated with UCP outcome in a small sample of infants with perinatal unilateral brain injury than other abnormal fidgety scores and we hypothesized that this asymmetrical pattern could be the expression of a miswiring of the sensorimotor system development in a specific time-window in which peripheral afferences and central projections are expected to shape one another in an activity-dependent process.

At the same age, data from an electrophysiological study in a population of infants with an early unilateral brain injury suggest that automated analysis of interhemispheric spindle power asymmetry (SPA) performed after a video-EEG recording, provides a potential biomarker of unilateral CP at a very early age. Taken together, these data could contribute to define potential biomarkers concerning the early neuromotor profile and early EEG signal in infants at risk for developing UCP to stratify the levels of intervention and give the parents reliable information about the functional prognosis, but further studies with larger samples are required.

In the second section of this PhD thesis which was addressed to early intervention, starting from the literature evidence that early parent-infant intersubjectivity should be considered as one of the highest forms of social enrichment, we hypothesized that early interactions may represent a valuable first field of intervention for infants at risk of CP and their parents. Our literature review shed light on the lack of detailed information on the characteristics of dyadic exchanges development in population of infants with CP and their parents, suggesting the need of new protocols specifically aimed at longitudinally investigating this dimension. Moreover, in a second study we proved that the direct application of an intensive family-centered approach such as the infant massage as a form of early intervention in a population of infants at high-risk of CP is highly feasible. This result would suggest to further test the efficacy of such approach compared to standard care by means of case-control studies.

In conclusion, current knowledge encourages to set up models of early intervention founded on the mechanisms of neuroplasticity taking advantage on the attitude of the young brain to be shaped by the experiences, but what strategy and what timing are "just the right ones" for each patient is still a matter of debate and a major clinical issue to be solved. Understanding the neurophysiological mechanisms underpinning each subjects' illness natural trajectory is therefore mandatory to build up personalized intervention aimed to assure to every patient the best quality of life.