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NOVEL CLINICAL AND NEUROPHYSIOLOGICAL TOOLS FOR THE EARLY DIAGNOSIS AND INTERVENTION IN INFANTS AT HIGH RISK OF NEURODEVELOPMENTAL DISORDERS

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CHAPTER 1

General introduction and outline of the thesis

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GENERAL INTRODUCTION

Neurodevelopmental disorders (NDDs) are a group of conditions originating from the disruption of developmental processes during foetal and early postnatal life due to a mix of genetic, metabolic and perinatal complications.[1] Examples of NDDs include cerebral palsy (CP), autism spectrum disorders, language difficulties and intellectual disabilities.

Large national surveys indicate that, in industrialized countries, more than one in ten infants can be considered at risk of NDDs with a recent significant increase in prevalence and possibly even higher rates in low- to middle-income countries.[2,3]

NDDs are associated with a substantial emotional, social, and economic burden, not only on subjects affected and their families, but also on national health services since patients often require intensive care, treatment, and social support throughout their entire lives.

Accumulating evidence indicates that infants at high risk of NDDs might benefit from timely participation in early therapeutic intervention programs during the first months of life, a time at which the brain shows the highest levels of plasticity.[4–6] Yet, early and accurate identification of the infants who may benefit from these interventions remains challenging. Early detection of NDDs is traditionally based on the combination of clinical history (i.e. preterm birth, hypoxic-ischemic encephalopathy), early brain MRI and clinical assessments of neurodevelopment such as the General Movement Assessment (GMA).[7] Nevertheless, a thorough screening of all infants with expensive brain imaging techniques is not plausible in many parts of the world and the correlation of early MRI with non-motor disabilities is far from being established. Also, GMA cannot be performed after 4 to 5 months of age. For all these reasons, there is a rising need to develop generalizable, scalable, objective, and effective tools for early

screening of NDDs which should ideally include clinical instruments and cost-effective assessments allowing an accurate diagnosis, prognosis, and early access to intervention programs.

Variation of motor behaviour as an early predictor of NDDs: the Infant Motor Profile.

The analysis of motor behaviour has always played a pivotal role in the early prediction of NDDs. Despite that, during the last decades, concepts of motor behaviour have largely changed. The earlier view of motor development as a mere sequence of milestones has been replaced by the notion that neural and motor development are non-linear processes with a multifactorial origin, phases of transition and a continuous intrinsic variability.[8]

Consistent with this idea, the Neuronal Group Selection Theory (NGST) has been developed.[9,10] The starting point of the NGST is the variability of neural behaviour. According to the NGST, neural development is characterized by two phases of variability: primary and secondary variability. Early development starts with a phase of *primary variability*, during which the spontaneous activity of the nervous system tries out all available functional options producing an abundance of sensorimotor experience. In terms of motor behaviour, this means that the nervous system explores all motor possibilities of its repertoire.[10,11] After the third month post-term, a phase of *secondary variability* begins, with the nervous system using the all self-produced sensorimotor experience for the selection of the behaviour that fits the situation best. This phase of secondary variability is characterized by movement selection, a process that is based on active trial-and-error experiences.

According to this theory, an early brain injury results in a reduction of the motor repertoire ultimately producing a less variable and more stereotyped motor behaviour.[10] These features of movement behaviour might be missed

by assessments solely based on *quantitative* motor performance and are better reflected by *qualitative* analysis of movement variability and complexity. Indeed, there is growing evidence that qualitative assessments of infant motor variation could reflect the integrity of brain networks in a much more subtle way than a traditional neurological evaluation based on accomplished motor milestones.[12]

The General Movements Assessment is a qualitative assessment of early motor behaviour in which features such as variation, distribution and complexity of early spontaneous movements are assessed contributing to early and reliable detection of atypical development.[13] However, because general movements disappear between 4 to 5 months corrected age, it is not possible to use this assessment in older infants.

On this ground, the Infant Motor Profile (IMP) has been developed.[14] The IMP, just like the GMA, is a standardized neurodevelopmental assessment based on the qualitative evaluation of motor behaviour. IMP is designed to be administered from 3 to 18 months of corrected age allowing repetitive and longitudinal assessments of infant motor development. The key feature of the IMP is the evaluation of the variation of motor repertoire (*variation* or primary variability) and the ability to select the best motor strategy (*adaptation* or secondary variability) which are assessed on a video recording of a semi-standardized play session of about 15 minutes. In addition to these two domains, the IMP contains other three traditional domains that describe movement symmetry, fluency, and motor performance. After its first publication in 2008, the IMP has demonstrated a strong correlation with other widely used infant assessments and has shown to be predictive of later cognitive and motor development in populations considered to be at low risk of NDDs.[15,16] Among the different domains assessed by the IMP, the variation domain has appeared to be the

most predictive for long term development confirming that variation of motor repertoire strongly reflects the complexity of the brain maturation.

Despite the increasing interest in this tool, the application of IMP in high-risk cohorts (i.e. infants with early brain injury) still needs to be explored. Should this predictive ability be demonstrated also in high-risk infants, it would confirm that the variation of motor repertoire is indeed a solid predictor of NDDs and it could pave the way for the implementation of this tool in clinical settings such as neurodevelopmental follow-up programs.

The contribution of neurophysiological tools to the detection of NDDs

The process of early detection of NDDs requires the integration of different clinical and instrumental tools that can help the clinician to guide diagnosis, predict developmental outcomes, and monitor treatment response. Neurophysiological tools hold both practical and theoretical advantages as clinical biomarkers of NDDs since they represent a unique window on the early phases of brain development.[17]

During the last decades, a substantial effort has been invested in the search for early electroencephalographic biomarkers. Conventional electroencephalogram (EEG) is a non-invasive and readily available cot-side measure of cortical function, traditionally used for seizure detection. The use of the EEG as a maturational assessment of brain activity has also been consolidated.[18] For instance, qualitative interpretation of EEG background and interictal features may provide valuable functional measures of the infant brain maturation. Also, evidence of EEG maturational abnormalities in the first month after birth has proven as an independent predictor of developmental delay and cerebral palsy .[18–20]

However, the accuracy of the EEG interpretation is strictly related to the expertise and training of the human expert, and it can be hardly guaranteed in all contexts with equal reproducibility. These problems have increased

the interest in the use of automated and quantitative analysis of the EEG signal, in search of possible objective and quantitative biomarkers of abnormal brain function. Among the explored biomarkers, the EEG analysis of sleep figures (e.g. sleep spindles) and structure is of particular interest. Studies on adults with hemispheric stroke have shown that sleep might play a role in synaptic plasticity underlying stroke recovery and the analysis of its features can be used as a marker of risk of functional outcome.[21,22] However, whether these data can be applied also to infants with perinatal brain injury to predict atypical neurodevelopment is still to be determined.

In the field of early detection of infants at risk, there are still open challenges that involve applications of other neurophysiological techniques. Among them, functional near-infrared spectroscopy (fNIRS) holds promise as an innovative and non-invasive method to investigate brain function in infants at risk. fNIRS allows a direct quantification of oxygen consumption in different regions of the brain cortex providing an indirect measurement of brain functional activity.[23] The possibility of assessing brain functional activation and connectivity, at a relatively low cost, in an ecological environment, in both physiological and pathological conditions, has made fNIRS an effective option for the study of infants at high risk of NDDs.

So far, clinical research on fNIRS has been mostly focused on the investigation of neural development and function. On the contrary, the contribution of fNIRS to provide useful biomarkers for NDDs is barely explored.

Recent encouraging data suggest that fNIRS can be useful to explore both task-triggered activation and functional connectivity in subjects with several NDDs, ultimately detecting patterns of atypical brain activity.[24,25] Most of the research data such as functional network efficiency and weighted separability are derived from complex algorithms extracted from fNIRS raw data by experienced bioengineers. This undermines the applicability and

translational value as a potential biomarker whose value greatly depends on its ease of use.

Considering these elements, it appears necessary to develop novel and reliable fNIRS paradigms which could be useful to identify atypical brain development ultimately providing potential biomarkers of NDDs.

Early intervention for infants at high risk of CP. State of the art and the role of telerehabilitation

Among the different NDDs, Cerebral Palsy (CP) is one of the most common and represents the most frequent physical disability in childhood. Accumulating evidence indicates that Early Intervention (EI) programs in the first months of life can promote motor and cognitive development of infants at high risk of CP.[26] However, despite the widespread consensus that EI in infants at high risk of CP can lead to a beneficial effect, evidence is still very limited for the effects of these interventions on motor development. This is mainly due to the extreme heterogeneity among the different EI programs (in terms of theoretical background, timing and duration) and the lack of high quality randomized controlled trials greatly limit conclusions.[27]

Given the recent flourishment of different EI programs, general guidelines have been developed establishing that, to be effective, an EI program needs to be prompt, intensive, personalized, and should target motor, sensory and cognitive activities in an integrated systemic approach.[28] It should also involve the whole family in the intervention, setting the program at home whenever possible and empowering parental capacity and experience.[29] Finally, it needs to be feasible and affordable for the families.

Such complex and sophisticated intervention programs could be extremely expensive and thus hardly affordable for a health care system, even in high-income industrialized countries. The growing availability of robotics and biotechnological solutions for telerehabilitation holds promise to provide an

EI program that fulfils the previously mentioned standards at a relatively affordable cost and in the most enriching and ecological environment of the infants' home.[29] In addition, the possibility of standardization of the intervention and the ability to collect quantifiable indexes of effectiveness has allowed the development of innovative EI programs.

On this ground, the CareToy (CT) has been developed. CT is a biomechatronic baby gym equipped with different types of sensors designed to provide through telerehabilitation, a home-based and personalized EI which is remotely managed by dedicated clinical staff.[30,31] CT has been previously validated in a multicentric RCT study involving preterm infants at low risk for cerebral palsy: results have shown that infants who received a 4 weeks training with CareToy presented a positive short-term effect on motor and visual development as well as a reduction of maternal levels of stress.[32,33]

Based on these results, the prototype version of CT has been structurally improved and adapted to meet the needs of infants at high risk of CP such as those with early brain injury.

To determine whether CT can be considered a valuable EI tool also in cohorts of high-risk infants, the efficacy of this system must be investigated in an RCT trial which should compare CT with other EI programs. Moreover, the acceptability and usability of this technology should be assessed to determine if CT could be indeed a useful resource for the EI in such a fragile population.

Infant Massage (IM) is an another EI program aimed at promoting neurodevelopment through systematic tactile stimulation of the infant often combined with other stimulations such as eye contact or talking.[34] Several studies have demonstrated that IM can positively influence brain development improving motor, cognitive development and visual acuity.[35] Unfortunately, most studies demonstrating the effects of IM on

neurodevelopment are settled in the neonatal intensive care units, mainly focused on preterm infants without brain injury and require a nurse or a therapist to administer the IM.[36] For these reasons, more studies are required to compare its efficacy with other EI programs and to determine if this intervention can be feasible also at home and in infants with early brain injury being at high risk for CP.

Aim and outline of the thesis

The main aim of this thesis is to explore novel frontiers in the field of early detection and intervention for infants at risk of neurodevelopmental disorders. The research plot is articulated in two main areas of research: the early detection and the early intervention.

Part I: novel clinical assessments and neurophysiological techniques have been investigated addressing the following questions:

1. Is variability of infants' motor repertoire assessed with the Infant Motor Profile predictive of atypical neurodevelopment? We evaluated the predictive validity of this new assessment tool in a population of infants at high risk of NDDs (**Chapter 2**)
2. Is it possible to develop early biomarkers of atypical motor development using automated quantitative analysis of sleep EEG? We developed a paradigm for automatic sleep spindles detection and for the quantification of asymmetry in spindles amplitude as a possible early predictor of neuromotor outcome (**Chapter 3**).
3. Can we derive new quantitative biomarkers of atypical neurodevelopment using fNIRS? We developed and tested an innovative stimulation protocol and evaluated the correlation of the hemodynamic response with the presence of autistic traits in a population of adults and children. (**Chapter 4)**

Part II: we explored the feasibility and efficacy of two home-based and parent-delivered early intervention programs in infants with perinatal brain injury, trying to address the following questions:

1. Is telerehabilitation with the CareToy system a feasible and acceptable option for parents of infants at high risk of CP? We assessed the feasibility, acceptability, and usability of an 8-week training in infants with perinatal brain injury using data collected from individual training and *ad hoc* questionnaires (**Chapter 5**).

2. Can a home-based, parent-delivered intervention with Infant Massage be considered a feasible and acceptable option also for high-risk infants? We collected and analysed the data regarding dosage, compliance, and acceptability of 8 weeks program of Infant Massage using dedicated questionnaires and structured interviews with parents (**Chapter 6**).
3. What are the effects of CareToy training on motor, visual and cognitive functions in infants with high neurodevelopmental risk? We explored the effects of a revised version of CareToy in a randomized control trial involving 39 infants with perinatal brain injury (**Chapter 7** and **Chapter 8)**.

The present thesis work supports the role of new clinical and neurophysiological tools for the early detection of infants with neurodevelopmental risk and explores the effect of novel early therapeutic interventions.

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CHAPTER 2

Concurrent and predictive validity of the Infant Motor Profile in infants at risk of neurodevelopmental disorders

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Abstract

Background: Preterm infants and infants with perinatal brain injury show a higher incidence of neurodevelopmental disorders (NDD). The Infant Motor Profile (IMP) is a clinical assessment which evaluates the complexity of early motor behaviour. More data are needed to confirm its predictive ability and concurrent validity with other common and valid assessments such as the Alberta Infant Motor Scale (AIMS) and Prechtl's General Movement Assessment (GMA). The present study aims to evaluate the concurrent validity of the IMP with the AIMS, to assess its association with the GMA, to evaluate how the IMP reflects the severity of the brain injury and to compare the ability of the IMP and the AIMS to predict an abnormal outcome in 5-month-old infants at risk of NDD.

Methods: 86 infants at risk of NDD were retrospectively recruited among the participants of two clinical trials. Preterm infants with or without perinatal brain injury and term infants with brain injury were assessed at 3 months corrected age (CA) using the GMA and at 5 months CA using the IMP and the AIMS. The neurodevelopmental outcome was established at 18 months.

Results: Results confirm a solid concurrent validity between the IMP Total Score and the AIMS (Spearman's ρ 0.76; p<.001) and a significant association between IMP Total Score and the GMA. Unlike the AIMS, the IMP Total score accurately reflects the severity of neonatal brain injury (p<.001) and proves to be the strongest predictor of NDD (*p*<.001). The comparison of areas under receiver operating characteristic curves (AUC) confirms that the IMP Total score has the highest diagnostic accuracy at 5 months (AUC 0.92). For an optimal IMP Total Score cut-off value of 70, the assessment shows high sensitivity (93%) and specificity (81%) (PPV 84%; NPV 90%).

Conclusions: Early motor behaviour assessed with the IMP is strongly associated with middle-term neurodevelopmental outcome. The present study confirms the concurrent validity of the IMP with the AIMS, its

association with the GMA and its ability to reflect brain lesion load, hence contributing to the construct validity of the assessment.

Trial registration: NCT01990183 and NCT03234959 (clinicaltrials.gov)

Keywords: Infant Motor Profile; Alberta Infant Motor Scale; Neurodevelopmental Disorder, General Movement Assessment

Background

Over the last decades, the increasing survival rates of preterm and high-risk full-term infants is becoming a reason for growing concern regarding their neurodevelopmental outcome. Consequences may include different forms of neurodevelopmental disorders (NDD). The term NDD includes a wide range of neurological and psychiatric conditions such as cerebral palsy (CP), social communication disorder, attention deficit hyperactivity disorder (ADHD), and brain malformations, resulting from a precocious disruption of functional brain connectivity.[1] Early detection of NDD is becoming one of the greatest challenges in developmental neurology since early evidence seems to indicate that response to an intervention is more effective if provided during early infancy, when brain plasticity is at its highest levels.[2]

It is widely accepted that standardized follow-up programs are crucial for the early detection of NDD; nevertheless, the identification of the right diagnostic instruments to be used at the right time is still a matter of debate. Indeed, an ideal clinical instrument should be able to detect early signs of atypical development and to predict the severity of the outcome. To date, a substantial number of neuromotor assessments have been proposed. Among them, Prechtl's General Movements Assessment (GMA) proved to be highly reliable in the prediction of long term neurologic dysfunctions such as CP during the first months of life.[3],[4] Accumulating evidence suggests that the GMA has the strongest accuracy in the prediction of later cognitive dysfunction, further supporting the use of this tool in the early assessment of infants at risk of NDD[5] [6]. The GMA is based on a standardized qualitative analysis of infant's spontaneous motor repertoire in which factors such as variability, distribution and complexity of movements reflect the pattern of typical and atypical development. However, after 4 to 5 months post-term age spontaneous general movements gradually fade-out, leaving room for a

new complex repertoire of intentional goal-directed movements. At that age the GMA cannot be performed and, for this reason, there is a need for other standardized assessment tools which will provide insight, not only into the presence of specific neurological signs but also into the quality and variability of motor behaviour.

A growing amount of literature seems to indicate that instruments assessing the *quality* of motor behaviour can provide more subtle information about the brain functioning of infants rather than a traditional neurological evaluation.[7] In general, the evaluation of quality and especially variability of the early motor repertoire seems to reflect brain functional integrity and connectivity in a much more accurate way. As a result, these kinds of qualitative assessments turned out to be useful, not only for the prediction of major motor disorders (such as CP), but also for the identification of early signs of other NDD.[4,8]

In this framework, the Infant Motor Profile (IMP) assessment has been developed.[9] The IMP is a video-based assessment of motor behaviour of infants from 3 months of corrected age (CA) until the age of autonomous walking (approximately 18 months).

The IMP was created in line with the Neuronal Group Selection Theory (NGST). According to this theory, infant motor development is characterized by two phases of variability: a first phase of abundant variation of movements and exploration of all motor possibilities, and a second phase during which infants learn to select the most adaptive strategies out of a motor repertoire based on trial-and-error experiences[10]. As a consequence, an early brain lesion results in a limitation of both phases leading to a reduction in the variation of the motor repertoire and to problems with the selection of the most adaptive motor behaviour.[11]

Consistent with this framework, the IMP has been developed on the assumption that qualitative aspects of movement are much more informative than the mere achievement of motor milestones.[12] A description of the IMP is provided in the Methods section. After the first report by Heineman et al (2008), the authors reported a strong correlation between the IMP and other widely used assessment tools such as the Alberta Infant Motor Scale (AIMS) and a satisfactory inter-rater reliability.[13] Subsequently, they explored the association between the IMP values and later cognitive and motor impairment. In 2011, they longitudinally assessed a group of preterm and full-term infants using the IMP at 4, 6, 10 and 12 months showing a high ability to predict CP at 18 months.[14] Recently, the same group demonstrated a clear relationship between developmental motor trajectories measured with the IMP and later outcome at school age.[15] These findings support the idea that the variability of an early motor repertoire could represent not only an early marker of major motor disorders but also of neurodevelopmental disorders as a whole. Nevertheless, these studies mainly involved infants being at relatively low risk for NDD (e.g. children of parents with reduced fertility or term infants with no additional risk factors)[12,14–16] raising the need to explore the relation between the IMP and outcome in high risk populations. Moreover, as neonatal brain ultrasound and MRI is becoming increasingly important in the prognosis of at-risk infants, the relation between the imaging findings and the IMP still needs to be fully elucidated. Finally, optimal cut-off scores have, as yet, not been established.

The aims of the present study were firstly to confirm the concurrent validity of the IMP with the AIMS in a selected population of infants at risk of NDD, secondly, to evaluate its association with the GMA, thirdly, to investigate how the IMP reflects the severity of the brain injury and finally to compare

the predictive ability of the IMP and the AIMS in a population of selected infants with an increased risk of NDD.

Methods

Participants

For the present retrospective study, we screened for possible inclusion, 99 participants of two clinical trials which included a population at risk for NDD (ClinicalTrials identifier NCT01990183, NCT03234959). Both trials were approved by the Tuscan Region Paediatric Ethics Committee. The first RCT (NCT01990183) investigated the effect of a 4-week-long intervention program with CareToy in preterm infants. The inclusion criteria were a gestational age between 28+0 weeks and 32+6 weeks, and an age at first assessment between 3 and 9 months. The exclusion criteria defined were: the presence of brain injury, infants born small for gestational age, history of seizures, severe sensory loss, and other polymalformative syndromes. The second RCT (NCT03234959) compared the effect of an 8-week-long intervention program with a revised version of CareToy (CareToy-R) and Infant Massage in infants with perinatal brain injury.[17] Infants with the following criteria were included: the presence of abnormal neurological signs at 2-4 months CA, the presence of early brain injury, severe sensory loss, progressive neurological disorders, malformation of CNS, polymalformative syndromes.

For the purpose of the present retrospective study, only those infants who fulfilled the following criteria were selected: age at GMA 3 months, age at the IMP and the AIMS assessments 5 months, follow-up visit at 18 months. Following the exclusion of 13 infants (9 infants from the first RCT and 4 from the second RCT) as they did not meet the inclusion criteria, a total number

of 86 infants (52 from the first RCT and 34 from the second RCT) were included in the present study. A flowchart showing the process of how the enrolment of the participants in the study was conducted is provided as Supplementary Material.

Data collection and measurements

All the subjects were recruited during hospitalization in the NICUs or during the follow-up programs for high risk infants at three different referral centres: the Neonatal Intensive Care Unit of the "University Hospital Santa Chiara" in Pisa, the Neonatal Intensive Care Unit of "Meyer Children's Hospital" in Florence and the Neonatal Intensive Care Unit of "Careggi University Hospital" in Florence. Written informed consent forms were signed by parents or the legal representative of the eligible infants.

After discharge from the NICU, all the patients were assessed at 3 months, 5 months and 18 months of CA. At 3 months of CA, Prechtl's Assessment of General Movements (GMA) of pre-recorded videos was performed independently by two experienced assessors certified by the GMs Trust (GC and AG). Physiologic fidgety movements were classified as normal (normal fidgety movements) or not normal (absent fidgety, sporadic fidgety, abnormal fidgety movements).[18] Whenever disagreement arose between the two assessors, the video was discussed until agreement on a final score was reached.

At 5 months CA, all infants were assessed with the IMP and the Alberta Infant Motor Scale (AIMS). The IMP allows to assess infant motor behaviour in different conditions. The assessment consists of a video-recording of approximately 15 minutes which is intended to evaluate spontaneous motor behaviour in different positions (supine, prone, sitting, standing and

walking). Subsequently, reaching, grasping and manipulations are assessed with the presentation of interesting objects in a supine and supported sitting position. No strict order of administration is required so that the assessment can adapt to the infant's age, preferences and interests.[9] 80 items are then scored off-line, based on the video-recording on a dedicated scoresheet. The items are classified into four qualitative domains (Variation, Adaptability, Fluency and Symmetry) and one quantitative domain (Performance). The first and second domains reflect the two phases of NGST: notably, the Variation domain refers to the size of the motor repertoire while the Adaptability domain refers to the ability of performing a selection of motor strategies from the entire repertoire. The Fluency domain contains items that assess the ability of the infant to adjust and calibrate movements and to fine-tune movements, the Symmetry domain investigates the presence of stereotyped asymmetric movements and the Performance domain is focused on achievements of motor milestones.

The AIMS is a standardized scale designed to evaluate gross-motor abilities in infants[19]. The assessment, which has a high sensitivity, specificity and accuracy in detecting motor deficits in infants[20],[21], consists of 58 items which assess motor skills in prone, supine, sitting and standing positions. Each item can be scored as 'observed 'or 'not observed'; the sum of the observed items provides a global score which is plotted on a percentile motor growth curve in order to determine motor performance percentiles compared to the normative sample of infants of the same age.

All the clinical assessments were video recorded and subsequently scored off-line by a trained assessor (VM) who was blind to the treatment. As previously described by Heineman and et al., since infants under the age of 6 months show limited ability to select appropriate strategies from the motor repertoire, the Adaptability domain was not assessed.[9],[11]

The final outcome was determined at 18 months CA after a clinical neurodevelopmental assessment was performed by a child neurologist (RR) who was blind to the assigned treatment. Additional clinical assessments (Bayley-III, ADOS-2 …) were individually chosen according to the clinical picture. The presence of a NDD was defined according to the DSM5 criteria by the presence of a significative impairment in motor, cognitive or social functions including CP, global developmental delay, social communication disorders, behavioural disorders, fine motor and coordination dysfunctions.[22]

Serial cranial ultrasound scans (cUS) were performed in the NICUs. When the cUS was suggestive of brain injury, the infants were further investigated with brain magnetic resonance imaging (MRI). Term and preterm infants who showed any sign of neurological diseases (hypoxic-ischemic encephalopathy, stroke, seizures…) were scanned with MRI as part of the standard clinical care. cUS and MRI images were evaluated in order to provide an overall stratification between: a) absence of lesions; b) mild/moderate brain injury (preterm white matter injury grade I-II,[23] intraventricular haemorrhages grade I-III,[24] hypoxic-ischemic injury with predominant watershed pattern,[25] ischemic stroke without basal ganglia involvement, small unilateral haemorrhagic infarction); c) severe injury (preterm white matter injury grade III, hypoxic-ischemic injury with predominant basal ganglia-thalami pattern, extensive bilateral haemorrhagic infarction, ischemic stroke with basal ganglia involvement or asymmetry of the posterior limb of the internal capsule).

Statistical analysis

A statistical analysis was performed using IBM SPSS Statistics 25.0 for Mac (IBM Corporation, Armonk, NY). Demographic and clinical summaries (sex,

gestational age, brain injury and GMA) were computed for each subgroup. The normality of data distribution was verified by Shapiro-Wilk's Test while non-parametric analyses were used to verify the non-normal distribution of the majority of the data. When conducting the concurrent validity analysis, Spearman's rank correlation coefficient (ρ) was calculated to examine the association between the IMP scores and the AIMS scores. Correlation was defined as strong for values of ρ >0.75, moderate for values of ρ 0.50-0.75, fair for values of ρ 0.25-<0.50, weak for values of ρ <0.25 .[26] The distribution of the IMP and AIMS values in relation to the GMA was evaluated with the Mann-Witney test. The association between the IMP scores and the severity of the brain injury was assessed using the Kruskal-Wallis test followed by a pairwise multiple comparison of mean ranks. Significance values were adjusted by the Bonferroni correction for multiple tests. Correlations between the IMP scores, the AIMS scores and the clinical outcome were tested for the prediction analysis with the Mann-Witney test; individual U coefficients were reported separately for each domain. A binary logistic regression model was used to estimate the ability of the IMP total score and the AIMS score to predict the outcome by applying the forced entry method. The Hosmer-Lemeshow test was used to determine the goodness of fit. The predictive power of the model was calculated from the Nagelkerke's R2 and the overall accuracy of the classification.

Finally, receiver operating characteristic (ROC) curves were computed to assess the individual predictive ability of both the IMP and the AIMS and to provide possible optimal cut-off points at 5 months CA for the prediction of NDD. Values of areas under the ROC curve (AUC) of 0.50 suggested no diagnostic accuracy of the test, values of 0.50-0.70 were considered to indicate poor discrimination, values of 0.70 - 0.80 were considered acceptable, 0.80 - 0.90 was regarded as excellent; values over 0.90 were

considered outstanding.[27] Differences and correlations with p<.05 were considered statistically significant.

Results

The mean gestational age of the study sample was 32 weeks (range 24+5 – 40+5; SD 3.9). The mean age at the IMP assessment was 4.9 months (range 4.0-6.0; SD 0.63). 34 infants presented perinatal brain injury (namely haemorrhagic infarctions, stroke or preterm white matter injury). The clinical characteristics of the study sample are presented in Table 1. At 3 months 33 infants (38.4%) showed sporadic or absent fidgety movements at the GMA; no abnormal fidgety movements were reported. A high interscorer agreement was reached among the assessors on the first evaluation of GMs (Cohen's kappa=0.80), while agreement was reached for the totality of the assessments following discussion. All the infants included in the study completed the follow-up at 18 months CA. At the end of the study 27 patients (31.4%) presented a NDD, and 59 patients (68.6%) were considered to be typical. Among the 27 infants with NDD, the prevalent diagnosis was CP in 14, followed by minor motor disorders in 6, cognitive impairments in 5, social communication disorders in 2.

Table 1. Demographic and clinical characteristics of the study sample.

	Typical, n (%) **n=59**	**NDD, n (%)** **n= 27**
Sex		
Male (n=46)	34 (73.9%)	12 (26.1%)
Female (n=40)	25 (62.5%)	15 (37.5%)

Gestational Age		
25-31 weeks (n=44)	33 (75.0%)	11 (25.0%)
32-36 weeks (n=27)	21 (77.8%)	6 (22.2%)
37-41 weeks (n=15)	5 (10.0%)	10 (90%)
Brain injury		
No brain injury (n=52)	49 (94.2%)	3 (5.8%)
Mild/moderate injury (n=14)	8 (57.1%)	6 (42.9%)
Severe brain injury (n=20)	2 (10.0%)	18 (90.0%)
GMA		
Normal Fidgety (n=53)	49 (92.5%)	4 (7.5%)
Not Normal (Absent / Sporadic) Fidgety (n=33)	10 (30.3%)	23 (69.7%)

Concurrent validity of the IMP with the AIMS

A clear and statistically significant relation between the IMP values and the AIMS total values was evident for the IMP total score and for almost all of the domain scores. The IMP Total and Performance domains showed a strong correlation with the AIMS (Spearman's ρ 0.76 and 0.89 respectively; p<.001) while there was a moderate correlation between the IMP Variation and Symmetry and the AIMS (Spearman's ρ .58 and .56 respectively; p<.001).

IMP assessment and GMA

The distribution of the IMP Total scores proved to be significantly different among infants with normal and not normal fidgety movements at the GMA (Mann-Whitney U = 83; p<.001) suggesting a strong association between the two assessments (Fig 1). The distribution of the AIMS values showed a weaker association (Mann-Whitney U = 235; p<.001).

Correlation between the IMP and the AIMS with neuroimaging data

Both the IMP Total (p<.001) and the AIMS (p<.05) scores correlated with the presence and severity of the brain injury at the neonatal brain MRI (Table 2). All of the IMP domain scores showed an individual correlation with the severity of the lesion load (Variation, Symmetry, Performance p=<.001; Fluency p<.05). The post-hoc analysis for each group showed a significant correlation for the IMP Total score only (p<.001).

Table 2. Distribution of scores among MRI severity classes

	No brain lesions median (interquartile range)	**Mild/Moderate injury** median (interquartile range)	**Severe injury** median (interquartile range)	***p* value**	
IMP Total Score	74.2 (4.7)	71.4 (4.2)	64.4 (7.0)	<.001	*
IMP Variation	71.0 (7.9)	70.1 (7.0)	62.5 (6.3)	<.001	
IMP Fluency	75.0 (-)	75.0 (-)	75.0 (-)	.007	
IMP Symmetry	100 (4.8)	91.9 (5.9)	77.0 (22.6)	<.001	

IMP Performance	55.1 (9.3)	49.9 (10.6)	45.9 (10.8)	<.001	
AIMS Total Score	14.0 (5)	13.0 (5)	10.0 (4)	<.05	

* Post-hoc pairwise comparison analysis significant at *p* <.05. IQR: interquartile range

Predictive validity of the IMP and the AIMS

Distribution of IMP and AIMS scores compared to the outcome at 18 months are reported in Table 3.

Table 3. Distribution of scores at 5 months in infants with typical development and neurodevelopmental disorders (NDD). *p* values and *U* coefficients of the Mann-Whitney *U* test.

	Typical **Median (interquartile range)**	**NDD** **Median (interquartile range)**	**p value**	**U coefficient**
IMP Total Score	74.0 (4.6)	65.6 (9.1)	<0.001	83
IMP Variation	71.4 (8.3)	62.5 (6.2)	<0.001	254
IMP Fluency	75.0 (-)	75 (-)	0.01	646

IMP Symmetry	100 (4.8)	83.3 (23.8)	<0.001	131
IMP Performance	54.8 (9.3)	45.9 (8.1)	<0.001	309
AIMS Total Score	14.0 (5.0)	11.0 (2.0)	<0.001	234

The IMP Total score at 5 months showed a highly significant relation with the neurodevelopmental outcome: infants with a typical development showed a substantially higher score (median 74.0; interquartile range 4.6) than infants with NDD (median 65.6; interquartile range 9.1)*; p*<.001 (see Fig.1). Furthermore, it was confirmed that Variation, Symmetry and Performance were individually correlated with the neurodevelopmental outcome (*p*<.001), as was the AIMS (*p*<.001*)*. In logistic regression, the IMP Total score was confirmed to be the best single predictor of NDD (*p*<.001): the model based on the IMP Total confirmed a good fit (Hosmer-Lemeshow's P=.67) and a good predictive power (Nagelkerke's R2 = 0.737) with an overall accuracy of classification of 88%. Fig. 3 shows a graphical representation of the probability to develop a NDD according to the model based on the IMP Total score values. A similar model based on the AIMS score showed a lower predictive power (Nagelkerke's R2 = 0.445).

The ROC curves generated from the IMP Total score and the AIMS Total score are reported in Fig. 4 summarizing the overall diagnostic accuracy of the two assessments. The Area Under the Curve (AUC) for the IMP Total score was outstanding (0.95; p<.001; CI95% 0.90-0.99) while the AUC for the AIMS score was lower (0.85; p<.001; CI95% 0.77-0.94) indicating that the accuracy of the IMP is higher in the early detection of NDD. The definition of an optimal cut-off point of 70 allowed us to obtain an overall sensitivity

of 93% and a specificity of 81% in the prediction of NDD (PPV 84%; NPV 90%).

Individual ROC curves were developed for each IMP domain: AUC values for the IMP Variation, Symmetry and Performance showed excellent accuracy whereas values for the IMP Fluency indicated poor prediction (see Table 4).

Table 4. Area under the ROC curves for IMP Total score and domains score.

	Area under the ROC curve (95% CI)	***p* value**
IMP Total Score	0.95 (0.90-0.99)	<.001
IMP Variation	0.84 (0.75-0.93)	**<.**001
IMP Fluency	0.59 (0.45-0.72)	.194
IMP Symmetry	0.92 (0.85-0.98)	<.001
IMP Performance	0.81 (0.69-0.92)	<.001
AIMS Total Score	0.85 (0.77-0.94)	<.001

Discussion

Our data confirm the excellent concurrent validity of the IMP and the AIMS. Values are in line with data previously published by Heineman et al.[9] confirming a maximal correlation for the IMP Performance and lower correlation for the IMP Fluency. The highest correlation between the IMP

Performance and the AIMS is explainable as both are focused on achievements of motor milestones. The association between the IMP and the GMA was also good, as evidence of the solid construct validity of the IMP. In fact, both assessments reflect the same qualitative elements such as variation, symmetry and fluency of movements.

In the definition of the prognosis of children at risk of NDD, the correlation between clinical and neuroradiological tools is pivotal. In our study, the IMP Total score reflected the presence and the severity of brain injury more accurately than the AIMS. This data supports the idea that any neurological condition which affects the complexity of brain connectivity results in a reduction of the complexity of the motor repertoire.[28] This subtle and complex process is better captured by qualitative assessments such as the IMP rather than performance-based tools such as the AIMS.

We compared the ability of the IMP and the AIMS to predict the neurodevelopmental outcome in a population of infants who had been specifically selected for being at risk of NDD. While both tests were confirmed to be significantly correlated to NDD, the IMP Total score proved to be the most accurate single predictor of an atypical outcome. At 5 months CA, after the identification of a cut-off value of 70, the IMP Total score predicted NDD with high sensitivity (93%) and specificity (81%). Among the different sub-scores, all the domains, except for Fluency, were significantly related to the outcome. IMP Fluency reflects the ability of infants to perform smooth and seamless movements in different conditions (e.g. sitting, supine, walking...). The domain is composed of only 7 items (6 for non-walking infants) which mostly investigate the presence of tremors and non-fluent movements during the assessment. Unlike previously published data,[15],[29] the majority of infants in our study sample scored the same low value on this domain (75 points). Moreover, the IMP fluency at 5 months

was poorly correlated to the presence of brain injury and showed no significant relation with the neurodevelopmental outcome. A possible reason for this might be related to the different characteristics of our study population which was largely selected among infants who experienced prolonged hospitalizations in NICU. Indeed, if on the one hand lack of fluent movements could be one of the first indicators of non-optimal neurologic development, it is also true that benign shudders, jitteriness and tremors are commonly seen during the first months of life, especially in infants with a prolonged stay in NICU.[30,31] Furthermore, the small number of items contributing to the IMP Fluency score resulted in a reduced variability of the values.

This is the first study to evaluate the predictive validity of the IMP in a population of at-risk infants, written by a group of researchers who are in no way connected to the developers of the scale. One of its strong points is the presence of three different video-based assessments which were scored by blind assessors, another being the fact that all the infants were recruited at the very early stages of life among infants at risk of NDD. Nevertheless, the study presents several limitations. First of all, the short duration of follow-up and the absence of a structured battery of assessments at 18 months may not have allowed us to identify milder conditions which require more time and standardized assessments for the diagnosis. Infants were retrospectively recruited among the participants of two clinical trials during which different kinds of early intervention programs were provided; a mild effect of these programs on the final outcome cannot be ruled out.[17,32] Furthermore, we provided a coarse classification of brain imaging since no widely used classification system of perinatal brain injury takes into account both term and preterm patterns of injury. Hence, our classification might not accurately reflect the actual severity of some patterns of brain injury. For all these reasons, and for the nature of the retrospective design, the

present findings cannot be generalized to all infants at risk of neurodevelopmental disorders. Further research should aim at assessing the predictivity of the IMP in prospective longitudinal studies including more homogeneous populations of infants at risk of NDD.

Conclusion

The accurate prediction of NDD during the first months of life is paramount in order to provide early access to rehabilitative intervention to children at risk. Literature supports the combined use of the GMA and brain MRI for an early prediction of NDD. However, starting from 4 to 5 months CA general movements gradually disappear, thus leading to the need to find other reliable qualitative assessments of early motor behaviour. The IMP represents a valid alternative; the high flexibility, the absence of need for expensive kit materials and its excellent psychometric performances make the IMP an extremely interesting tool in the evaluation of infants at risk of NDD. In this sense, a greater integration of the IMP among the clinical tools used during the follow-up programs will be useful. Also, the use of the IMP as an outcome measure in clinical trials will provide data on the possible use of this instrument to reflect the effect size of a treatment.

The present study shows that the IMP has a high concurrent correlation with two of the most used clinical assessment tools in early infancy (the AIMS and the GMA). Furthermore, we demonstrated that the IMP accurately reflects the degree of early brain injury and that there is a clear relationship between early motor development assessed with the IMP and neurodevelopmental outcome. These findings support the idea that at the early stages of development, qualitative aspects of motor behaviour may reflect the complexity of cerebral connectivity, thus representing a strong indicator of a future diagnosis of NDD.

Additional observational trials with prospective cohorts of at-risk infants should further elucidate the relationship between early motor behaviour and neurodevelopment, particularly by investigating how different patterns of brain injury affect the different IMP domains.

List of abbreviations: Attention Deficit Hyperactivity Disorder (ADHD); AUC: Area Under the Curve; GMA: General Movement Assessment; IMP: Infant Motor Profile; MRI: Magnetic Resonance Imaging; CP: Cerebral Palsy; NICU: Neonatal Intensive Care Unit; NDD: Neurodevelopmental Disorder

Declarations

Ethics approval and consent to participate

This trial was approved by the Tuscan Region Paediatric Ethics Committee. Written informed consents were signed by parents or the legal representative of eligible infants.

Consent for publication

Informed consents for data publication were signed by parents or the legal representative of eligible infants.

Availability of data and materials

The dataset analysed during the current study is available from the corresponding author to researchers on reasonable request.

Competing interests

The authors report no competing interests.

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Authors 'contributions

RR gave a substantial contribution to the clinical assessment of infants, the analysis of data and the drafting of the paper. VM, VB and EB administered and scored all the clinical assessments. MLC, AC, MG selected and recruited newborns at high risk and were also in charge of the traditional clinical follow-up. GC and GS are responsible for the study design and the approval of the submitted version of the paper. All the authors had complete access to the study data of this work.

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CHAPTER 3

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

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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 (UCP).

METHOD This was a multicentre retrospective study of 40 infants with unilateral brain injury. Sleep-spindles were detected and quantified with an automated algorithm from electroencephalograph records performed at 2-5 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 UCP (N=13; 33%), with the most robust interhemispheric difference seen in the central spindles. The best individual-level prediction of UCP 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 UCP.

INTERPRETATION Our data suggest that automated analysis of interhemispheric SPA provides a potential biomarker of UCP at a very early age. Such functional biomarker holds promise for guiding and targeting early therapeutic interventions in infants with a perinatally identified brain injury.

WHAT THIS PAPER ADDS

- Unilateral perinatal brain injury may affect the development of EEG sleep spindles
- Interhemispheric asymmetry in sleep spindles can be quantified with automated EEG analysis
- Spindle Power Asymmetry (SPA) can be a potential biomarker of unilateral cerebral palsy

A common neuroanatomical substrate for the development of 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[37]. Neonatal stroke is a well-known cause of adverse neurodevelopmental outcomes, including unilateral cerebral palsy (UCP). The global prevalence of neonatal stroke is around 1/3000 live births, and over half of the affected children develop long-term disabilities, including UCP.[38,39] Growing evidence supports the importance of early identification of infants at high risk for CP, as early detection allows for timely delivered therapeutic interventions aiming to exploit the neuroplastic potential of the developing brain.[7,40]

Sleep spindles are hallmarks of non-rapid eye movements (NREM) sleep, representing a stereotypical, widely coordinated neuronal activity in the thalamo-cortical and cortico-cortical networks. [41–43] 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.[44–49] 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 do not exist until the first months of life, which is long after the occurrence of the structural brain lesion.[42,43] Hence, the newborn brain lesion does not affect the sleep spindle activity *per se*, as in adults, but rather the brain networks that will later support sleep spindle occurrence.

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 pathologic network reorganization after unilateral brain injury that leads to UCP.

METHOD

Study design and participants

A multicentre cohort (N=40) 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 (1 January 2011 to 31 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 (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).

Table 1. Demographics

	All groups n=40	UCP n=13	Typical n=27
Females (%)	19 (48%)	6 (46%)	13 (48%)
Lesion side - left (%)	22 (55%)	8 (61%)	14 (52%)
Median age at EEG (months)	3.0 [1.5-5.0]	3.0 [1.5-5.0]	3,0 [2.0-5.0]
Median age at follow up (months)	24.0 [18.0-117.0]	35.9 [19.0-117.0]	22.5 [18.0-84.0]
Median Gestational Age (weeks)	40.1 [36.6-42.3]	40.4 [37.6-41.9]	40.0 [36.6-423]

Inclusion criteria were: a) a diagnosis of unilateral brain injury such as focal ischemic or haemorrhagic injury confirmed on neonatal MRI; b) at least one sleep-EEG recording between weeks 8 and 20 after term age, with at least 5 minutes of clear N2 sleep, and c) clinical follow-up of at least 18 months.
Exclusion criteria were: a) premature birth (before the 36th week of GA); b) bilateral brain injury; c) a known other brain malformation; d) isolated hypoxic-ischemic brain injury; e) epidural or subdural hematoma; f) intraventricular haemorrhage 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 Paediatrics Ethics Committee (SPACE2020; nr.187/2020) and for the Finnish cohort, the study protocol was approved by the Institutional Research Review Board at HUS Medical Imaging Center, Helsinki University Hospital. In both countries, a waiver of consent was granted due to 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 (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 centers, 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 images. For the Finnish cohort, as 11 infants were the same as in Nevalainen 2019,[50] 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 2008 and Goveart 2009 and recently adapted by Nevalainen 2019.[50]–[52] 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 non 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; 10. Intracerebral lobar Haemorrhage.

EEG acquisition

EEG recordings of the identified patients were retrospectively collected from the hospital archives. All the subjects 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 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, USA). Recordings were

sampled at 256 Hz, 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 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 70 Hz and a high-pass filter with a cut-off of 0.3 Hz was applied.

Sleep epochs were visually identified (by VM, PN, RR, FM), and the N2 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 (MATLAB 2018B, The MathWorks Inc., 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.[41,45,53] 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,[53] we then categorized the spindles depending on their respective frequency ranges as slow (11-13 Hz), fast (13-15 Hz) or full-band (11-15Hz). The mean spindle power was then computed for each of these frequencies and for every bipolar channel: fronto-central (FC), fronto-temporal (FT), centro-temporal (CT), centro-occipital (CO), and temporo-occipital (TO).

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 SPA indexes were obtained depending on the region and the frequency assessed: FC-full-SPA, FT-full-SPA, CT-full-SPA, CO-full-SPA, TO-full-SPA, FC-slow-SPA. FT-slow-SPA, CT-slow-SPA, CO-slow-SPA, TO-slow-SPA, FC-fast-SPA, FT-fast-SPA, CT-fast-SPA, CO-fast-SPA, TO-fast-SPA.
Details of the spindle selection procedure and steps of power analysis and interhemispheric asymmetry are reported in the Supplementary Material section (Figure S1).

Motor outcome

As part of the standard care for infants with brain injury, all patients were included in the neurodevelopmental follow-up program performed by the paediatric neurologists of the four tertiary centres. Motor outcome (classified as: typical motor outcome and UCP) was collected after retrospective review of the medical record (authors VM, PN, CA, RR) of the last follow-up visit (range 1.5- 9.8 years; minimum 18 months).

Statistical analysis

Statistical analysis was performed with IBM SPSS Statistics software v. 23.0 (IBM SPSS, Chicago, IL, USA, RRID: SCR_002865). Demographic characteristics were compared for infants with and without UCP by X2 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 subjects). We chose $p < 0.05$ as the level of statistical significance.
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 (PPV and NPV) and area under receiver operating characteristic curve (AUC) for the SPA index that was most accurate for predicting CP based on the logistic regression model.

Data availability

All relevant data are within the paper and its supplemental information. 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).

RESULTS

Thirteen out of 40 neonates developed UCP (32.5%). There were no significant differences in the demographic variables between infants with vs without UCP (see Table 1).

Neonatal MRIs were classified according to the stroke patterns. Most of the lesions (n=27, 67.5%) occurred in the territories of the MCA: 10 (25.0%) infants had lesions involving the proximal tract of the MCA, 5 (12.5%) the distal branches, 3 (7.5%) the anterior trunk, 7 (17.5%) the posterior trunk, 2 (5.0%) the Lateral Lenticulostriate Arteries territory. In 2 infants (5.0%) lesions occurred in other arterial territories than MCA. Of the remaining cases, 6 (15.0%) presented periventricular venous infarction, 3 (7.5%) intracerebral haemorrhage and 2 (5.0%) periventricular haemorrhagic infarction. In our sample, all infants who developed UCP had unilateral brain

injury involving the MCA territory, while those having a positive motor outcome were distributed across all MRI classification groups.

Spindle power asymmetry (SPA) and outcome

We first examined whether early SPA significantly differed between the clinical outcome groups. The infants with eventual UCP had a greater SPA in their wideband spindles (11-15 Hz) in the centro-temporal and centro-occipital regions (CT-full-SPA p<0,001, CO-full-SPA p<0,001). Frequency wise analysis showed the group difference to be frequency specific: the slow spindles (11-13 Hz) differed between clinical outcome groups in the same centro-temporal and centro-occipital derivations (CT-slow-SPA p<0,001, CO-slow-SPA p<0,001), while the fast spindles (13-15 Hz) showed a group difference also in the fronto-central derivations (FC-fast-SPA p=0,014, CT-fast-SPA p<0,001, CO-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.

Table 2. Regional Spindles Power Asymmetry (SPA) indices

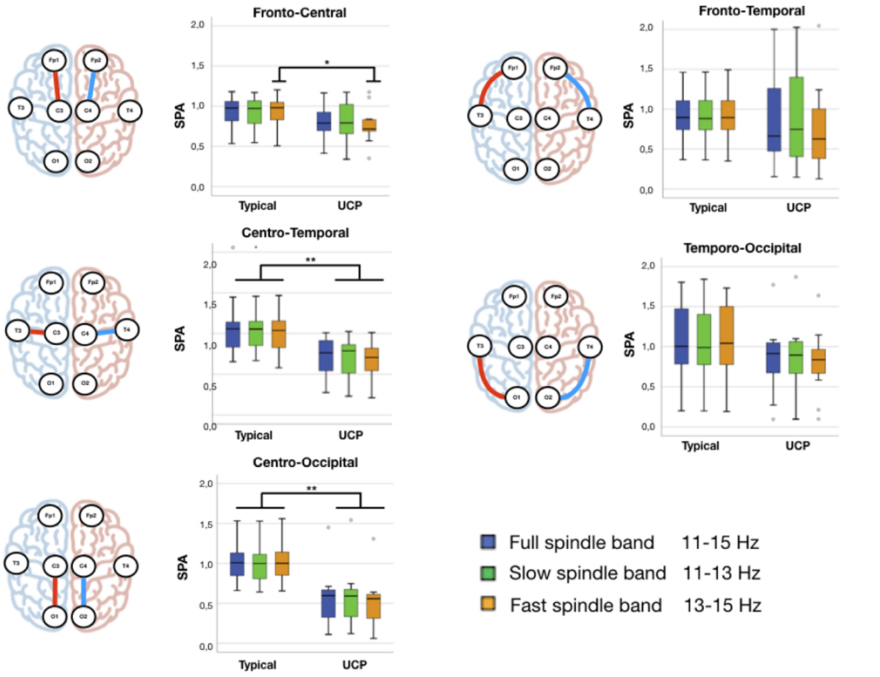
Asymmetry		MEDIAN	IQR	Mann-Whitney U	*p*-value
FC-full-SPA	Typical	0.98	0.28	111.00	0.064
	UCP	0.79	0.26		
FT-full-SPA	Typical	0.89	0.38	151.00	0.493
	UCP	0.66	0.90		
CT-full-SPA	Typical	1.06	0.31	61.00	<0.001
	UCP	0.76	0.45		
CO-full-SPA	Typical	1.01	0.31		

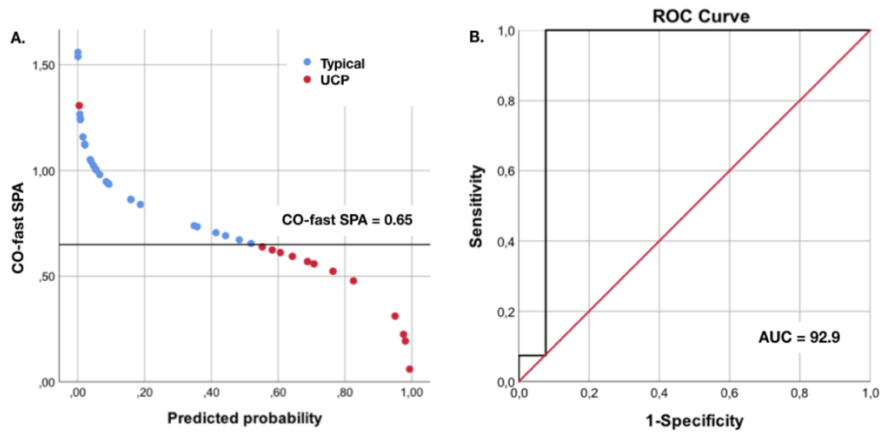
	UCP	0.60	0.39	33.00	<0.001
TO-full-SPA	Typical	1.00	0.73	135.00	0.252
	UCP	0.92	0.43		
FC-slow-SPA	Typical	1.97	0.32	125.00	0.151
	UCP	0.79	0.40		
FT-slow-SPA	Typical	0.88	0.37	157.00	0.608
	UCP	0.75	1.06		
CT-slow-SPA	Typical	1.05	0.32	62.00	<0.001
	UCP	0.79	0.44		
CO-slow-SPA	Typical	1.00	0.33	39.00	<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		

Abbreviations: FC = fronto-central; FT = fronto-temporal; CT = centro-termporal; CO = centro-occipital; TO = temporo-occipital

We then tested the ability of SPA to predict UCP at individual level by using a binary logistic regression model. An asymmetry between fast spindles over centro-occipital regions (CO-fast SPA) was found to provide the best prediction, explaining 95% of the outcome variability (CO-fast-SPA

Wald=08.61, p=0.003). The AUC of the ROC curve for CO-fast-SPA was 0.93 (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 UCP.





DISCUSSION

Our results show that unilateral brain injury in newborns links to interhemispheric asymmetry of sleep spindles in those infants that later will develop UCP. Our work extends prior literature 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 infant 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.[45,48]

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.[42,53] The brain networks sustaining sleep spindles show rapid activity-dependent growth and organization during the early development of brain circuitry.[54,55] 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 (13-15 Hz) peak over the central regions while the slow spindles (11-13 Hz) become more dominant over the frontal regions, after the second year of life.[53] 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 hence considered to reflect the integrity of thalamo-cortical circuitry.[42,55,56] Studies in adult patients showed that the occurrence and synchrony of spindles are greatly reduced after an injury affecting the sensory-motor cortex,[44,46,49] the thalamic nuclei or the thalamocortical projections.[41,48,57] Moreover, after hemispheric stroke, amplitude asymmetry of spindles significantly correlates with long-term motor outcome of adult patients,[44,49] indicating that post-stroke sleep

changes could be used as early biomarkers of abnormal motor development.[45],[48] 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 thalamo-cortical networks, leading to the long-term neurodevelopmental consequence, UCP. Recent studies on structural imaging are in line with a key role of thalamocortical pathways in the emergence of both unilateral and bilateral types of cerebral palsy after perinatal stroke.[58]–[60] Moreover, abnormalities in the thalamo-cortical projections are even a stronger predictor of motor outcome as compared to findings of the corticospinal tract.[58]

Early prediction of UCP is crucial for identifying infants to early intervention programs, as well as to adequately support and counsel caregivers. Current clinical recommendations support the combination of structural brain MRI with the assessment of GMA to estimate the risk of UCP before the 5th month of age.[7] The assessment of sleep spindles could provide an independent predictive measure to this age range. Previously, clinical qualitative interpretations of neurophysiological recordings were used to predict outcome following perinatal brain injury.[50],[61]–[63] 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 to complement the combination of neuroimaging and neurological assessments.

Our study has some limitations that may affect the practical conclusions. First, this work is based on a retrospective data collection from specialized medical centres, which might lead to selection bias towards higher risk populations; however, the incidence of UCP in our cohort was comparable

to the existing literature.[64] 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 Assessments)[7] or identical neuroradiological protocols were not available for all subjects 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 datasets. 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 is to 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 is 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 spindle power asymmetry (SPA) holds promise as an early, easy to obtain and totally automated biomarker for predicting UCP. Should these results be confirmed, SPA could represent a valuable tool for the follow-up of infants at neurodevelopmental risk.

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Health (NET - 2013-02355263; Ministero della Salute, Ricerca Corrente Stella Maris; VM, AG and CA). AG, VM and CA were supported by BornToGetThere grant – Horizon2020). VM was also supported by a research scholarship from the International Federation of Clinical Neurophysiology (IFCN). CA was also partially funded by PRA_2018_71, University of Pisa. The funders did not have any involvement in study design, data collection, data analysis, manu- script preparation, and/or publication decisions. The authors have stated that they had no interests that might be perceived as posing a conflict or bias.

SUPPLEMENTARY MATERIAL

Spindle extraction pipeline. Spindles were detected for each bipolar channel by power and duration criteria applied on a band-pass filtered signal (11-15 Hz; roll-off 3 dB at 10 and 16 Hz; Chebyshev Type II filter; for details see D'Atri et al 2018)[53]. We applied Hilbert transform to each channel to compute the instantaneous amplitude of the filtered signal (see Sarasso et al. 2014)[65]; spindle events were detected by setting two amplitude thresholds defined on the mean amplitude of every channel: 1. Spindles peak threshold: set at mean + 3 standard deviations (SD); 2. spindles start/end threshold: set at the mean amplitude of each channel. Adjacent events occurring within the 0.5 s were merged; only events with a duration of between 0.5 s and 10 s were further considered spindles.

For every detected event we obtained the spindle oscillation frequency, calculated as the maximal power within the spindle frequency range of the spectrogram using short-time Fourier transform (±2 s around a spindle detection, 1-second windows, 99% overlap and a resolution of 0.2 Hz); according to Andrillon et al., 2011.[41] Based on the spindle oscillation frequency, we then divided the selected spindles between "fast", if the

selected frequency was between 13 and 15 Hz and "slow", if the selected frequency was between 11 and 13 Hz, according to D'Atri et al, 2018.[53] See Figure S1 for details.

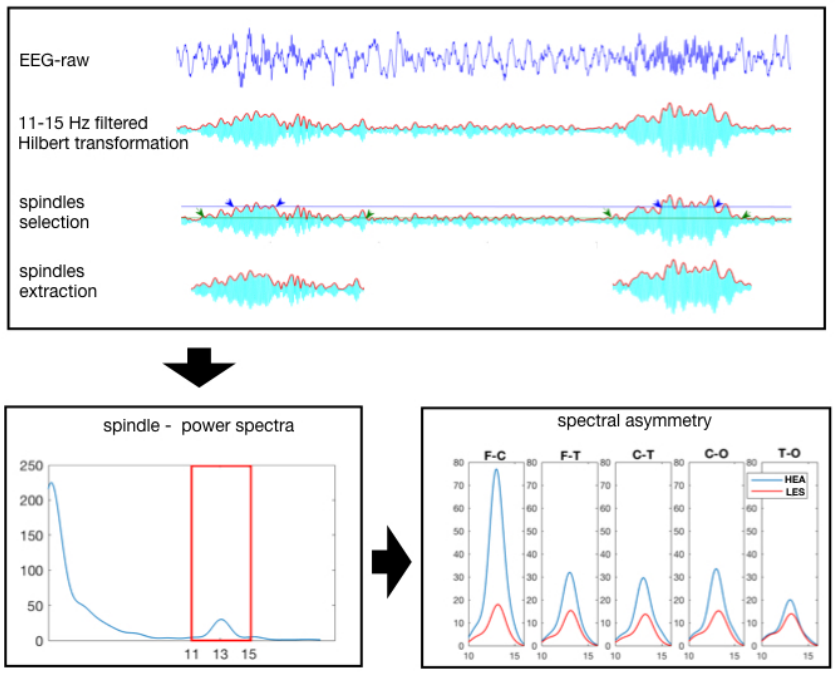

Figure S1. Sleep Spindle detection and Spindle Power Asymmetry (SPA) procedure. A) I. representative raw sleep EEG trace (filters 0.3– 70 Hz) showing NREM 2 phase activity detected from the bipolar montage. II. and III. Steps of the spindle detection procedure (amplitude and duration criteria): the sleep-EEG trace in I. was band-pass filtered between 11 and 15 Hz (−3 dB at 10 and 16 Hz) using a 2nd order Chebyshev filter. The instantaneous amplitude of this signal was computed via the Hilbert transform (red trace). A detection threshold (blue line) was set at mean + 3*mean and a start/end threshold (green line) was set at mean of the amplitude of the channel activity during sleep. The blue and green arrows indicate the start/end and the centre of the detected event, respectively

(same in panel A). IV. Only events crossing the detection threshold and whose duration was between 0.5 s and 5 s were considered spindles and further analysed. B) Power spectra was then accounted for every selected event, considering only spindles band frequencies. C) Mean spindles power was then accounted for every channel and Spindles Power Asymmetry (SPA) was then regionally calculated as the ratio between the spindle spectral power of the lesioned and non-lesioned hemisphere.

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CHAPTER 4

The amplitude of fNIRS hemodynamic response in the visual cortex unmasks autistic traits in typically developing children

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ABSTRACT

Autistic traits represent a continuum dimension across the population, with autism spectrum disorder (ASD) being the extreme end of the distribution. Accumulating evidence shows that neuroanatomical and neurofunctional profiles described in relatives of ASD individuals reflect an intermediate neurobiological pattern between the clinical population and healthy controls. This suggests that quantitative measures detecting autistic traits in the general population represent potential candidates for the development of biomarkers identifying early pathophysiological processes associated with ASD. Functional near-infrared spectroscopy (fNIRS) has been extensively employed to investigate neural development and function. In contrast, the potential of fNIRS to define reliable biomarkers of brain activity has been barely explored. Features of non-invasiveness, portability, ease of administration and low-operating costs make fNIRS a suitable instrument to assess brain function for differential diagnosis, follow-up, analysis of treatment outcomes and personalized medicine in several neurological conditions. Here, we introduce a novel standardized procedure with high entertaining value to measure hemodynamic responses (HDR) in the occipital cortex of adult subjects and children. We found that the variability of evoked HDR correlates with the autistic traits of children, assessed by the Autism-Spectrum Quotient. Interestingly, HDR amplitude was especially linked to social and communication features, representing the core symptoms of ASD. These findings establish a quick and easy strategy for measuring visually-evoked cortical activity with fNIRS that optimize the compliance of young subjects, setting the background for testing the diagnostic value of fNIRS visual measurements in the ASD clinical population.

I

INTRODUCTION

Autism spectrum disorder (ASD) is a heterogeneous developmental condition that involves persistent challenges in social interactions, restricted/repetitive behaviours, and the lack of behavioural and cognitive flexibility[1]. Since the pioneering work by Lorna Wing[2], increasing epidemiological evidence indicates that autistic traits are continuously distributed across the general population[3,4]. This is due to the complex genetic and epigenetic inheritance pattern of ASD, where multiple candidate loci contribute to the pathogenesis of the disease[5,6]. Milder autistic traits have been termed the extended or broader autism phenotype (BAP), with BAP features being particularly prevalent in first- and second-degree relatives of individuals with ASD[6–9]. Much research is currently focused on siblings of children diagnosed with ASD, namely high-risk infants (HR), because around 20% of them receive a diagnosis of ASD within the third year of life[10] and a further 20-30% develop neurodevelopmental conditions[11]. Moreover, a high rate of BAP symptoms has been documented in this population[12]. The prospective study of HR children allows to detect behavioural risk signs or biomarkers of neurodevelopmental disorders at a very early age, before the full-blown clinical expression, while the investigation of BAP features in the general population might be helpful to dissect clinical subtypes and set-up personalized intervention strategies according to developmental stages.

Over the last decade, the biological dimension of ASD has been largely explored, thanks to the growing availability of advanced tools to explore brain correlates of neurological disorders, including high-density EEG, magnetoencephalography, positron emission tomography, magnetic resonance imaging (MRI), and functional near-infrared spectroscopy (fNIRS)[8,13,14]. A number of studies reported defective neuroanatomical and

neurofunctional features in individuals with ASD, suggesting that a dysfunction of specific brain areas might underlie the core symptoms of ASD[15-19]. Interestingly, relatives of autistic probands, even when not behaviourally impaired, display neurostructural and neurofunctional patterns significantly different from healthy controls and correlated to BAP features[8]. Since ASD and broader autistic manifestations share common genetic variants and neurobiological susceptibility factor[20], the general population emerges as a suitable testing bed for the development of quantitative measures detecting hallmarks of autism.

FNIRS is an optical imaging technique that allows quantifying oxygen consumption in different regions of the cerebral cortex, providing an indirect measure of neuronal activity[21,22]. This blood-oxygen-level-dependent (BOLD) signal is similar to that detected with functional MRI (fMRI)[23]. However, fNIRS is more tolerant to motion artifacts than fMRI, and the development of robust methods for motion detection and correction allowed to avoid sedation in children[24,25]. Furthermore, fNIRS has the advantage of being totally non-invasive, low-cost, portable, noiseless, and endowed with high experimental flexibility and no setting constraints. This methodological strength provides the fNIRS with a high ecological value for investigating neural circuit maturation either in typically developing children or clinically relevant populations[24,26].

Although the use of fNIRS in autism research is still an emerging area, a number of studies aiming to decipher the neuronal mechanisms and circuits underlying ASD evaluated different aspects of brain function and organization, including resting-state and task-evoked responses[27,28]. Coherence analyses of resting-state hemodynamic activity showed weaker local and interhemispheric functional connectivity in different cortical regions[29-34]. Moreover, individuals on the autism spectrum present patterns of atypical activity, including reduced hemodynamic responses within

specific brain regions, bilateral differences in neuronal activation and the lack of cortical specialization, in tasks ranging from sensory perception[35] to executive functions[36], social perception[37-40], joint attention[41-43], imitation[44,45], facial and emotional processing[46-49], speech perception and language[50-53]. The majority of studies targeting evoked brain activity was focused on the prefrontal and the temporal cortex[28], where symptom severity seems to be inversely correlated with the degree of cortical activation[45,46].

Growing evidence suggests that fNIRS might be a candidate biomarker for several neuropsychiatric disorders, including ASD[32,54-58]. In particular, functional network efficiency[32], weighted separability of NIRS signals[59], multi-layer neural networks and sample entropy of spontaneous hemodynamic fluctuations[57,60] have been proposed as auxiliary diagnosis indexes for ASD. However, all these approaches require complex algorithms to extract high-level features from the fNIRS raw data, while fitness, applicability and translational value of biomarkers greatly depend on their ease of use. In this framework, the analysis of visual phenotype has become an important model to evaluate cortical processing in different neurodevelopmental conditions[61-66]. Indeed, electrophysiological measurement of visually evoked responses has been introduced as a quantitative method to assess brain function in Rett syndrome[61,67], and hemodynamic responses (HDR) emerged as a potential longitudinal biomarker for CDKL5 Deficiency Disorder and Creatine Transporter Deficiency in murine models[65,66].

Since clinical studies suggested a dysregulation of sensory processing and functional connectivity in the visual cortex of ASD subjects[68-70], and atypical visual processing has been implicated in the neurobiology of autism[71,72], we hypothesized that fNIRS visual measures might represent a tool to quantitively assess inter-individual differences in autistic traits. The typical

stimulation protocol used in most fNIRS and electrophysiological studies quantitively assessing visual responses in both typical and clinical populations consisted of reversing black and white checkerboard patterns spaced by baseline intervals with a grey isoluminant screen[67,71-75]. The same paradigm has been also employed to measure visually evoked potentials in autistic individuals[71,72]. The entertaining value of the checkerboard/grey alternation, however, is quite low, possibly reducing the experimental compliance of children with intellectual and neurodevelopmental disorders. Thus, this work had three specific aims: 1) to set up a novel standardized procedure to assess HDR in the occipital cortex; 2) to test the feasibility and reliability of fNIRS measurements in typical adults and children using this innovative stimulation protocol; 3) to investigate the correlation between HDR and broad autism dimensions,evaluated with the Autism-Spectrum Quotient (AQ[76,77]), in the general population

RESULTS

An animated cartoon-based stimulus is able to evoke visual responses in the adult cortex

We measured the cortical HDR function[78] elicited by a reversing checkerboard pattern in the adult population. In agreement with the previous literature[69,79], we obtained a significant activation of the occipital cortex in response to different conditions of visual stimulation (Fig. 1 and Fig. S1). Grand averages across adult participants (see Table 1 for demographics) of Total Hb (THb), OxyHb (OHb) and DeoxyHb (DHb) concentration changes are plotted in Fig. 2. Using a classic mean-luminance grey screen as baseline, statistical analysis revealed a significant main effect of the checkerboard stimulus (S) with respect to the blank presentation for all HDR metrics (Radial Stimulus condition, RS, Fig. 2A; see Table S1 for statistical details).

To increase the entertaining quality of our experimental paradigm, we devised an innovative visual stimulation protocol blending the checkerboard pattern with an isoluminant commercial cartoon, thus serving as a reference baseline (Fig. 1 and Fig. S1). We found a significant increase of THb and OHb, with a parallel reduction of DHb concentration, in response to S appearance, reflecting the functional activation of visual areas in this condition as well. The cortical response was independent from the cartoon employed as baseline: a comparable HDR, indeed, was clearly elicited both when the baseline movie was fixed a priori by the experimenter (Cartoon Fixed condition, CF; Fig. 2B, Table S1) and when the cartoon was freely selected by the tested subject (Cartoon Chosen condition, CC; Fig. 2C, Table S1). Interestingly, a significant pattern of correlations emerged among HDR metrics recorded with different stimulating conditions (Fig. S2; see Table S1 for statistical details), indicating that the quality of visual input does not quantitatively impact HDR. The amplitude of cortical activation was only slightly smaller in response to CF and CC, with the range of OHb and DHb fluctuations being significantly lower with respect to that evoked by RS (Fig. 2D; Table S1).

Within the CF condition, we also established that the baseline cartoon does not affect the degree of visual activation: indeed, a comparable modification of THb, OHb and DHb concentrations was recorded using “The Lion King”, “The Powerpuff Girls”, “Peppa Pig” or “Kung Fu Panda” (Fig. S3A; Table S1). Furthermore, no differences of visually evoked responses were detected modulating the contrast level of the baseline cartoon: THb, OHb and DHb fluctuations, indeed, were comparable when a fixed baseline cartoon was presented at 20%, 40%, or 80% of contrast (Fig. 2E; Fig. S3B-D; Table S1). Finally, the response latency was homogenous in RS, CF, and CC conditions (Fig. S4A; Table S1).

Altogether, these results demonstrate the validity of this innovative stimulation procedure to evoke a significant and reliable response in the occipital cortex preserving inter-subject variability.

The cartoon paradigm was reliable in eliciting cortical responses in children

We measured cortical responses in typically developing children (see Table 2 for demographics) viewing the radial checkerboard blended with the animated cartoon. We compared three different conditions: each subject, indeed, was asked to select two cartoons of their preference for the baseline and the first choice was employed for the low-contrast (cartoon 1 low contrast, 20%, L1) and the high-contrast (cartoon 1 high contrast, 80%, H1) stimulation, while the second cartoon was presented only at low-contrast (cartoon 2 low contrast, L2; Fig. S1).

Our data showed a significant activation of the visual cortex, with a prominent change of THb, OHb and DHb concentration in response to the S with respect to the blank for all conditions tested (Fig. 3A-C; see Table S1 for statistical details). The amplitude of elicited cortical responses was comparable following L1, H1 and L2 (Fig. 3D; Table S1), proving that the HDR is independent from the cartoon narrative selected for the baseline and the contrast level of baseline presentation in children as well. Small differences were observed for response latency among L1, H1 and L2 conditions (Fig. S4B; Table S1). A highly significant pattern of correlations among different HDR indexes recorded in the diverse conditions was detected (Fig. S5; see Table S1 for statistical details).

In agreement with previous literature[79], a maturational trend of cortical responsivity was recognized, with children showing significantly higher HDR amplitude with respect to adult subjects (Fig. 3E; Table S1). On the contrary,

age-dependent effects were not identified for response latency (Fig. S4C; Table S1).

These findings establish a novel method for measuring visually-evoked cortical activity with fNIRS that ensures an elevated compliance of young subjects and high-quality reliability of measurements, suggesting a valuable tool for studying visual cortical processing in typically developing children, but also in clinically relevant populations.

Negative correlation of HDR amplitude with AQ score

Despite no effects detectable in adults (Fig. 4A-C; Table S1), the amplitude of visual responses was highly correlated to AQ scores in children (Fig. 4D-E; see Table S1 for statistical details). Consistent with a recent work[57], the correlation was specific for THb, with higher AQ score being associated with a lower amplitude of THb visually-evoked signals (Fig. 4D-E).

Interestingly, HDR amplitude was especially linked to social and communication autistic traits (Fig. 5, S6; Table S1): indeed, assessing separately the five AQ subscales[77] we found a significant correlation of THb and OHb with the Social Skills subscale (AQ_S, Fig. 5A-B), while only THb modulation was related to the Communication subscale (AQ_C, Fig. 5C-D) and no significant interaction was observed testing the other three AQ subscales (AQ_A, AQ_D and AQ_I; Fig. S7). Given the reliability across different visual tasks (L1 and H1), the strongest interaction was between THb and AQ_S (Fig. 5A-B).

DISCUSSION

We measured hemodynamic responses in the occipital cortex while subjects viewed a reversing checkerboard pattern on a grey isoluminant baseline or the same stimulus blended with a commercial animated cartoon. In all participants, the patterned stimulus elicited a significant change of cortical

Hb (upwards for THb and OHb, down for DHb) independently from the reference baseline, while no response was detected following blank presentation. Since HDR consists of an initial increase in oxygen rich blood followed by a smaller depletion of deoxy-haemoglobin, with a tight interrelation among total, oxygenated and reduced Hb levels, reporting all data allows for a more accurate physiological interpretation of the results[78]. Interestingly, the level of occipital cortex activation did not depend on either the movie selected as reference baseline or the baseline contrast, with only a slight reduction of OHb and DHb change in response to the stimulus blended to animated cartoons with respect to the classic RS condition. These data demonstrate the reliability of this novel procedure with high entertaining and ecological value in eliciting cortical activity. Thus, our approach might be helpful for studying cortical function in children with an atypical trajectory of brain development, commonly showing a reduced compliance in experimental environments.

The magnitude of HDR modulation, and in particular of THb, was inversely correlated with AQ scores in children. Indeed, we found that the higher were the AQ scores of subjects the lower was the amplitude of THb response to visual stimulation, suggesting that visually-evoked fNIRS responses are able to capture the dimension of autistic traits in the general young population. Our findings are consistent with previous studies showing that cortical activation measured with fNIRS and the performance in visual psycophysics negatively reflected ASD symptom severity[45,46,71,72,80-83]. Moreover, these data reinforce the concept that THb changes could provide richer discriminative information for classifying between typically developing children and ASD subjects[57]. In our experiment, the THb index describes about 45% of the variance in AQ scores. This correlation is remarkably high, considering that it is detected across two separate visual stimulating procedures, i.e., the vision of low- and the high-contrast blended RS-

animated cartoons. Since levels of THb reflect the relative changes of both OHb and DHb concentrations over the visual cortex[21], we surmise that the better correlation of THb with AQ scores might be due to a combinatory effect of OHb and DHb variables. The information about OHb increase and DHb reduction for each subject, indeed, converges in the modification of THb levels[78].

A tentative explanation of reduced HDR in children with stronger autistic traits might be found in the difference of perceptual styles in the general population[84,85]: the preference for focusing on local details vs. the global stimulus configuration, indeed, is a defining feature of ASD[86] and locally centered perception could be less effective in activating neural circuits of visual cortex. Interestingly, a recent study established a vascular link to ASD, showing early dysfunction of endothelial cells and impaired endothelium-dependent vasodilation in a mouse model of 16p11.2 deletion[87]. Since the HDR measured with fNIRS strongly relies on neurovascular coupling, this suggests that lower neurophysiological activity may stem in part from endothelial-dependent vascular factors.

In contrast, we failed to detect any correlation between HDR and AQ scores in adult subjects. This is likely due to the different output range of variables measured in children and adult participants. Accordingly, the 4-point Likert scale used for questionnaires in child population changes the range of AQ scores from 0-50 to 0-150, potentially revealing a different variability of inter-individual traits. Moreover, the maturation of neural and vascular networks over brain development affects the pattern of hemodynamic responses[88,89]. Indeed, our data showed that the amplitude of fNIRS visually-evoked responses is significantly higher in children compared to adults, with a parallel broader distribution of recorded signals.

Although the autistic questionnaire for children is well-validated, showing good test-retest reliability and high internal consistency, not all items have

the same validity and factor analysis identified five subscales, named 'Social Skills', 'Communication', 'Attention to Detail', 'Imagination' and 'Attention Switching'[77]. We observed variability in the strength of correlation between HDR and AQ subscales, with the highest correlation for the 'Social Skills' and 'Communication' subscales. Interestingly, 'Social Skills' and 'Communication' are the subscales with higher construct validity performance in differentiating individuals with or without ASD[90,91], while 'Attention to Detail' was the poorest classifying domain[92]. It is also worth stressing that among the items composing the 'Attention to Detail' subscale, only four actually relate to sensory perception (e.g. 'My child usually notices details that others do not'), the others being more focused on cognitive functions (e.g. 'My child is fascinated by numbers'). Accordingly, the first pilot report of the AQ questionnaire showed lower internal consistency (measured by Cronbach's alpha coefficient) of 'Attention to Detail' items compared to other subscales[76].

Although primarily affecting social functioning, there is a growing body of evidence showing that ASD is also associated with abnormalities in multiple sensory domains, fluctuating between hyper- and hypo-sensitivity to sensory stimuli[80,93]. In addition to a higher incidence of refractive errors and strabismus[94], anomalies in visual processing, visual attention and visual-motor integration have been described in ASD population[71,72,74,80,95]. Interestingly, sensory symptoms are correlated with the severity of the disorder, at least in children[96]. Moreover, commonly observed alterations in social skills might have a visual component[80] and perception deficits could impact with cascading effects on the maturation of cognitive and social domains[95].

It has been recently suggested that an early assessment of pupil size modulation and visual behaviour might improve the diagnostic process of ASD[74,84,95,97]. Currently, ASD diagnosis and follow-up almost entirely rely on

phenotypic information collected via clinical measures and parental input that are highly prone to subjective bias[98]. Moreover, the late appearance of some behavioural autistic traits often delays the diagnosis until mid-childhood[99,100]. Thus, the identification of solid brain biomarkers early predicting ASD pathophysiology is a critical step to anticipate tailored interventions, leading to better outcomes for patients and possibly even the prevention of certain behaviours typically associated with ASD. Objective biomarkers have also the potential to be helpful in the management of patients, allowing the classification of disease severity and monitoring response to treatments[14]. A recent systematic review highlighted that both functional and structural neuroimaging features might predict ASD diagnosis in the early pre-symptomatic period[101,102], but further studies are needed to validate the promising performance of such biomarkers[14]. Lately, resting-state fNIRS measurements have been suggested as candidate biomarkers for ASD[32,57,59,60]. As stressed above, fNIRS offers significant advantages with respect to other neuroimaging tools, including non-invasiveness, ease of use, no need of sedation, tolerance to movements and portability, making it a child-friendly approach. However, the extraction of metrics with diagnostic value from resting-state recordings involves complex algorithms.

In contrast, our analysis of visually evoked responses is quick, easy and requires only thatchildren pay attention to a short movie of their choice. Since our stimulating strategy has been studied to optimize the compliance of young subjects, we believe that our results might set the background for testing fNIRS visual measurements in ASD individuals. Moreover, screening for autistic traits in the general population may be helpful in epidemiological research because it may provide a large sample size to investigate the correlation between autism phenotype severity and other pathophysiological processes[91].

Although our study provides a first step towards the use of fNIRS for empowering early detection of autistic traits, some limitations need to be discussed. First, the quantification of AQ scores for children according to parent questionnaires might introduce a response bias in the dataset[103]. Despite the high test-retest and reliability coefficients of AQ-Child[77], future studies might examine autistic traits in the child general population with an integrated approach consisting of direct behavioural observation and administration of multidimensional questionnaires. Moreover, testing a larger and gender-balanced sample will allow not only to confirm the validity of our results, but also to potentially highlight gender differences in fNIRS measurements and to stratify the child population in different age groups. Finally, combined fNIRS-EEG recordings are needed to dissect whether the sensitivity of fNIRS to autistic traits is determined by neural or vascular processes.

MATHERIALS AND METHOD

Subjects

We recruited a total of 40 adult subjects (20 women, age: 31.05 ± 3.94 (SD) years) and 19 children (5 girls, age: 7.20 ± 3.01 (SD) years). All participants reported normal or corrected-to-normal vision and had no diagnosed neuropsychiatric condition. Experimental procedures on children were authorized by the Regional Pediatrics Ethics Board (Comitato Etico Pediatrico Regionale-Azienda Ospedaliero-Universitaria Meyer-Firenze, Italy; authorization number 201/2019) and were performed according to the declaration of Helsinki. Written informed consent was obtained from all adult participants and from the parents of each child, authorizing the use of anonymized data for research purposes. Assent was also obtained from the children involved in the study before participation.

AQ score

Adult participants filled in the Autistic-Spectrum Quotient (AQ) questionnaire, a 50-items self-administered report validated for the Italian version[76,104]. The items consist of descriptive statements assessing personal preferences and typical behaviour. For each item, participants respond on a 4-point Likert scale: ''strongly agree'', ''slightly agree'', ''slightly disagree'', and ''strongly disagree''. The items are grouped in five subscales: Social Skills, Communication, Attention to Details, Imagination and Attention Switching. All the questionnaires were scored by a neuropsychiatrist blinded to subject data: 1 point was assigned when the participant's response was characteristic of ASD (slightly or strongly), 0 points were attributed otherwise. Total scores range between 0 and 50 (0-10 for each subscale), with 32 being the clinical threshold for autism risk[76]. No subjects scoring above 32 points were recorded. The mean (min-max) of the scores was 15.1 (3-32) with SD of 6.5 (Table S2). The children's version of Autism-Spectrum Quotient (Italian version of AQ-child) was completed by parents[77]. This version of the AQ questionnaire includes 50 items as well, grouped in the same subscales described above, and parents were required to report for each statement the degree of consistency with their child's behaviour. Scores range from 0 to 150 (0-30 for each subscale), since the response scale is treated as a 4-point Likert scale with 0 representing definitely agree; 1 slightly agree; 2 slightly disagree; and 3 definitely disagree. Items were reverse scored as needed. The threshold score is 76[77]. All subjects scored below 76 points. The mean (min-max) of the scores was 32.1 (17-49) with SD of 10.7 (Table S3).

Apparatus and montages

To measure changes in total Hb (THb) concentration and relative oxygenation levels (OHb and DHb) in the occipital cortex during the task, we

used a continuous-wave NIRS system (NIRSport 8x8, NIRx Medical Technologies LLC, Berlin, Germany). Our NIRSport system consists of 8 red light-sources operating at 760 nm and 850 nm, and 7 detectors which can be placed into a textile EEG cap (EASYCAP, Herrsching, Germany), forming an array of 22 multi-distant channels[105]. Textile EEG caps of different sizes were used. The probe arrangement was fixed in each of the caps using grommets, optode stabilizers, colored labels and holders in order to assure comparable probe mapping over all subjects. For data recording, the Aurora Software 1.4.1.1 (NIRx Medical Technologies LLC) was employed. The sampling rate was 10.2 Hz. Visual areas were identified according to the craniocerebral topography within the international 10-20 system and the placement of the optodes was done using fOLD v2.2[106] and NIRSite 2.0 (NIRx Medical Technologies LLC) softwares. Sources and detectors were symmetrically distributed to define 22 channels around the region of interest, each adjacent pair of sources and detectors defining one channel (min-max source-detector separation: 20-44 mm for adults, 22-30 mm for children; Fig. S2).

Experimental Design and Visual Stimulation

Prior to the experiment, adult participants (or parents for children) filled in the AQ questionnaire. Then, subjects were asked to sit on a comfortable chair and the fNIRS cap was positioned. Optodes were placed into the cap and the calibration of light coupling between sensors and detectors was performed. All experimental sessions lasted 30 minutes. Visual stimuli were generated using Python 3 and Psychopy3[107] and displayed with gamma correction on a monitor (Sharp LC-32LE352EWH, 60Hz refresh rate, 45 cd/m^2 mean luminance, resolution of 800×600 pixels) placed 70 cm from the subject. Cortical hemodynamics in response to full-field, reversing, square wave, radial checkerboard, with abrupt phase inversion (spatial frequency:

0.33 cycles per degree, temporal frequency: 4 Hz; Fig. 1A) was evaluated in the time domain by measuring the peak-to-baseline amplitude and latency. To have an internal control with blank stimulation, we used an event-related design consisting of: i) 20 cycles of 5 seconds stimulus 'on' (reversing checkerboard, 90% of contrast) followed by 10 seconds stimulus 'off' and ii) 20 cycles of 5 seconds mock stimulus 'on' (reversing checkerboard, 0% of contrast) followed by 10 seconds stimulus 'off'. The two stimulating conditions were pseudo randomly interleaved for each subject during the recording. Blocks lasted 10 minutes and participants were permitted to take rest between recordings. Fig. 1D shows a schematic representation of the experimental procedure. Visual events were synchronized with NIRSport over wireless LAN communication through the Python version of LabStreamingLay.

Recordings in adult participants- Experiment 1 for adults (exp1) aimed to understand whether a reliable hemodynamic signal could be recorded in response to the radial checkerboard merged with an animated cartoon. Thus, exp1 started with a 10-minutes recording using the reversing checkerboard as stimulus 'on' and the grey screen as stimulus 'off' (RS condition), and continued with the vision of two different blended animated cartoons, where the stimulus 'on' was a merge between the reversing checkerboard and the movie, whereas the stimulus 'off' was the grey-scale isoluminant cartoon (CF and CC conditions; Fig. S1). The main purpose of using a cartoon was to increase the entertaining value of visual stimulation. The checkerboard presentation was needed to ensure a standardized episodic stimulation allowing event-related transient analysis. We decided to merge the checkerboard with the movie in order to avoid possibly distracting interruptions of the storyline and to facilitate screen fixation in children. The merging procedure was achieved using Python3 OpenCV[108]. During the appearance of the stimulus each frame was filtered using an

automatic Canny edge detection algorithm (), then the filtered cartoon was blended with the radial checkerboard. Each pixel of the animated cartoon with the same color of the corresponding pixel of the radial checkerboard was inverted, to obtain a fully visible image. The result was a RS with an overlayed cartoon frame (Fig 1B). The first cartoon was randomly selected by the operator within a group of 4 ("The Lion King", "The Powerpuff Girls", "Peppa Pig" or "Kung Fu Panda"; CF), whereas the latter was a free choice of the subject (CC). Exp2, aiming to dissect the contribution of baseline contrast to visual responses, was performed in a subset of adult participants (n = 15). Exp2 consisted of 3 consecutive recordings of a CF ("Peppa Pig"; "Hide-and-seek", "Fly the kite", "Polly parrot" episodes) with the modulation of the baseline contrast (20%, 40%, 80%; Fig. S1). The presentation order of different contrast levels was randomly shuffled.

Recordings in children- To confirm that the baseline movie and its contrast do not affect the emergence of visual responses to the radial checkerboard in children, we measured hemodynamic signals in response to 2 different blended RS-animated cartoons freely decided by the subject: cartoon 1 was presented at both low (20%, L1) and high (80%, H1) contrast, while only low contrast was recorded for cartoon 2 (L2; Fig. S1). In this case, the presentation order was decided by the child, in order to maximize subject compliance.

During the experimental sessions, data were quickly analyzed and visualized using nirsLAB software (NIRx Medical Technologies LLC, v2019.4).

Signal Processing and Statistical analysis

Data preprocessing was completed using the Homer3 package (v1.29.8) in MATLAB (R2020a). We created a processing stream tailored on recent guidelines for analysis of fNIRS data[25]. First, the raw intensity data were converted to optical density (OD) changes (*hmR_Intensity2OD*). Then,

channels showing very high or low optical intensity were excluded from further analyses using the function *hmR_PruneChannels* (dRange: 5e-04-1e+00, SNRthresh: 2; SDrange: 0.0-45.0). Motion artifacts were then removed by a multistep rejection protocol. After a step of motion artifact detection using the *hmR_MotionArtifactByChannel* function (tMotion: 1.0, tMask: 1.0; STDEVthresh 13.0; AMPthresh: 0.40), motion correction was performed with a combination of Spline interpolation (*hmR_MotionCorrectSpline*, p: 0.99) and Wavelet filtering (*hmR_MotionCorrectWavelet*, iqr: 0.80) functions[25]. The remaining uncorrected motion artifacts were identified using the *hmR_MotionArtifactByChannel*. A band-pass filter (*hmR_BandpassFilt: Bandpass_Filter_OpticalDensity*, hpf: 0.01, lpf: 0.50) was applied to decrease slow drifts and high-frequency noise, and the OD data were converted to Hb concentration changes using the modified Beer–Lambert law (*hmR_OD2Conc*, ppf: 1.0 1.0 1.0). Finally, trials of each subject were block-averaged for every stimulating condition and channel (*hmR_BlockAvg: Block_Average_on_Concentration_Data*, trange: -2.0 20.0)[25]. The resulting txt file was imported in Python as a Pandas DataFrame. For each subject, only the channel with the highest response amplitude was analyzed. The peak response was identified as the maximal value for THb and OHb and the minimum value for DHb. A grand average was taken of the 20 trials of data per stimulating condition and differences between visual stimulation 'on' (reversing checkerboard) and 'off' (blank) were compared. All data were normalised with respect to the blank-evoked response using a subtraction method. Statistical analysis was carried out using *pingouin* Python library[109] and the following functions: *pingouin.ttest* (paired and two-sided t-test), *pingouin.rm_anova* (one-way repeated measures ANOVA), *pingouin.pairwise_ttests* (post-hoc analysis), *pingouin.pairwise_corr* (Spearman correlation), *pingouin.regplot* (Linear regression). T-test, ANOVA

and post-hoc analysis were used to assess differences in fNIRS peak responses following different stimulating conditions, whereas we tested the interaction between the amplitude of fNIRS measures and AQ scores with Spearman correlation. Total AQ scores, and AQ_S, AQ_C, AQ_A, AQ_D and AQ_I subscale scores were used for correlational analysis. We employed the Linear regression to plot such correlations. For the correlational analysis adjustments for multiple comparisons were performed using the Benjamini/Hochberg false discovery rate (BH-FDR) correction. The effect size calculated for the ANOVA was the generalized eta-squared. All the plots have been generated using *Matplotlib* Python library[110]. All statistical metrics and details are reported in Supplementary Table 1 (Table S1).

Data availability

The datasets generated during the current study and scripts used for visual stimulation are available, respectively on Zenodo http://doi.org/10.5281/zenodo.5101912) and GitHub website (https://github.com/raffaelemazziotti/FNIRS_code).

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Figure legends

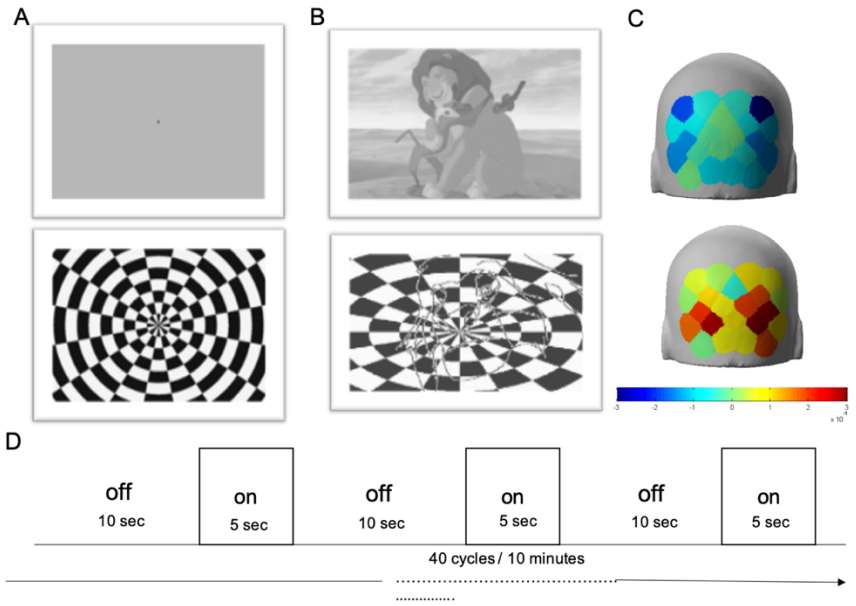

Fig.1: Visual stimulation and experimental paradigm. A: Representative frame of baseline grey screen (upper row, stimulus 'off') and reversing checkerboard (lower row, stimulus 'on') for RS condition. The small black square indicates the fixation point. **B:** Representative frame of low-contrast (20%) grey-scale baseline animated cartoon (upper row, stimulus 'off') and blended checkerboard-cartoon (lower row, stimulus 'on') for CF and CC conditions. **C:** Representative HDR in the occipital cortex during the stimulus 'off' (upper row) and stimulus 'on' activation phase (lower row) according to the output of nirsLAB software. The Look Up table is reported under the images. **D:** Experimental protocol showing that the cycles of visual stimulation were structured in blocks of 40 trials (20 trials with the reversing checkerboard and 20 trials with the 'mock' stimulus) for a total duration of 10 minutes.

Fig 2

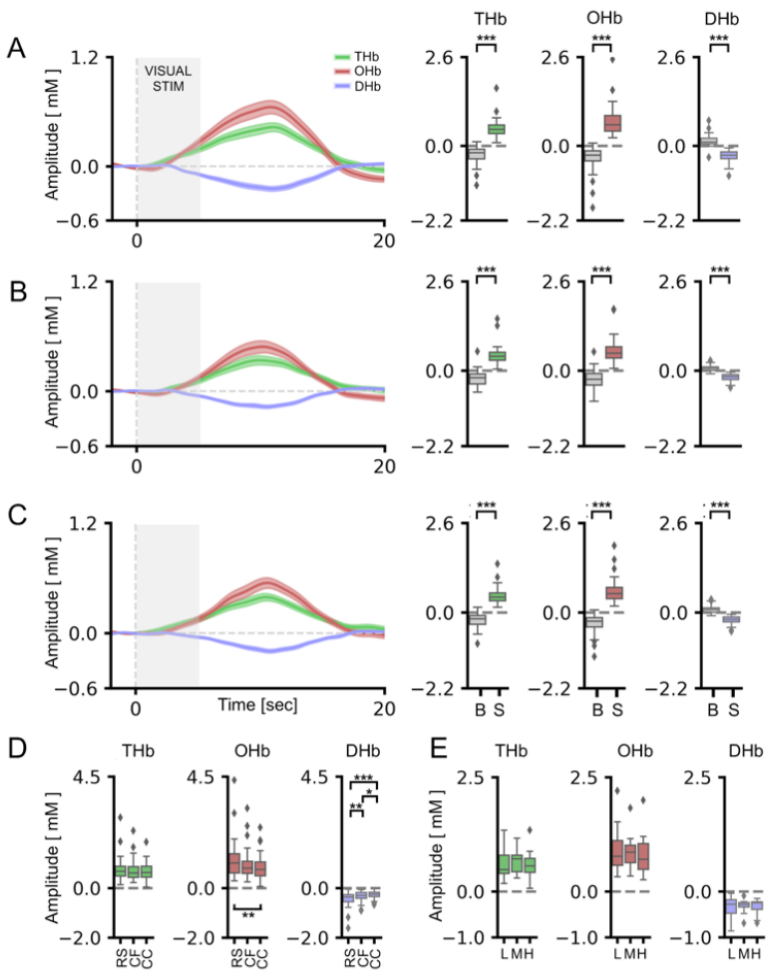

Fig. 2: **HDR was reliably detected in adults using both RS and blended RS-animated cartoons.** For all panels, values in the y-axis are multiplied for 10^4. **A:** On the left, the average time course for THb (green line), OHb (red line) and DHb (blue line) in response to the RS are shown. The three plots on the right depict the average peak response to the stimulus (S) vs. the blank (B) across all the adult subjects. The stimulus-driven signal was significantly different from the blank for all the conditions (t-test, $p < 0.001$ for all comparisons). **B:** Same plots as above for the CF condition. On the left, the average time course of the evoked HDR is depicted. On the right,

the graphs showed that the HDR amplitude was significantly higher in response to S with respect to B for THb, OHb and DHb (t-test, $p < 0.001$ for all comparisons). **C:** CC condition. Also in this case the S elicited significantly higher responses for THb, OHb and DHb with respect to the B (t-test, $p < 0.001$ for all comparisons). **D:** Comparison among different visual stimulations (RS: Radial Stimulus, CF: fixed cartoon, CC: chosen cartoon) shows no differences in evoked amplitudes for THb, whereas a significant difference was detected between RS and CC for OHb (One-way RM ANOVA, $p < 0.01$, post hoc BH-FDR, RS vs. CC $p < 0.01$) and a more complex pattern of differences emerged for DHb (One-way RM ANOVA, $p < 0.001$, post hoc BH-FDR, RS vs. CF $p < 0.01$, RS vs. CC $p< 0.001$, CF vs CC $p < 0.05$). **E:** No differences of evoked responses were detected with different contrast levels of the baseline movie (L: low, M: medium, H: high). For statistical metrics and details, refer to table S1. Data are shown as average ± s.e.m. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Fig 3

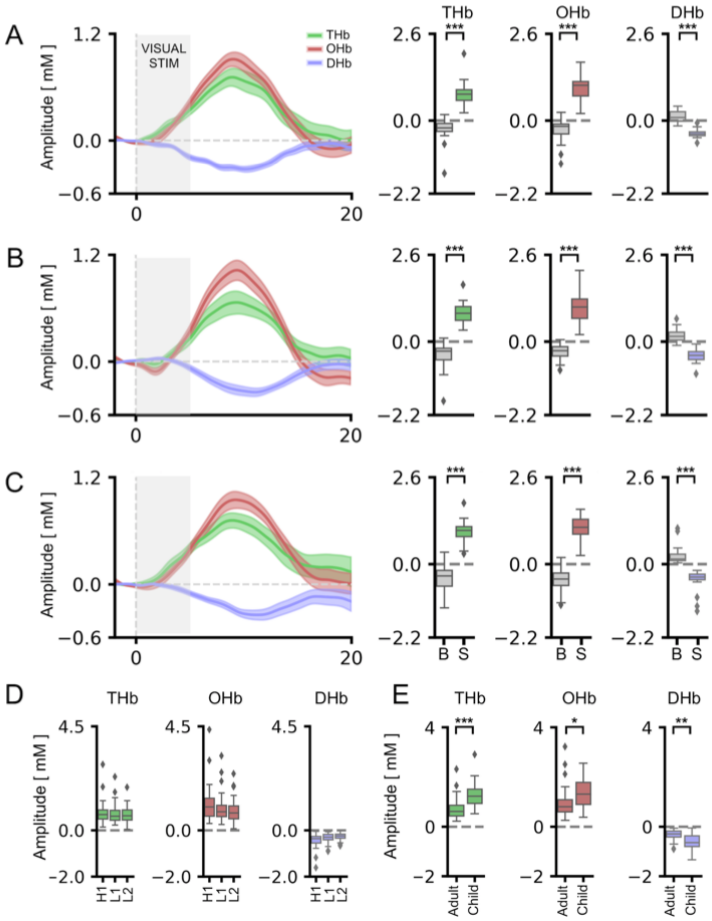

Fig. 3: **HDR signal was reliably detected in children using blended RS-animated cartoons with low and high contrast.** For all panels, values in the y-axis are multiplied for 10^4. **A:** On the left, the average time course for THb (green line), OHb (red line) and DHb (blue line) in response to high-contrast (80%) blended RS-animated cartoons is shown (CH1). On the right, the graphs represent the average amplitude of the evoked HDR following the stimulus (S) and the blank (B). A significantly different response to S with respect to the B was detectable for all metrics (t-test, $p < 0.001$ for all

comparisons). **B:** The average time course of the HDR to low-contrast (20%) blended RS-animated cartoons is shown (CL1). Here, the baseline cartoon is the same as the experiment described in panel A, but a different part of the movie was used. THb, OHb and DHb showed a significantly higher deflection to the S with respect to the B in this condition as well (t-test, $p < 0.001$ for all comparisons). **C:** On the left, the average time course of HDR following the second low-contrast blended RS-animated cartoon selected by the subject (CL2). On the right, the analysis of peak amplitudes revealed significantly higher responses during S compared to B for THb, OHb and DHb (t-test, $p < 0.001$ for all comparisons). **D:** Comparison among different contrast levels of the baseline cartoon revealed no differences in the amplitude of HDR. **E:** Response amplitudes for low-contrast blended RS-animated cartoons in adults and children. More specifically, we compared the response to CF condition of adults with CL1 condition for children. The average amplitude of HDR was significantly higher in children (t-test, $p < 0.001$ for THb, $p < 0.05$ for OHb, $p < 0.01$ for DHb). For statistical metrics and details, refer to table S1. Data are shown as average ± s.e.m. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Fig 4

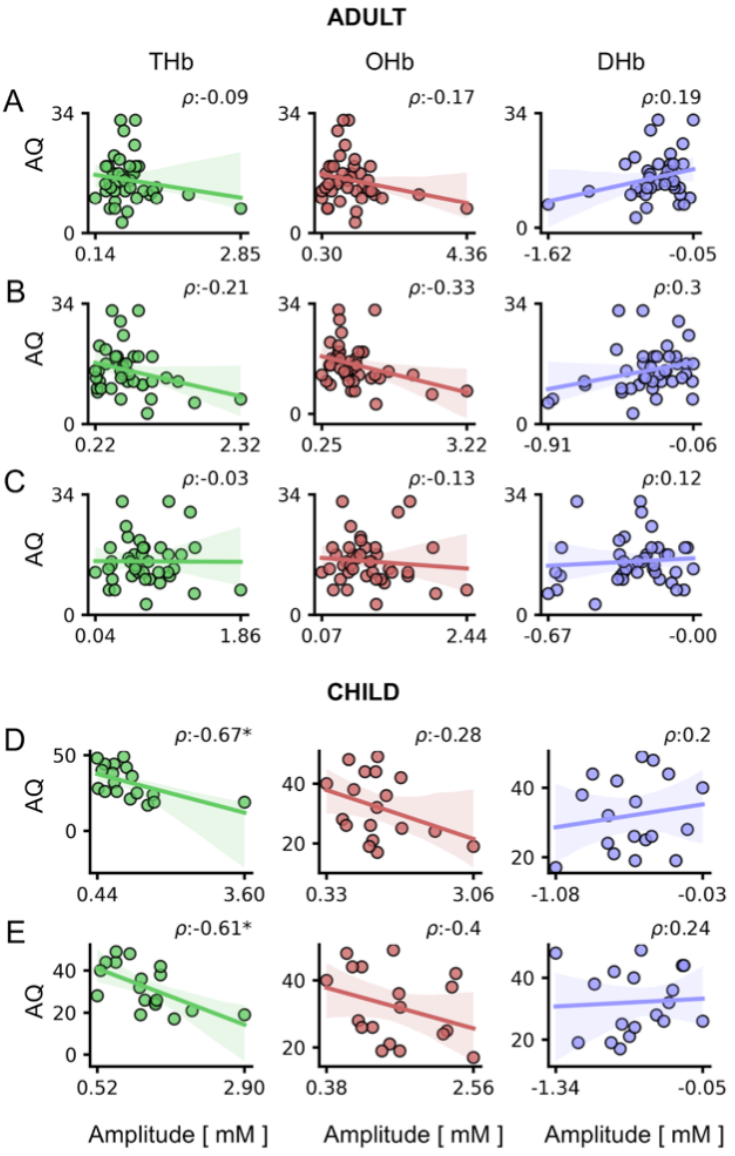

Fig. 4: **Correlation between HDR and AQ scores.** For all panels, values in the x-axis are multiplied for 10^4. The ρ (rho) index in each plot indicates the Spearman correlation value. **A-C:** Correlation between HDR and AQ scores in adults, for amplitudes obtained using RS (**A**), CF (**B**), and CC (**C**). No

significant correlations were detected for adult participants. **D-E:** Correlation between HDR and AQ scores in children, for amplitudes obtained using high (**D**), and low (**E**) contrast baseline cartoons. A significant correlation was found between THb and AQ scores for both high- and low-contrast blended stimuli ($p < 0.05$ for both cases). Circles are individual values, lines represent the linear regression model fit and shaded regions are the 95% CI.

Fig 5

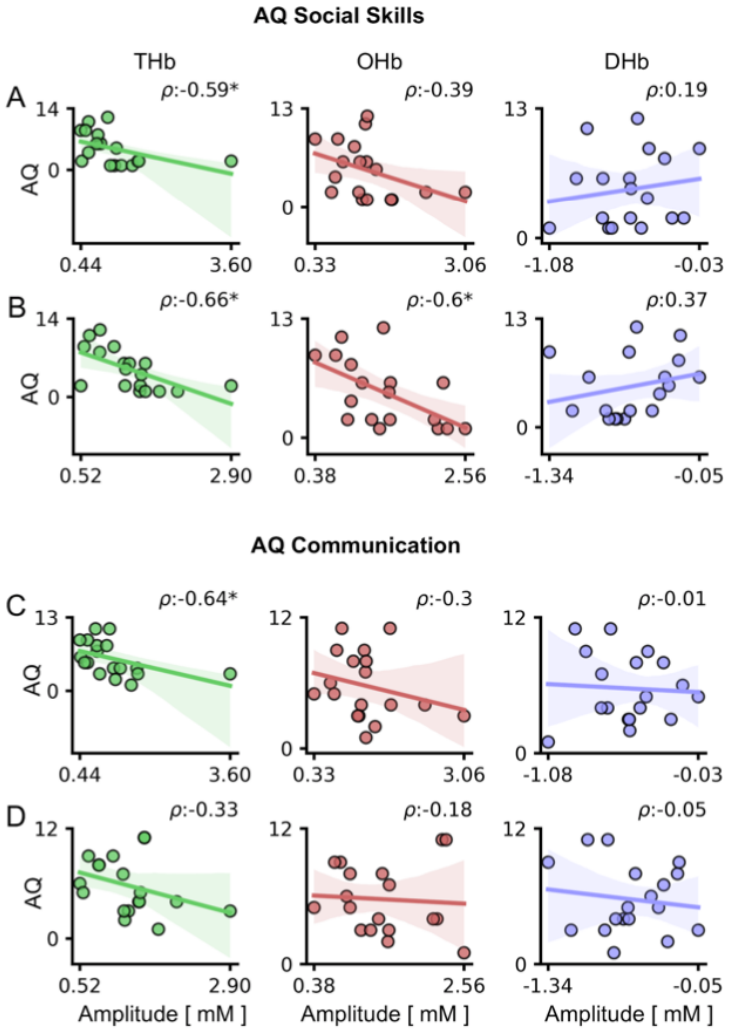

Fig. 5: **Correlation between HDR and AQ subscales in children.** For all panels, values in the x-axis are multiplied for x10^4. The ρ (rho) index in each plot indicates the Spearman correlation value. **A-B:** Correlations between HDR and AQ Social Skills (AQ_S) subscale. A significant correlation between THb and AQ_S was detected using both high- (A) and low-contrast blended stimuli (B; $p < 0.05$ for both cases). In addition, OHb recorded in response to

the low-contrast blended RS-cartoon was significantly correlated with AQ_S (B; $p < 0.05$). **C-D:** Correlations between HDR and AQ Communication (AQ_C) subscale. THb amplitude in response to the high-contrast blended RS-cartoon was significantly correlated with AQ_C ($p < 0.05$). Circles are individual values, lines represent the linear regression model fit and shaded regions are the 95% CI.

Table 1: Demographic characteristics of adult subjects. Age (years), gender, head circumference (head, cm), cap size (cm), total AQ score (AQ), AQ subscale scores (AQ_S, AQ_C, AQ_A, AQ_D, AQ_I) and the movies used for visual stimulation (CF and CC according to the experimental protocol) are listed for each participant. For movies, production company, release date and episode title are indicated as well.

Table 2: Demographic characteristics of children. Age (years), gender, head circumference (head, cm), cap size (cm), total AQ score (AQ), AQ subscale scores (AQ_S, AQ_C, AQ_A, AQ_D, AQ_I) and the movies used for visual stimulation (C1 and C2 according to the experimental protocol) are listed for each participant. For movies, production company, release date and episode title are indicated as well.

CHAPTER 5

Feasibility of Early Intervention Through Home-Based and Parent-Delivered Infant Massage in Infants at High Risk for Cerebral Palsy

Valentina Menici [1], Camilla Antonelli [1,2,3], Elena Beani [1], Alessandra Mattiola [4], Matteo Giampietri [4], Giada Martini [1], Riccardo Rizzi [1,2], Alessandra Cecchi [5], Maria Luce Cioni [6], Giovanni Cioni [1,3], Giuseppina Sgandurra [1,3] and Caretoy-R Consortium

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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 min, 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. Nineteen 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 min and a total of 21.04 ± 8.49 h. 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).

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) (2), since it is considered as a valid model of environmental enrichment (3) 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 toward the studies supporting this last finding. However, a meta-analysis by Wang and colleagues (4) 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 et al. (5) and Lu et al. (6) 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 (7).

In two other systematic review of literature (7, 8) 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 (7). Álvarez et al. (8) 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 (9, 10).

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 (11) 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 (10, 12–21). 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 (11, 22–28).

A study by Ferber et al. (26) proposed to compare 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 (29, 30) and those of infants born at term (31–33).

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 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 1,000 in high-income countries (34). 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-centered and affordable for families and the health service (35). 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 (36). 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 (37).

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 (Beani et al., 2020). The present study aimed to assess the feasibility, acceptability and usability of IM as a new home EI program.

Materials and methods

This feasibility study is part of a larger CareToy-R RCT study described in detail by Sgandurra and colleagues (Sgandurra et al., 2018). 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.

2.1 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.

2.2 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) (Heineman et al., 2013, Heineman et al., 2008), a video-based assessment of motor behaviour in infancy that can also be used to assess infants at high risk of CP(Heineman et al., 2011, Rizzi et al., 2021). Secondary measures included Peabody Developmental Motor Scales – Second Edition (PDMS-2) (Wang et al., 2006; Provost et al., 2004), Bayley Scales of Infant Development Cognitive subscale (BSID-III) (Bayley et al., 2005), standardized video-recordings of parent-infant interaction (Greenr et al., 2002; Biringen, 2009), Teller Acuity Cards® (Teller et al., 2008) and Actigraphic analysis (Motionlogger Microwatch) (So et al., 2007). Moreover, parents were also asked to fill in the BSID-III Social-Emotional Scale

(Greenspan, 2004) and the Parenting Stress Index questionnaire (PSI) (Abidin, 1995).

After the intervention period, families were asked to fill in a questionnaire on the feasibility of their intervention: "CareToy-Revised Questionnaire Parent-Infant Experiences" (Beani et al., 2020) 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 breakout.

2.3 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.

2.4 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 (Leon et al., 2011; Thabane et al., 2010; Verhelst et al., 2017; Orsmond and Cohn, 2015).

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 (Davis, 1985; Dillon and Morris, 1996) and usability (Wixon and Wilson, 1997; Abran, 2003; Jokela et al., 2003) 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 (Beani et al., 2020).

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.

2.5 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.

RESULTS

3.1 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.

3.2 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.

3.3 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.

3.4 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.

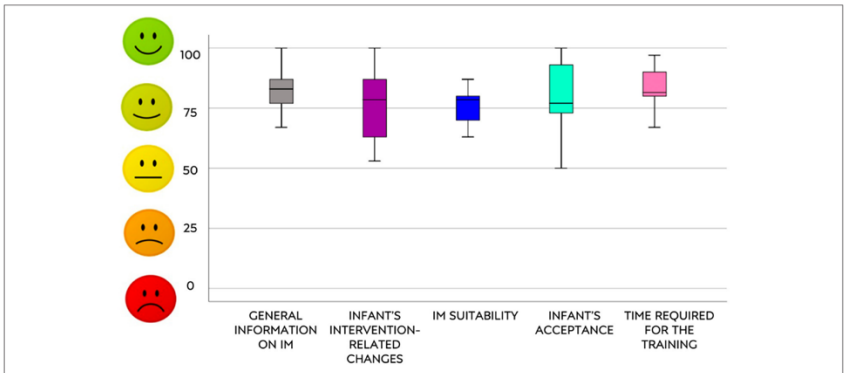

FIGURE 1 | Answers of IMQPE sections.

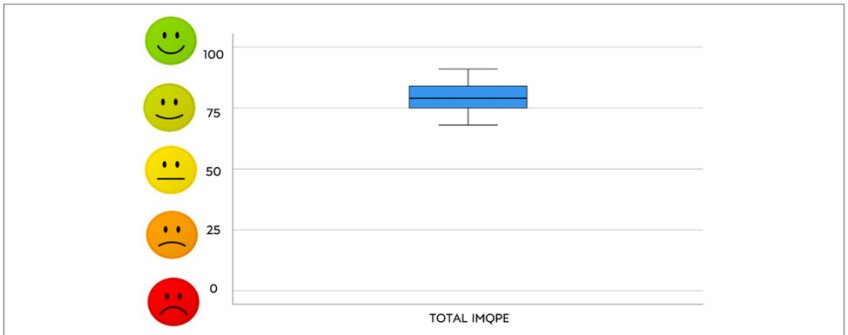

FIGURE 2 | Total answers to the IMQPE.

3.5 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.

3.6 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.

3.6 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.

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 (Vickers et al., 2004; Badr et al., 2015; Juneau et al., 2015; Álvarez et al., 2017; Wang et al., 2013).

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 (Cioni et al., 2016; Dirks et al., 2011; Novak et al., 2017; Spittle et al., 2018).

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 environmental enrichment (Treyvaud et al.,2012; Lai et al., 2016). 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, being based on parents 'empowerment and can be performed in the NICU as well as at home, after the hospital discharge. However, its efficacy has to be proven also for a population at high risk for CP that may include pathological conditions other from prematurity alone (Spittle et al.,2016).

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 (Afand et al., 2017; Feijó et al., 2006; O'Higghins et al., 2008; Midtsund et al., 2019). 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 (Beani et al., 2020; Beani et al., 2020; Corti et al., 2018) so in this study the feasibility evaluation criteria were customized to a home intervention conducted without the use of technological tools (Leon et al., 2011; Thabane et al., 2010; Verhelst et al., 2017; Orsmond and Cohn, 2015).

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 our 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 an early diagnosis and of effective communication strategies for diagnosis disclosure to the parents (Guttmann et al.,2018; Dagenais et al.,2006). 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 correlations 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 (Beani et al., 2020) 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 interventions.

Tables

Table 2: sample characteristics

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%)
Mothers' 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
Mothers' educational level according to the ISCED: n° (%)	Level 1-2: 5 (26.31%) Level 3: 8 (42.10%) Level 6-7: 3 (15.79%) Level 8: 3 (15.79%)

PSI: Parent Stress Index, PD: Parental Distress; P-CDI: Parent-Child Dysfunctional Interaction, DC: Difficult Child, TS: TOTAL SCORE; ISCED: International Standard Classification of Education

Table 3: mothers' characteristics and the amount of IM

	R^2	Estimate	SE	t-value	p
PT Legs and Feet					
Mothers' nationality (Italian)	47.50%	-0.67	0.29	-2.29	0.04
Arms and Hands					
Mothers' nationality (Italian)	28.80%	-9.53	3.63	-2.62	0.02
PT Arms and Hands					
Mothers' nationality (Italian)	33.10%	-9.80	3.38	-2.90	0.01

SE: Standard Error; PT: post training

Table 4: variables of model between mothers' characteristics and the amount of IM

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

		PT Arms and Hands

PT: post training

Table 5: infants' characteristics and the amount of IM

	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					
Siblings	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

SE: Standard Error; IM: Infant Massage

Table 6: variables of model between infants' characteristics and the amount of IM

	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
Siblings	1	Total Hours IM

IM: infant massage

Table 7: IMQPE questionnaire and the amount of IM

	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

SE: Standard Error; IM: infant massage; PT: post training

Table 8: Variables of model between IMQPE questionnaire and the amount of IM

	N° variables of model	Variables
Infant's 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

IM: infant massage; PT: post training

Table 9: IMQPE questionnaire and mothers' characteristics

	R^2	Estimate	SE	t-value	p
Infant's intervention related changes					
PSI-PD	44.60%	-0.72	0.23	-3.05	<0.01

SE: Standard Error; PSI: Parent Stress Index; PD: Parental Distress

Table 10: variables of model between IMQPE questionnaire and mothers' characteristics

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

PSI: Parent Stress Index; PD: Parental Distress

Table 11: IMQPE questionnaire and infants' characteristics

	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					
Siblings	56.20%	-3.09	1.00	-3.08	<0.01
Lesion IVH or PVL		-6.58	2.54	-2.59	0.02

SE: Standard Error; IM: infant massage; IVH: intraventricular hemorrhage; PVL: periventricular leukomalacia

Table 12: variables of model between IMQPE questionnaire and infants' characteristics

	N° variables of model	**Variables**
Twins	1	IM suitability
Siblings	1	Time required for the training
Lesion IVH or PVL	1	Time required for the training

IM: infant massage; IVH: intraventricular hemorrhage; PVL: periventricular leukomalacia

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions: GS and GC conceived the idea for this original research, and all other authors contributed to the conception and the design of the study. RR, AC, MLC, MG carried out the enrolment of all infants for the study and RR did the baseline neurological assessment and eligibility evaluation. VM, EB and GS designed and realized the questionnaire. VM and GM performed the IM training to the parents. AM administered the IMQPE questionnaires. CA, VM, GS, and GC conceived and prepared the manuscript. All the authors read, critically revised, and approved the final manuscript.

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CHAPTER 6

Feasibility Analysis of CareToy-Revised Early Intervention in Infants at High Risk for Cerebral Palsy

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ABSTRACT

Infants with perinatal brain injury are at high risk for Cerebral Palsy (CP). Progresses in detection of early signs of brain injury and of CP allow Early intervention (EI) programs for improving the outcome of these infants. CareToy system (CT), developed within an European project (Trial Registration: NCT01990183) allows to provide, by means of tele-rehabilitation, a highly personalized, family-centred, home-based EI for young infants, remotely managed by clinicians. CareToy, already used with preterms without brain injury, has been adapted for high-risk infants in a project funded by the Italian Ministry of Health and the CareToy-Revised (CareToy-R) has been realized (Trial registration: NCT03211533 and NCT03234959). Before assessing its efficacy, it was crucial to evaluate acceptability, usability and feasibility of CareToy-R EI.

19 high-risk infants with perinatal brain injury, aged 5.95± 2.13 months (range 3.12-10.78 months), carried out a 8 weeks training with CareToy-R at home, performing customized playful activities with their parents, tailored on their rehabilitative needs, remotely managed by clinicians. The feasibility of training and study procedures were assessed through criteria derived from literature; acceptability and usability have been analyzed from data about individual training and an ad-hoc questionnaire.

All CareToy-R trainings were planned by the clinical staff with a daily personalized use per each infant between 30 and 45 minutes (mean 34.37). The amount of executed training by the infants was very high (daily mean 30.30 minutes), with no differences related to infant age, sex and gestational age. All the 9 feasibility criteria were achieved, family compliance to the project was very good, data collection was completed and CareToy-R system worked properly and easily for parents. The answers of the questionnaire had a total mean score of 84.49% and they ranged from a

minimum of 81.05% (in “easy to use” area) to a maximum of 86.49 (“changes due to the training” area),with no differences related to nationality or familiarity with technology of the mothers.

This study reports preliminary evidence to the feasibility of an home-based EI with CareToy-R system in infants at high-risk for CP. Results of the RCT will provide data about the potential effectiveness of this approach.

INTRODUCTION

Perinatal brain injury expose infants to a high risk of developing Cerebral Palsy (CP), the most common cause of physical disability during the developmental age (Oskoui et al., 2013). Despite recent evidence of a genetic contribution to the pathogenesis of CP, the presence of an early brain injury still represents the most important causative factor (Nelson, 2008).

Typical care pathways for infants born preterm or with congenital brain injury consists of dedicated neuroradiological and clinical follow-up programs which can establish if the infant is at high risk of developing a CP (Novak et al., 2017). Indeed, the combined predictive power of Magnetic Resonance Imaging (MRI) and clinical assessment tools such as the General Movement Assessment (GMA) according to Prechtl or the Hammersmith Infant Neonatal Examination (HINE) allow to establish a diagnosis of CP as early as 3-5 months with high sensitivity and specificity (Novak et al., 2017; Einspieler et al., 2019; Romeo et al., 2008). An early and accurate diagnosis of CP is crucial as it allows a prompt and individualized access to a rehabilitative intervention program in a critical period for brain development; this promptness allows to maximize the effectiveness of intervention, exploiting a window of maximal plasticity for many different developmental domains (motor, visual, cognitive…) (Cioni et al., 2016).

In order to be maximally effective, an early intervention program (EI) should be intensive, personalized, family-centred and affordable both for families and health services. Moreover, EI should include multi-axial activities targeting motor, cognitive, sensory and social functions in an integrated systemic approach (Morgan et al., 2013).

The recent availability of tele-rehabilitation tools has allowed to apply this rehabilitative approach to the home setting which is the most enriching and ecological environment for the infants (Novak and Berry, 2014). Moreover,

the possibility to standardize a methodology of intervention and to remotely acquire quantitative measure during the EI program, thanks to biomechatronic toys and telemonitored systems, has created a promising opportunity for developing innovative EI programs. The home-based concept and the tele-rehabilitation architecture provide significant added value to EI programs and both represent the pillars of the CareToy (CT) system.

The CareToy system was created in the framework of a multicentric international project (www.caretoy.eu, Trial Registration: NCT01990183). It is a biomechatronic baby gym equipped with different types of sensors designed to provide a highly personalized, family-centred home-based intervention for young infants, remotely managed by dedicated clinical and rehabilitation staff. CareToy has been validated in a RCT study involving a sample of Italian and Danish preterm infants at low risk for Cerebral Palsy (Sgandurra et al., 2017): results showed an improvement of the visual and motor development, as well as a maternal reduction of levels of stress (Sgandurra et al., 2019). In a feasibility study, an EI program using CareToy has been carried out in a small population of infants with Down Syndome showing the good adaptability of the system to different populations (Inguaggiato et al., 2019).

Basing on this experience, an EI program using a revised version of CareToy system (CareToy-Revised, CT-R) has been implemented in an ongoing RCT involving high-risk infants with perinatal brain injury (Trial registration: NCT03211533 and NCT03234959) (Sgandurra et al., 2018).

Before analysing the clinical efficacy of the CareToy-R system on this population, the present study aims to investigate the rate of acceptability, usability and feasibility of an EI based on CareToy-R in families with infants at high-risk of Cerebral Palsy.

MATERIALS AND METHODS

This feasibility study is focused on the upgraded version of CareToy, the biomechatronic smart baby gym, that is the CareToy-R, designed and adapted for infants with perinatal brain injury, at high risk for developing a CP.

The study protocol of the RCT project and the detailed description of the system have been published elsewhere (Sgandurra, et al., 2018). The wide CareToy-R RCT study is multi-center, paired and evaluator-blinded study with two investigative arms of two EI: Infant Massage and CareToy-R training for a duration of 8 weeks, in which eligible infants are allocated randomly at baseline (T0). Details are shown above.

The study has been approved by Tuscany Pediatric Ethics Committee (84/2017), and it was registered (NCT03234959) on Clinical Trials.gov.

Before comparing the effects of the Infant Massage and CareToy-R training, the feasibility, acceptability and usability of the CareToy-R system need to be investigated.

Participants

Families of the subjects involved in this study were approached during the hospitalization in the Neonatal Intensive Care Units or during the neurodevelopmental follow-up programs in three different University Hospitals: the “Santa Chiara Hospital” in Pisa, the “Meyer Children’s Hospital” and the “Careggi General Hospital”, both in Florence.

For the CareToy-R study, infants with any sign of perinatal brain injury at the neonatal brain UltraSonography (US) or Magnetic Resonance Imaging (MRI) were considered eligible. After 3 months corrected age, all the infants were checked for the presence of atypical clinical signs (absence of physiological fidgety movements or abnormal score at the Hammersmith Infant Neurological Examination, HINE). The presence of both the clinical and the

radiological criteria was considered mandatory in order to establish the high risk of CP and to offer the participation to the trial. The presence of polymalformative syndromes, cerebral malformations, severe sensory impairments (retinopathy of prematurity grade > II, deafness or blindness) were considered exclusion criteria.

Parents or legal representatives signed the informed consent forms to accept the inclusion of the infant in the study. Recruitment for this preliminary study on feasibility started after the approval of the research project by the Ethics Committee. Intervention could start when the infant reached some pre-established motor skills (starting from the initial head control), which are expressed by the cut-off score of gross motor area of the Ages & Stages Questionnaire (last additional criterion).

For this feasibility study, eligible families were those of infants randomized to the CareToy-R arm of the project.

Study design and procedures

The recruitment of the whole CareToy-R project has started on September 2017 and has been completed on June 2020.

The minimum sample size required was 19 for the CareToy-R group.

After the enrolment, infants were allocated in one of the two investigative arms: CareToy-R training or Infant Massage (for details see Sgandurra et al., 2018). Both interventions lasted 8 weeks and during this phase infants continue to receive standard care provided by National Health System.

Assessments, already described in the study protocol (Sgandurra et al., 2018) were performed by a child neurologist and a therapist at the following times:

i) T0, the baseline, in the week before CareToy-R training or Infant Massage

ii) T1, the primary endpoint, within a week after the end of the training

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

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

The Infant Motor Profile (IMP, Heineman, 2008; Heineman, 2013), primary outcome measure of the RCT CareToy-R study, is a video-based assessment of motor behaviour of infants. Peabody Developmental Motor Scales – Second Edition (PDMS-2, Wang, 2006; Provost 2004), Bayley Scales of Infant Development (BSID-III, Bayley, 2005) Cognitive subscale, standardized video-recordings of parent-infant interaction (Crittenden and Claussen, 2002; Biringen, 2009), Teller Acuity Cards® (Teller, 1986) and Actigraphic analysis (Motionlogger Microwatch, So, 2007) were included as secondary measures. In addition, two questionnaires were administered to parents at all assessments time: BSID-III social-emotional scale (Greenspan,2004) and Parenting stress index (PSI, Abidin, 1995).

These assessment tools were administered at all assessments time.

Finally, families allocated in the Caretoy-R arm of the study were asked to fill in two questionnaires the familiarity with technology one (Faett et al., 2013) and "CareToy-Revised Questionnaire Parent-Infant Experiences" (see details below).

This last questionnaire, related to the feasibility aim, will be the measure reported in this study.

Intervention

CareToy-Revised system

CareToy is a technological smart system equipped with sensorized toys, developed as a telerehabilitation tool for home EI. As described in literature (Cecchi et al., 2010; Sgandurra et al., 2014), it is a biomechatronic gym,

composed by: i) two feedback walls containing button, wires for toys, lights and speakers; ii) a wall with a screen where specially developed pictures and videos are shown; iii) a wall for the positioning of sitting posture modules; iv) sensorized toys with different shapes; v) an arch with lights and wires for toys; vi) videocameras for recording infant's behavior; vii) a sensorized mat; ix) a kit of wearable sensors; x) a laptop with an ad-hoc software. As described in the study design paper (Sgandurra et al., 2018), in order to be adapted to the new population of infants, while maintaining the flexibility and variability of the proposals, the clinical staff planned to make some small but essential modifications to the CareToy system, creating the CareToy-R system. First of all, due to the crucial postural needs of these infants, a postural modular system was added in the gym. An ad-hoc kit of Velcro-strap pillows was realized, using Siedo & Gioco facilities (Fumagalli, Italy), allowing a safe and comfortable positioning of infants in supine, prone, sitting, or side position, with different facilities (see Fig. 1).

Together with this structural modification also the content of the goal-directed activities, called CareToy scenarios, was changed. In details, thanks to the possibility to set the audio-visual stimuli, duration, intensity and features of lights and sounds presented as stimulations and/or feedback have been modified.

Moreover, the video library was changed, adding some pictures and short movies with high contrasted images and customized features, useful for those infants with visual impairments.

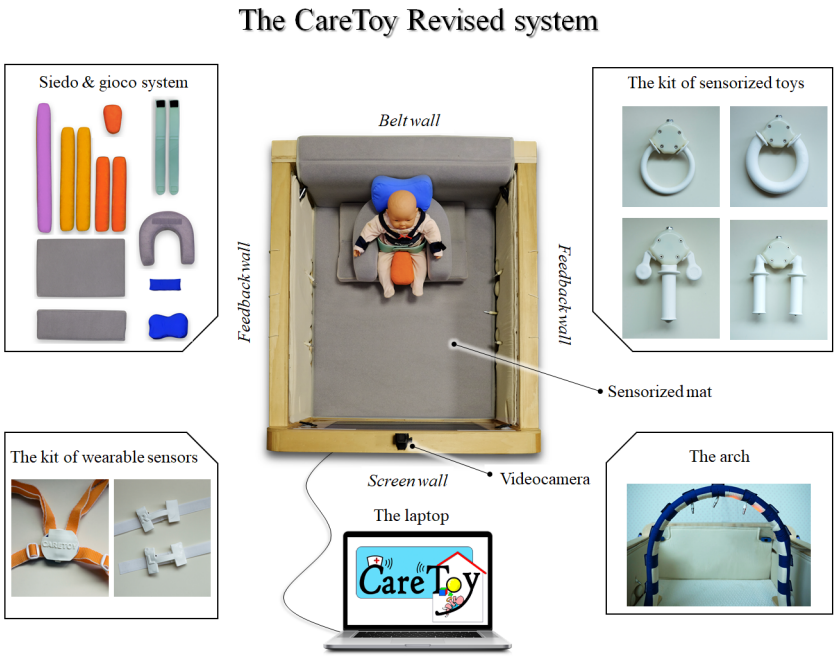
The scenarios library was also modified and the clinical and rehabilitative staff made it more functional for the target population. Then, as in the previous projects, CareToy scenarios were further adapted to the individual developmental needs. Indeed, the training can be designed with high complexity and variability depending on the activated modules and features of lights, sounds, videos and feedback. Scenarios could be planned to train

the infant in different positions (supine, sitting, prone, on side), remotely chosen and periodically updated by rehabilitative staff according to infant's needs and capabilities, to promote the personalized goals.

After the T0 assessment, the CareToy-R system was delivered at home of infants randomized to the CareToy-R group; families who did not have an available internet connection were provided with a portable wi-fi router.

In general, the training was planned for 8 weeks, with daily activity between 30 and 45 minutes, organized in different scenarios (with different goals) lasting from 2 up to 10 minutes each. The first days of training were planned based on the baseline assessment; then, therapists from remote periodically updated the training, according on infant's behavior and/or progresses.

Clinical and rehabilitative staff, mainly composed by child neurologists and paediatric physical therapists, followed infants and their families during the whole training period, planned and monitored the customized goal-directed rehabilitative activities, called Caretoy scenarios; parents were trained on how to use the system and how to play with their infant during the first days of CareToy-R intervention with face-to-face visits with therapists. During the whole training the research team remotely monitored infants and performed on-site visits on weekly basis. Additional assistance, with supplementary visits or video-calls managed by clinicians together with the technical assistance supplied by bioengineers when necessary, was arranged as required, in order to provide hardware and software support and any additional advices on how to manage the training.



The CareToy-R project was active also during the COVID-19 pandemic period with three trainings provided during the forced lockdown; in these cases, on-site visits were replaced by tele-visits and regular staff meetings were performed on-line.

Outcome measures

The feasibility of CareToy-R training has been evaluated several measures.

Feasibility criteria

The criteria, based on relevant recommendations for conducting research on feasibility, have been taken from the literature (Leon et al., 2011; Thabane et al., 2010; Verhelst et al., 2017). The feasibility measures were grouped on the basis of their focus which could be the training intervention or the procedures and study design.

These criteria have been adapted for the CareToy-R study; in details measures have been established as follows:

Feasibility of Intervention:

- ✓ Accessibility: intelligibility of information of the scenarios in terms of preparation (use of pillows, toys, etc) and execution (how to stimulate infant), showed on parents' interface of the laptop
- ✓ Training compliance: required days for completing the 80% of the planned training (at least 8 weeks, that is, the fixed interval of the training)
- ✓ Technical smoothness: good functioning of the CareToy-R system, defined as the quantity of technical issues and malfunctioning experienced
- ✓ Training motivation: motivation and reported effort in carrying out the training.

Feasibility of Study Design and Procedures:

- ✓ Participation willingness: rate of acceptance of the participation in the study
- ✓ Participation rates: number of dropouts
- ✓ Loss to follow-up: recording of all data from all outcome measures
- ✓ Assessment time scale: required interval for collecting all outcome measures (at least 1 week)
- ✓ Assessment procedures: loss to follow-up rates.

The definition and measurement of CareToy-R feasibility criteria are shown in the table 1.

The questionnaires:

Considering the crucial role of parents in CareToy-R intervention, it was necessary to assess their ability to use the system and their perception

about system usage and training effectiveness. For this reason, all families were asked to fill in two questionnaires, aimed to understand their familiarity with technology and their opinions about the training.

The first tool is a questionnaire already available in literature, that is the Information Technology (IT) Familiarity Questionnaire, developed by Geyer (Faett et al., 2013). It has been used to evaluate the participants' familiarity with Information Technology. It consists of 8 questions in 3-points Likert scale (1=daily use, 2=seldom use, 3=never used), investigating the frequency of use of IT. The total score was the average of the scores of the eight questions. This first brief questionnaire was aimed to understand the familiarity with technology of parent who mainly carried out the training.

The second tool is an ad-hoc questionnaire called 'CareToy-Revised Questionnaire Parent-Infant Experiences (CRQPE)', developed from the acceptability questionnaire of the first CareToy project (Inguaggiato et al., 2015; see supplement 1). It is organized in 44 questions, divided in 5 areas: general features of CareToy-R system, changes due to the training, easy to use, infant participation, time dedicated to the training. It is mainly composed by Likert scale answers in 5 points (were 1 meant "not at all", up to 5 which meant "yes") and there are also some open answers in which parents can express their thoughts. All the items of CRQPE were developed, specifically for CareToy-R project, out of the standard definitions of usability (Wixon and Wilson, 1997; Abran, 2003; Jokela et al., 2003) and acceptability (Davis, 1985; Dillon and Morris, 1996).

The two questionnaires were administered in the post-training assessment (T1).

Data collection

The clinical staff remote management of the training was possible thanks to the software CareToy Admin, which allows to plan the training choosing

scenarios from the library and/or to modify them for a more suitable use for the single infant. Moreover, CareToy Admin collects all the training sessions and automatically provide a detailed report which includes planned and details of executed scenarios. Clinicians have the possibility to check all data of the modules (e.g. sensorized toys, mat, etc.) and the videos of infant play, for specific analyses, detecting the results and planning further training.

At the end of each training, a Microsoft Excel sheet is created, summarizing planned and executed scenarios together with the duration of each scenario, each session (training day) and of the whole training.

The questionnaires were administered to families immediately after the end of the CareToy-R training period (T1) in a face-to-face interview with the parent who was mainly in charge of the training (or both). This allowed an easier administration, as the interviewer was free to explain the questions when necessary and, above all, it gave the opportunity to parents to explain their opinions.

Statistical analysis

Clinical data were analyzed by means of Statistical Package for Social Sciences (SPSS, vers. 20.0). Descriptive analyses were used to show the demographic data of infants and of their mothers and the results of questionnaires, for the different areas and the total. Next, multivariate analyses were carried out to explore the differences of treatment planning (i.e. Mean daily CareToy-R training planned in minutes and Total planned training in hours) and of treatment execution (i.e. Mean daily CareToy-R training executed in minutes and Total executed training in hours) and of the total scores and the different domain scores of the CRQE in relation to the infant's and mother's factors. Specifically, for infants we considered the age at T0 as covariate and the sex (male/female) and the gestational age (prematurity/at term) as fixed factors. For the mothers, we considered the values of "familiarity with technology" as covariate and the nationality

(Italian/Foreign) as fixed factor. Moveover, as exploratory analyses, Mann-Whitney test was carried out to compare the hours of total training planned and executed in the infants that did the CareToy-R training during the lockdown period respect to the others.

RESULTS

Participants

Among the 20 eligible families randomized to the CareToy arm of the RCT, 19 families agreed to participate to the intervention. All participants included in the CareToy-R intervention investigative arm of the CareToy-R project (total: 19 families) accepted to fill in the two questionnaires.

14 families were Italian while five from foreign countries (2 Polish, 1 Albanian, 1 Peruvian and 1 Ukrainian). Trainings were carried out in different districts of Tuscan region with a mean distance of 79,8 km from IRCCS Fondazione Stella Maris, ranging from Pisa (15 km, the nearest place from Stella Maris) to Arezzo (165 km, the farthest).

The sample of infants was composed by 11 males and 8 females, with a mean age at the beginning of the study of 5.95 ± 2.13 months (range 3.12-10.78 months).

The trainings were assisted exclusively by mothers and their mean age was 32.95 ± 6.49 years (range 18-41 years). Most mothers were employed at the time of the training and had a high school education.

Demographic characteristics of participants (infants) and mothers are shown in table 2.

Feasibility outcome

Feasibility criteria were all achieved as follow.

First of all, the Feasibility of Intervention:

- ✓ Accessibility: all participants completely understood instructions presented with software and written on printed manual; there were

no further requests of clarification. Furthermore, variables related to mothers, as the nationality or the results of the questionnaire "familiarity with technology" did not impact the dose of executed training (Table 5).

- ✓ Training compliance: the clinical staff, on the basis of each infant's developmental need and personal goals, scheduled all the trainings for a total duration of 33 to 55 days, planning a mean daily training which ranged from 27.90 to 39.27 minutes (mean 34.37 ± 3.15 minutes) (Table 3). The period of forced lockdown (#2, #12 and #19) due to the emergency of COVID-19 pandemic did not change the way to plan and deliver the training and, even if not significantly, the total planned and executed training (hours) were higher in the 3 cases carried out during COVID-19 (28.29 ± 1.53 and 26.42 ± 2.92, respectively) respect to the others (24.47 ± 4.69 and 21.60 ± 5.28, respectively).

Considering different variables as corrected age of infant at the beginning of the training , sex and gestational age (expressed as preterm or at term), there were no significant differences in the planned number of days of training and in the planned daily mean duration of the training (Table 3).

All participants completed the training with a total duration of 31 to 55 days (mean 43.21 ± 7.67), carrying out a mean daily training which ranged from 21.34 to 35.83 minutes (mean 30.30 ± 4.42). The mean total duration of the performed training was 22.40 ± 5.15 hours (Table 4).

All participants completed the 80% of training in 8 weeks; only 4 participants needed one extra week to complete the training (total duration: 9 weeks) and this was mainly related to holiday periods.

Moreover, when considering the executed amount of training, there were no significant differences related to the variables: age at the beginning of the training, sex, gestational age.

- ✓ Technical smoothness: the CareToy-R system experienced two kinds of hardware issues: in one case it was necessary to replace during the training the laptop of the Caretoy system and in 12 families one of the toys. The software presented some technical issues during 3 trainings. Nevertheless, all these issues were fixed with a remote assistance by a dedicated team of engineers; after a quick replacement of the malfunctioning item all participants could resume the training after a stop of about a half day.
- ✓ Training motivation: 12 subjects executed the 100% of the planned training days and the other 7 subjects between 96% and 99% of the planned training days.

Concerning the feasibility of Study Design and Procedures:

- ✓ Participation willingness: only one eligible family did not give the consent in participating to the project, because they have not the required space in their house for positioning the CareToy-R system
- ✓ Participation rates: No dropouts were reported.
- ✓ Loss to follow-up: it was possible to record all data of all outcome measures and there were no missing data.
- ✓ Assessment time scale: follow-up measurements of all participants were collected within one week after the end of the training (range 0–7 days, mean= 4.53 ± 3.31 days). Only three follow-up measurements were collected between 8 and 15 days after training, because of holiday period (mainly summer holiday and Christmas).

- ✓ Assessment procedures: all participants who started the training intervention completed the post-training assessments.

The questionnaire

All 19 families accepted to fill in the questionnaires and the interview carried out by therapist during the assessment after the training (T1).

Overall, the answers of the CRQPE questionnaire had a total score all above 110 points (corresponding to 73.33%) with a range of 110-138 points and mean total score of 126.74 ± 8.43 points (84.49%).

Regarding the five sections: "Infant participation" had a range of raw scores between 19 and 30 points, "Easy to use" had a range of raw scores from 21 to 27; the section "General features of CareToy-R system" presented a range from 22 to 29 and "Changes due to the training" showed a range from 21 to 30 and "Time dedicated to the training" between 20 and 29.

In the subsections the percentage of mean raw scores resulted in increasing order: 81.05% in "easy to use" (low percentage obtained), 84.74% in "general features of CareToy-R system", 84.91% in "time dedicated to the training", 85.26% in "infant participation" and 86.49% in "changes due to the training" (higher percentage obtained).

Median and 95% confidence interval of scores in the questionnaire (both total and section scores) are shown in Table 6 and Figure 2 and 3.

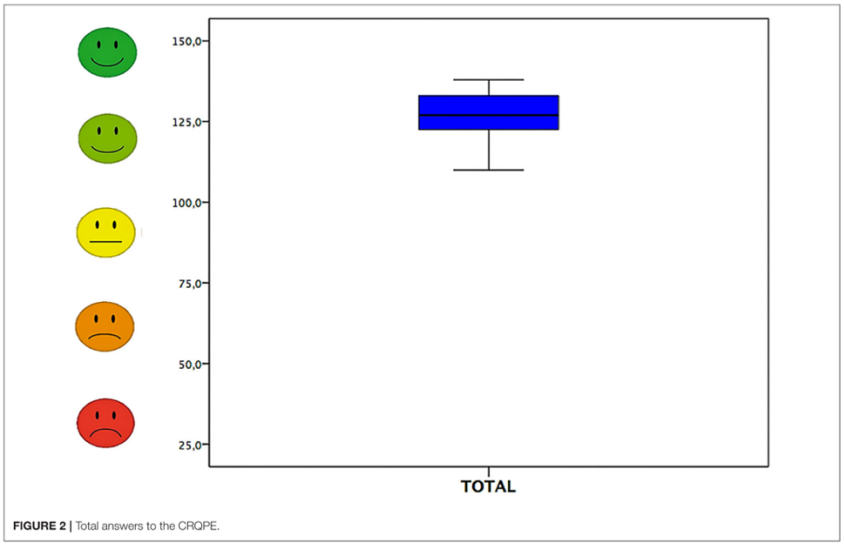


FIGURE 2 | Total answers to the CRQPE.

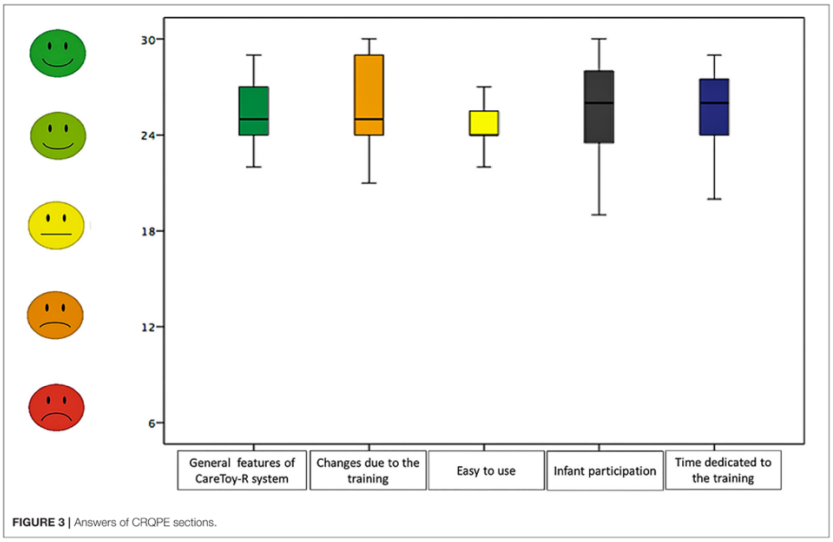


FIGURE 3 | Answers of CRQPE sections.

Furthermore, the answers of (IT) Familiarity Questionnaire (Faett et al., 2013) had a raw score range between 8 and 21 points (33.33%-87.50%) and mean total raw score of 12 points (51.32%). The total mean presented a range between 1.00 and 2.63 points.

There were no significant effects in the total scores and different sections of CRQPE respect to the sex of the subjects, the age at the beginning of the training, or the gestational age (Table 7).

Likewise, there were no differences in the total scores of answers related to mothers' characteristics: nationality and the level of familiarity with technology (Table 8).

DISCUSSION

The present study is the first one in the literature that investigate the acceptability, usability and feasibility of CareToy-R EI in families with infants at high-risk of CP, evidencing the first milestone in the field of the use of new medical devices in tele-rehabilitation. It is crucial that a new device that is addressed to the home use is feasible in its use, because even if it is effective but not feasible, it is hard to be used and translated in the clinical practice. With the current study we have shown for the fist time the applicability of the CareToy-R system and its relevance for home-based early intervention programs in high risk infants.

For our purpose we referred to the literature for the indication about the methods to assess usability and acceptability of technologies for home-based rehabilitation (Sgherri et al., 2019) and the criteria based on relevant recommendations for conducting research on feasibility, already used in studies (Leon et al., 2011; Thabane et al., 2010; Verhelst et al., 2017; Beani et al., 2020).

The data of this feasibility study highlight different achievements and are in line which the principle of high customized training of the CareToy concept. Since the first CareToy has been created, many studies have been dedicated to test its effects on preterm infants (Sgandurra et al., 2017) and its feasibility in other population (Inguaggiato et al., 2019); the current study presents the first results concerning its feasibility in another delicate population, namely infants with perinatal brain injury. As compared to the previous CareToy studies, this project not only involves a new and critical

population, but also introduces some change in the CareToy system and the extention up to 8 weeks of the training duration.

The high rate of acceptance of the CareToy-R project is the first interesting data, which means that the proposal has been agreed and the majority of families share this approach and trust the clinical staff in the importance of this EI approach. In one case, a family refused to participate to the project for the lack of enough room to install the CareToy in their house; this could represent a limit to overcome. The scale in which the CareToy-R system was realized was a compromise between the need of including many different sensors and technology and the need to guarantee enough space for the infant move freely; in a future perspective the use of smaller sensors and hardware components could reduce the size of CareToy making it more suitable also for families who live in small apartments.

The use of tele-rehabilitation, together with the already known advantages of overcoming the limit of distances and maintaining patients in close contact with their therapists (Peretti et al., 2017), allowed to guarantee the prosecution of the rehabilitation intervention also during the lockdown due to the COVID-19 pandemic emergency, without reducing the dose of the proposed training. The tele-rehabilitation architecture has recently gained much attention due to the possibility of delivering rehabilitative intervention also in periods in which access to healthcare facilities is limited as during the sanitary lockdown.

The results of this feasibility study on CareToy-R training confirmed the good functioning of the tele-rehabilitation architecture, that in this specific project did not present the typical limit of the unstable or malfunctioning connection (Eriksson et al., 2011), thanks to the possibility of delivering a portable router which supplied internet access to those who did not have a personal one or whose connection did not have the required speed. Indeed,

connectivity barriers often influence the experience of tele-rehabilitation of patients and clinical staff (Tyagi et al., 2018).

Within this project, only families who lived in Tuscany participated to the CareToy-R intervention and this was due to the recruitment, carried out in the three Neonatology Units of the main Hospitals of this region. An interesting perspective could be to create a more national network, including several Neonatology Units, in order to offer the possibility to join the CareToy-R project also to infants who live in other regions, with the aim of standardizing the methodology and giving the same opportunity of a home-based EI also to infants who live far away from the main clinical centers.

Another first index of CareToy-R feasibility was represented by the clinical and rehabilitative staff management and planning of CareToy-R training and, in particular, by the possibility to program an 8-weeks training with daily session of a minimum of 30 minutes. All the planned scenarios were addressed to meet each specific rehabilitation need; the absence of differences in the amount of planned training among infants who presented different clinical pictures confirms the appropriatenes and the high personalization of CareToy-R scenarios.

On their side, families were very compliant also in performing the training. The familiarity of technology, generally high for our sample, seemed not to impact the amount of executed training; this means that the CareToy-R system has been shown as an easy to use platform and the experience in using technological devices does not play a role in the usability of the system.

The high quantity of executed scenarios, not influenced even by the infants' characteristics (sex, gestational age and age at T0) further support the high customization and the focus on each single developmental need and goal of each participant.

As we know, the personalization is of critical importance and the underlying heterogeneity of many disease processes suggests that the strategies for treating an individual with a disease, and possibly monitoring or preventing that disease, must be tailored to match that individual's unique biochemical, physiological, and behavioral profile. This precision medicine, which is one of the new challenges of the research, is not a whole new approach, but it can represent an enhancement of an already used concept aimed to identify, assess, organize and analyze multiple variables to generate a precise and tailored approach. This is not an end-point process, but it includes a number of feedback loops which need ongoing efforts to become ever more precise. Patient data are used to develop clinically relevant models, and the results of these analyzes then address the further assessment of patients, as an example of precision medicine as an evolving result (König et al., 2018; Khoury, 2015; Robinson, 2010; Hamburg and Collins, 2010).

In this framework, the CareToy approach showed to have a modular concept and it allows to be further upgraded for becoming a model of precision medicine, by adding specific and detailed quantitative measurements for each infant allowing a personalized functional profile of infants with different and specific needs.

It is also interesting to consider the dose of performed training together with users' satisfaction in using the system, because they are crucial factors to increase the motivation and the compliance. On the basis of the CRQPE results, the whole sample of 19 families willingly accepted to fill in the questionnaires and gladly accepted the interview, showing a very good level of acceptability and usability.

CRQPE has demonstrated the possibility of systematically and quantified parental opinions on different features of the CareToy project and the CareToy-R system.

The section “infant participation” had the lowest score but the score meant that the involvement of the infant in CareToy scenarios was high; together with the high result of the section “changes due to the training” and the data about the amount of training, these data could confirm the suitability of the planned activities to each infant. The areas of the CRQPE relative to the “easy to use” and “general features of CareToy-R system” could be linked to the appreciation of the CareToy-R by families, which had experienced a simple use of the system and liked its features, both in terms of functionality and appearance. Furthermore, despite the variability of expertise in the use of IT, all families reported an easy use and returned positive feedbacks about the feasibility of CareToy-R training, and this further confirmed the usability and acceptability of CareToy-R system. Finally, the area “time dedicated to the training”, which included questions related to the role of the parent while performing the training, had the highest score of all areas. This means that mothers (who mainly executed CareToy-R training) felt free and confident using the system; moreover many of them reported that they thought to be empowered after the training. In this sense, the remote guide and support by an expert clinical staff seemed to yield benefits to the parental role.

Looking at the additional comments, the CareToy-R system showed to be, from the parental perception, a useful and innovative tool to promote the development of infants at high neurological risk. The CareToy-R system was indeed widely appreciated in several features by parents, who considered it useful in enhancing and promoting specific skills of their infants on the basis of their individual profile. Parents felt theirselves totally involved in their infant’s rehabilitation project and they shared activities and playful sequences that allowed them to discover their infant’s abilities and potentialities.

Thanks to families' opinions raised from the CRQPE, the CareToy can be improved and optimized for different populations and it could be further empowered in terms of acceptability and usability. This should support the motivation, encourage parents to perform the training with their infants and, as a consequence, maximize the efficacy of the intervention.

The results of the questionnaires have given an interesting insight directly from the families who participated to the project; this has been useful in order to get feedback on the adequacy of the system and on the appropriateness of the rehabilitation proposals. This vision will serve as an input in order to further improve the CareToy system, correcting those features which considered less acceptable.

Out of the importance of this feasibility study, some limitations need to be underlined. First of all, concerning the study design, the participation of families in a RCT study and the previous positive results of the CareToy approach in low risk infants can represent a bias, because their expectations could affect the answers in the questionnaire. Another bias could be related to the administration way of the CRQPE questionnaire that was carried out by the assessor therapist, blind to the intervention in terms of duration and progressions, as an interview. We chose this way to administer interviews for make parents more confident in talking about their experiences and adding personal comments to the Likert answers but a positive bias could be raised on. Moreover, we have investigated only the parents' point of view but there is the lack of having the feedbacks of all other end-users opinions than parents, e.g. it would be interesting to create a tool respecting the standard definition of usability and acceptability, aimed to collect data about the point of view of the rehabilitation staff. Moreover, it has not foreseen a detailed cost analysis in order to assess a cost-effectiveness analysis, crucial for estimate the real possibility that CareToy training could have a contribution to reduce the costs of health services and could become

relatively inexpensive and can expand the accessibility of rehabilitation to infants at high risk for CP.

Beside these limitations, the current study constitutes the basis of the feasibility of the CareToy-R intervention in high risk infant and results of the RCT will show the efficacy of this approach in improving the outcome of this population.

Table 2: sample characteristics

ID	Gestational Age (weeks)	Infant Age at T0 (months)	Sex	Mother age (years)	Mother Nationality
#1	40^{+5}	4.34	female	34	Italian
#2	37^{+5}	4.67	female	41	Polish
#3	26^{+0}	10.59	male	36	Italian
#4	40^{+0}	6.48	male	35	Italian
#5	32^{+4}	6.12	male	41	Italian
#6	40^{+0}	7.56	female	36	Italian
#7	40^{+0}	5.75	female	28	Italian
#8	26^{+0}	6.84	female	35	Italian
#9	36^{+3}	4.50	male	31	Italian
#10	33^{+0}	5.00	female	29	Italian
#11	40^{+0}	8.68	male	37	Italian
#12	37^{+0}	4.54	male	26	Italian
#13	39^{+0}	4.96	female	24	Polish
#14	27^{+0}	3.12	male	27	Albanian
#15	40^{+0}	4.27	male	39	Italian
#16	26^{+6}	5.92	male	39	Italian
#17	29^{+0}	10.78	male	18	Peruvian
#18	40^{+0}	4.27	female	29	Italian
#19	40^{+0}	4.64	male	41	Ukrainian
Group results	*35.16 ± 5.68*	*5.95 ± 2.13*	*11 males 8 females*	*32.95 ± 6.49*	*14 Italian 5 foreign*

Table 3: Infants characteristics and training

	Age at T0			Sex (male and female)			Gestational age (preterm or term)		
	F test	df	p-value	F test	df	p-value	F test	df	p-value
Mean daily CT-R training planned *(minutes)*	1.402	1	0.256	0.004	1	0.951	0.208	1	0.656
Mean daily CT-R training executed *(minutes)*	0.318	1	0.582	2.250	1	0.156	0.100	1	0.756
Total planned training *(hours)*	1.592	1	0.228	0.069	1	0.797	1.217	1	0.289
Total executed training *(hours)*	1.049	1	0.323	0.637	1	0.438	0.358	1	0.559

Table 4: CT-R training data

ID	CT-R Training days planned *(N°)*	CT-R Training days executed *(N°)*	training executed *(%)*	Mean daily CT-R training planned *(minutes)*	Mean daily CT-R training executed *(minutes)*	Total planned training *(hours)*	Total executed training *(hours)*
#1	42	38	96%	35.72	23.06	25.01	16.14
#2	55	55	100%	32.20	31.35	29.51	28.74
#3	33	33	100%	39.26	35.64	21.59	19.60
#4	48	48	100%	38.44	35.83	30.75	28.66
#5	33	31	98%	33.52	29.84	18.43	16.41
#6	35	34	99%	27.90	21.34	16.28	13.31
#7	51	51	100%	34.62	31.10	29.43	26.43
#8	43	43	100%	37.56	28.60	23.59	20.49
#9	50	50	100%	34.46	34.34	28.72	28.62
#10	46	43	97%	31.65	26.52	24.26	20.33

#11	50	50	100%	36.36	33.73	30.30	28.11
#12	50	50	100%	31.88	27.77	26.57	23.14
#13	44	42	98%	39.27	34.67	29.45	26.00
#14	50	50	100%	33.79	32.30	28.16	26.92
#15	41	39	98%	28.85	22.40	19.72	15.31
#16	35	35	100%	35.99	34.46	20.99	20.10
#17	33	33	100%	35.07	31.97	19.29	17.58
#18	45	43	98%	33.99	29.85	25.49	22.39
#19	53	53	100%	32.59	31.02	28.78	27.40
Group results (mean ±SD)	*44.05 ± 7.28*	*43.21 ± 7.67*	*99% ± 1%*	*34.37 ± 3.15*	*30.30 ± 4.42*	*25.07 ± 4.54*	*22.40 ± 5.15*

Table 5: Mothers characteristics

	Nationality (Italian or foreign country)			Questionnaire "familiarity with technology"		
	F test	df	p-value	F test	df	p-value
Mean daily CT-R training planned *(minutes)*	0.043	1	0.839	0.146	1	0.708
Mean daily CT-R training executed *(minutes)*	1.230	1	0.284	0.014	1	0.907
Total planned training *(hours)*	2.032	1	0.173	3.416	1	0.083
Total executed training *(hours)*	2.890	1	0.108	1.661	1	0.216

Table 6: Results of CRQPE questionnaire

	Sample	
	Median [95% CI]	
	Raw Score	%
General features of CareToy-R system	25.42 [24.49 - 26.35]	84.74% [81.65 – 87.83]
Changes due to the training	25.95 [24.53-27.36]	86.49% [81.78 – 91.20]
Easy to use	24.32 [23.53-25.10]	81.05% [78.43 – 83.68]
Infant participation	25.58 [24.19-26.97]	85.26% [80.62 – 89.91]
Time dedicated to the training	25.47 [24.15-26.79]	84.91% [80.52 – 89.31]
TOTAL	126.74 [122.67-130.80]	84.49% [81.78 – 87.20]

Table 8: Mothers' characteristics and CRQPE answer questionnaire

	Nationality (Italian or foreign country)			Results of the questionnaire "familiarity with technology"		
	F test	df	p-value	F test	df	p-value
General features of CareToy-R system	0.094	1	0.763	0.45	1	0.835
Changes due to the training	0.056	1	0.816	0.889	1	0.360
Easy to use	0.440	1	0.517	0.498	1	0.490
Infant participation	0.644	1	0.434	0.553	1	0.468
Time dedicated to the training	1.213	1	0.287	0.228	1	0.639
Total CRQPE	0.350	1	0.562	0.011	1	0.918

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

GS and GC conceived the idea for this original research, and all other authors contributed to the conception and the design of the study. CA, CM, GM, GS, GC, and RR carried out the enrollment of all infants for the study and their baseline neurological assessment and eligibility. EB, VM, and GS designed and realized the questionnaire. VM performed all the motor assessments and carried out the questionnaires. GS performed the statistical analysis. EB GS, VM, GC and RR conceived and prepared the manuscript. All the authors read, critically revised, and approved the final manuscript.

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CHAPTER 7

Early intervention at home in infants with congenital brain lesion with CareToy revised: a RCT protocol

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ABSTRACT

Congenital brain lesions expose infants to be at high-risk for being affected by neurodevelopmental disorders such as cerebral palsy (CP). Early interventions programs can significantly impact and improve their neurodevelopment. Recently, in the framework of the European CareToy (CT) Project (www.caretoy.eu), a new medical device has been created to deliver an early, intensive, customized, intervention program, carried out at home by parents but remotely managed by expert and trained clinicians. Reviewing results of previous studies on preterm infants without congenital brain lesion, the CT platform has been revised and a new system created (CT-R).

This study describes the protocol of a randomised controlled trial (RCT) aimed to evaluate, in a sample of infants at high-risk for CP, the efficacy of CT-R intervention compared to the Infant Massage (IM) intervention.

Methods/design

This RCT will be multi-centre, paired and evaluator-blinded. Eligible subjects will be preterm or full-term infants with brain lesions, in first year of age with predefined specific gross motor abilities. Recruited infants will be randomized into CT-R and IM groups at baseline (T0). Based on allocation, infants will perform an 8-week programme of personalized CareToy activities or Infant Massage. The primary outcome measure will be the Infant Motor Profile. On the basis of power calculation, it will require a sample size of 42 infants. Moreover, Peabody Developmental Motor Scales-Second Edition, Teller Acuity Cards, standardized video-recordings of parent-infant interaction and wearable sensors (Actigraphs) will be included as secondary outcome measures. Finally, parents will fill out questionnaires (Bayley Social-Emotional, Parents Stress Index). All outcome measures will be carried out at the beginning (T0) and at end of 8-weeks intervention period, primary endpoint (T1). Primary outcome and some secondary

outcomes will be carried out also after 2 months from T1 and at 18 months of age (T2 and T3, respectively). The Bayley Cognitive subscale will be used as additional assessment at T3.

Discussion

This study protocol paper is the first study aimed to test CT-R system in infants at high-risk for CP. This paper will present the scientific background and trial methodology.

Trial registration NCT03211533 and NCT03234959 (www.clinicaltrials.gov).

Keywords: Early intervention, High-risk infants, Randomized clinical trial, Tele-rehabilitation, Information and communication technology, Neurodevelopmental bioengineering, Cerebral palsy, Infant massage

BACKGROUND

Cerebral Palsy (CP), a clinical outcome linked to pre- or perinatal brain injury, represents the main chronic condition of disability in childhood [1, 2]. Disorders and disabilities associated with CP determine a relevant social, financial and emotional influence, on CP subjects, on their relatives, and also on health services, since these patients require continuous care, treatment and social support throughout their entire lives [3, 4]. The combined use of assessment tools such as the General Movement Assessment (GMA) according to Prechtl (GMs) and brain Magnetic Resonance Imaging (MRI) have shown high sensitivity and specificity in CP identification in infants since their first months of life [5]. In particular, a recent literature review has shown that brain MRI associated with GMA or neurological examination (Hammersmith Infant Neonatal Examination, HINE) performed at around full-term age determines the greatest predictive power of CP in high-risk newborns [6]. Early diagnosis has considerable importance because it allows for an early medical response and intervention which, as indicated by literature, can improve developmental outcome of high-risk children [7]. Moreover, early intervention (EI) is crucial because it targets brain plasticity, which for many functions has a maximum expressivity in an early limited time window or "critical period" [8, 9].

Several EI programs, based on Environmental Enrichment (EE) and Goal Directed approaches, have been used in clinical settings with positive results on neurodevelopment, but these findings are not conclusive due to high heterogeneity of clinical studies and applied intervention models [10–13].

In general, EIs should include certain essential characteristics to be effective and more specifically they should be early, intensive, personalized, multi-axial, family-centred and affordable for families and health services. In this context, biotechnologies and tele-rehabilitation appear to be promising approaches that can help achieve these standards [14].

Recently, in a European project (www.caretoy.eu, Trial Registration: NCT01990183), the CareToy (CT) system has been created. It is a technological and modular system able to provide, by means of tele-rehabilitation, a home and personalized intervention for very young infants (for further details see [15, 16]). CT allows subjects to carry out an early, intensive, individual EI carried out at home by parents consisting of highly customized exercises (rehabilitative packages called scenarios) remotely monitored by a clinical staff [15–18].

CT has been recently validated as a EI tool through a RCT study (CareToy training vs Standard Care), preceded by a pilot study, which involved a total of 61 in preterm infants born from 28 to 32 weeks of gestational age, without brain lesions and therefore considered at low risk for neurodevelopmental disorders. Children recruited in the pilot and RCT studies were divided into CT and Standard Care groups. Children allocated to the CareToy group followed a 4 weeks training with CT system, at the same time children allocated to the control group performed only Standard Care. All children were assessed with specific and standardized scales and questionnaires, immediately before and immediately after treatment period. Results of the study showed that CareToy training has a positive effect on promoting short-term visual and motor infant's development [19, 20].

Based on these findings, the purpose of this study is to compare, through a Randomized Controlled Trial (RCT), the effects of two types of EI (CareToy training vs Infant Massage) on neurodevelopment of a group of at least 42 children at high risk for CP. The general hypothesis is that the CareToy system, with some adaptations, could be a useful EI tool also in brain-lesioned children at high risk for CP, effectively promoting motor, cognitive and perceptual development. In order to provide an EI to all children participating in the study, it was decided to offer Infant Massage (IM) as a

valid alternative to CareToy. The choice of proposing IM was favoured by its ever-increasing application in Neonatal Intensive Care Units (NICU) [21]. The general hypothesis of IM intervention is that tactile stimulation (parent-infant) is able to promote neurodevelopment, emotional regulation of behavioural states and parent-infant relationship [22]. The rationale underlining these mechanisms seems attributable to increased metabolic efficiency and reduction of stress hormone synthesis. A recent systematic review [22] highlighted the effectiveness of IM on promoting neurodevelopment of preterm babies [23]; moreover, although studies on IM in infants with early brain damage are still limited, it seems to have positive effects on muscle tone and general motor development [24].

Based on this state of the art, the main purpose of this study is to evaluate the effects of CareToy EI (with a revised version of CareToy designed for this purpose), compared to those of Infant Massage, on neurodevelopment of infants at high risk for CP.

METHODS/DESIGN

This paper presents the protocol of an RCT which compares the effects of CareToy training to those of Infant Massage on neurodevelopment of infants at high risk for CP. Details of the two treatment protocols are described below.

Ethical considerations

Tuscan Region Paediatric Ethics Committee (Italy) approved this study (no. 84/2017). Before the signing of informed consent, a dedicated personnel will verbally inform all parents of eligible infants about the trial, giving them also written informative material. Two informed consent forms during two different phases of the study will be provided. The first form allows for an observational phase related to a standardized GMA from the writhing period up to the fidgety one. Then, a second consensus form related to the

intervention phase (CareToy or IM) will be given only to parents of infants with fidgety absent. There is a dual purpose for offering two treatments: to compare the two EIs and, for ethical reasons, to allow all eligible infants to carry out an EI.

Primary objective

The main aim of the present trial is to explore the effects of CT training on neurodevelopment of infants at high risk of CP and then compare these effects to those of IM.

Three hypotheses have been specified:

- CareToy Revised (CT-R) is a useful rehabilitative medical device for young infants (< 1 year) at high risk for CP
- Infants receiving CT-R training will develop motor, perceptual and cognitive abilities faster than infants receiving IM
- Improvement in visual and motor abilities will be faster during CT-R training than during IM.

The secondary aim is to investigate the different impact of CT-R on neuromotor development, both quantitatively (postural, motor and manipulation competences) and qualitatively (motor repertoire and adaptive abilities). Moreover, another purpose is to measure the efficacy of CT-R training on visual and cognitive development, parent-infant interaction and sleep-waking pattern.

The two phases of this project will be preceded by a small pilot study in which feasibility of EI effects of CareToy Revised system (CT-R training) compared with Infant Massage (IM) will be evaluated.

Study design

To make a comparison between the effects of CT training and IM in brain-damaged infants, a multicentre, evaluator-blinded, paired RCT will be carried out.

The involved clinical centres are the Department of Developmental Neuroscience, IRCCS Fondazione Stella Maris in Pisa; the Neonatal Intensive Care Unit of University Hospital "Santa Chiara" in Pisa, the Neonatal Intensive Care Unit, Department of Perinatal Medicine of University Children's Hospital "A. Meyer" and the Division of Neonatology, Careggi University Hospital, in Florence.

There will be two investigative arms: CT-R and IM training. Both training programs will last 8-week.

All the infants will be clinically assessed at T0 (baseline, in the week preceding CT-R training/IM) and at T1 (i.e. in the week after the end of the 8-week programme of CT-R training/IM). T1 will be the primary endpoint. Then, all infants will be followed at T2 (i.e. 8 weeks after the end of intervention period) and at T3 (i.e. at 18 months of age). Figure 1 shows the detailed timeline.

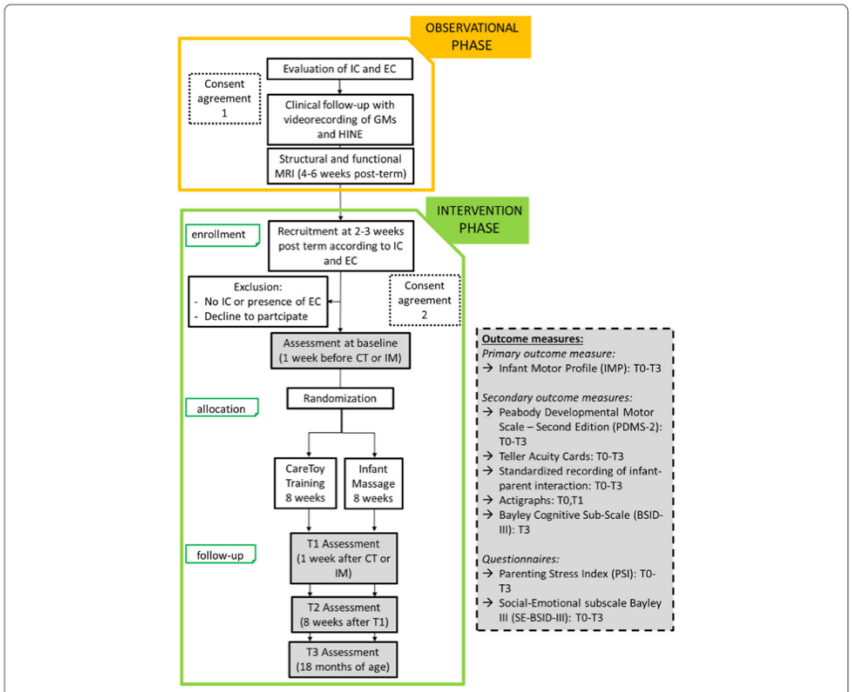

Fig. 1 Study design

This study will be structured in two parts: an observational phase and an intervention one.

The observational phase will be aimed to early detect the brain-lesioned infants at high risk for CP through standardization of traditional clinical procedures, based on the most updated existing criteria. The following intervention phase will be aimed at verifying and comparing, with a RCT, the efficacy of two EI models (CareToy-Revised and Infant Massage) in promoting neurodevelopment of high-risk infants.

Observational phase

The observational phase will consist of a standardized follow-up for monitoring development of infants through ultrasound evidence of brain injury verified at the two Neonatology Units involved in this project. Monitoring will also include neurological assessment (HINE) and recording of spontaneous activity according to Prechtl (GMA) [25, 26].

These examinations will be performed at around term period and also 2–3 months after post-term age, in the Neonatology Unit designated for regular clinical follow-up examinations.

Moreover, as clinical recommended for high-risk infants, a brain MRI during spontaneous sleep will be performed within 6 weeks post-term. MRI protocol foreseen by this project includes structural images, already present in clinical protocols adopted by the involved clinics and necessary for definitive inclusion in the second intervention phase, and a brief period of functional MRI acquisition [27–29]. It will include two further series of images (GRE-EPI, TR / TE = 3000/50, FA = 90 °, FOV = 240 × 240 mm, matrix = 96 × 96, thickness = 3 mm), for a total duration of 4 minutes, according to a block diagram, with an alternation of visual stimulus presentation suitable for assessing visual system integrity that is used in movement perception (MT area of the parietal cortex). More specifically, the passive stimulus consists of image presentations, through appropriate non-magnetic glasses, for MRI that simulates coherent or incoherent movement.

Intervention phase

In the interventional phase, infants will proceed with an early intervention, which could be CareToy-R or Infant Massage, according to allocation group. Each intervention will last 8 weeks, 5 days a week; during this experimental phase all infants will continue to receive standard care foreseen by National Health System. All other specific interventions (such as physical therapy or other treatments) will be recorded in a specific diary. Therapy protocols are described below.

Study sample and recruitment

Participants will be recruited at Tuscany Neonatology Units in Pisa (Santa Chiara University Hospital) and in Florence (Meyer and Careggi Hospitals). The neonatological staff will assess eligibility of infants on the basis of

inclusion and exclusion criteria prior to hospital discharge and staff will inform parents about study. If a family expresses interested in participating in the study, they will receive an introductory letter and an informative flyer. Recruitment must take place within the first year of age of the infant and, because of the characteristics of the interventional setting and exercises, before the complete acquisition of the trunk control in sitting position. If parents are willing to participate, they will be asked to sign a first agreement for admission to the observation phase and another one to partake in the intervention phase. As indicated above, Tuscan Region Paediatric Ethics Committee approved the clinical trial. Moreover, CT-R, as the previous CT, has been classified as a medical device without an EC mark, so an approval of a new clinical trial was requested and obtained by the Italian Ministry of Health.

Inclusion criteria will be the following:

- Abnormal or specific neurological signs at neurological examination of GMA or HINE, associated with one of the following conditions
- Persistent brain ultrasound periventricular hyper-sonority
- Evidence of cerebral haemorrhage
- Evidence of Periventricular Leukomalacia (PVL)
- Cerebral stroke
- Moderate or severe asphyxia

Exclusion Criteria:

- Polymalformative syndrome or cerebral malformations
- Severe retinopathy (III-IV degree)
- Severe sensory disturbances (deafness or blindness)

Moreover, to be enrolled in the intervention phase, additional inclusion criteria will be:

- Persistence of abnormal Spontaneous GMs evaluation according to Prechtl at 2–3 months post-term (absent or abnormal FMs)
- Persistence of abnormal/specific signs according to HINE

Finally, the inclusion criterion for the start of intervention phase (CareToy-R or Infant Massage) is based on motor requirements defined on the basis of Ages & Stages Questionnaire scores. In general, skills required to start EI range from initial head control to complete trunk control in sitting position. In other words, infants who have no initial head control or have acquired the sitting position cannot be admitted to the intervention phase. In the first case, it is necessary to wait until the infant can control the head within the first year of age, in the second case they will not allocated to the intervention phase.

To monitor motor progress, families will receive the section on "gross motor skills evaluation" of Ages & Stages Questionnaire (ASQ-3; gross motor [30]); as soon as the child reaches some pre-established motor skills (see below). Treatment can then start according to assignment group.

- score ≥ 10 from 3 to 4 months
- score ≥ 5 < 50 from 5 to 6 months
- score ≥ 10 < 30 from 7 to 8 months

The exclusion criterion is represented by a worsening of general clinical situation for intercurrent medical conditions or an onset of epileptic seizures.

Sample size

Based on the chosen primary outcome measure, Infant Motor Profile (IMP [31],), and on IMP results of a pilot study and of previously conducted RCT in preterm infants without congenital brain lesions [15, 19, 20] and considering a 80% power and a significant level of 0.05 and a effect size of at least 0.6, a sample of 38 children is required. However, considering also a 10% of drop-out rate, a minimum of 42 infants will be required. In order to facilitate parental work and participation in the project, eligible twins will be allocated into the same group.

Randomisation

Randomization will be done after enrolment: participants will be randomly assigned to CT-R training or IM group by the use of an automatic generation of 1:1 sets. A third party blind to clinical aspects of trial will manage these sets, sealing them in numbered envelopes.

Blinding

Parents, therapists and clinical staff will be aware about the allocation group of infants. Assessors who will evaluate infants with outcome measures (IMP; Peabody Developmental Motor Scales - Second Edition (PDMS-2); Bayley Scales of Infant Development III Edition, BSID-III) will use the video-recording of the assessment session so they will be absolutely blind.

Therapy protocols

CareToy-R training

As in a previous study [20], the clinical staff, mainly composed by child neurologists and paediatric physical therapist will organize customized goal-directed rehabilitative activities (i.e. CareToy scenarios) to be done in three different possible position which could be sitting, supine or prone, mainly focused on: postural abilities (e.g. postural control, rolling...), manipulation

capabilities (e.g. reaching, grasping, manipulation…) and visual functions (e.g. visual attention and orientation…).

The new CT system, i.e. the revised version (CT-R), has been planned and designed in order to adapt the CareToy system to this new population by integrating a support for posture maintenance. Based on postural needs of brain-lesioned infants, a modular system called Siedo & Gioco (Fumagalli, Italy) has been incorporated. Siedo & Gioco system is a soft multifunctional system which allows the infant or a small child to be placed in many different confortable and safe positions (e.g. supine, prone, sitting or on one side). The Siedo & Gioco system is composed a set of soft and coloured modules with different shapes which can be attached to a mat by Velcro straps placed on modules and on mat. Technical specification for optimize suitability of Siedo & Gioco to CT system have been provided and an ad-hoc set of modules has been created in order to offer postural and perceptive stimuli. Thanks to the flexibility of this integrated system, the modularity of CT platform in this revised version (CT-R) has been maintained.

According to the initially established activities and modules (the activities and consequently the modules will be periodically updated), the CT-R system will be customized and delivered to the family's home. CT-R training will be structured in 8 weeks, in which there will be about 30–45 min of daily planned activities, consisting of different scenarios lasting 2–10 min each.

Parents will be instructed to carry out the daily training during the wake and active periods of their infants. It will be remotely supervised by the clinical staff, who will have the possibility to remotely manage the CT scenarios based on each infant rehabilitation requirement and improvement during the 8-weeks training. Each scenario will be scored at the end by the parents on the basis of a questionnaire recording the infant's acceptance and compliance.

Rehabilitation staff will train parents to use the system and to interact with their infant during the first 5 days of training and once a week in the following weeks.

Infant massage

Infant massage consists generally of a systematic touching by hands on different parts of the body and is characterized by slowness and gentleness [22]. IM is often associated to other forms of "contact" with the infant, e.g. kinaesthetic (arm and leg passive extension/flexion), auditory, verbal or visual stimuli.

Families assigned to IM group will perform a training course of 4–5 sessions lasting about one hour each. The massage will be done on different body areas and illustrated material on how to perform massage sessions at home will be provided to families. Parents will be required to perform IM 5 days a week, depending on the child's willingness, for 8 weeks, and to record massage frequency in a dedicated diary.

Outcome measures

As previously reported, infants will be assessed according to the timeline (Fig. 1). The primary outcome measure, assessing the motor development, will be the IMP. The selected secondary outcomes will be the: PDMS-2, BSID-III Cognitive subscale, standardized video-recordings of parent-infant interaction, Teller Acuity Cards® and Actigraphic analysis (Motionlogger Microwatch). Due to multiple outcomes and variable willingness of infants, each evaluation time would be completed, if necessary, in two successive days to guarantee greater compliance.

Primary outcome measure

Infant motor profile (IMP)

IMP is a video-recorded motor evaluation of the infant placed in different positions (supine, prone, sitting, standing while grasping and manipulating objects). It is composed at first by observation of spontaneous activity and then by stimulation of motor abilities and manipulation of objects in different position. It consists of 80 items, subdivided in five motor domains, which allow for a calculation of a total IMP score. IMP does not only evaluate motor performance in quantitative terms, but also provides qualitative assessments, such as movement variability and fluency and adaptability of motor strategies.

It is suitable for infant born at term and preterm from 3 months of age to until the child has acquired good autonomous skills (which is approximately 18 months).
Previous studies with CT have had the IMP as the primary outcome measure. It will be carried out at all assessments as shown in Fig.1.

Secondary outcome measures
According to secondary goals, the following measures have been selected.

Motor assessment

Peabody developmental motor scales - second edition (PDMS-2)

It evaluates fine and gross motor movements from birth to 5 years. The scale is made up of 6 subtests (reflex, sitting, walking, manipulation of objects, grasping and visual-motor integration), whose results are combined into 3 global motor performance quotients: Gross Motor, Fine Motor and Global Motor [32]. It will be performed at all time-points (T0, T1, T2 and T3).

Cognitive assessment

BSID-III-cognitive subscale

It is a standardized scale for the cognitive development assessment in 1–42 months old children [33]. It will be performed at T0 and T3.

Visual function assessment

Teller acuity cards®

It is a test widely used in young infants and non-collaborative subjects to estimate visual acuity. The trained assessor proposes a series of black and white stripes cards with different widths on one side and neutral stimulus (grey background) on the other side to the infants, judging his/her visual attention. It evaluates the skill to look at a visual target. The estimated visual acuity is the thinnest width of stripes that the child is able to fix and prefer to the neutral grey area. The test is based on the "preferential looking" concept; response indicators are on a spontaneous behaviour basis, i.e. head positioning and eye directing to stimulus. The test is highly reliable, versatile, and it can be executed in a few minutes [34, 35]. It has been used in scientific studies for evaluating the results of training on visual development [36]. It will be performed at T0, T1, T2 and T3. A further complementary visual assessment will be carried out by means of an eye-tracker (CareToy C) [37] that allows a measurement of fixation and pursuit.

Parent-infant relationship assessment

Standardized video-recordings of parent-infant interaction

A video of a free play interaction of about 3–5 min between parent and child will be carried out. Videos will be classified by expert certified raters according to Child – Adult Relationship Experimental Index (CARE – Index) [38] and/or Emotional Availability (EA) Scales [39], blinded to allocation group. They will be carried out at T0, T1, T2 and T3.

Study of organization and maturity of sleeping

Movement recording with Actigraphs

Actigraphs is the simplest and least invasive measurement tool to evaluate sleep which allows for protracted monitoring (from days to months) [40]. It uses accelerometric sensors similar to a wristwatch and it is generally worn on the non-dominant side (hand or wrist). Motionlogger Microwatch will be used and combined with Sadeh algorithm for sleep evaluation. Clinical data such as total sleep time (TST), waking after sleep onset (WASO), and sleep efficiency (SE) will be calculated during the training. It will be worn on an infant's ankle for 1 week at T0 and T1.

Questionnaires

BSID-III social-emotional scale

This is a subscale of the Bayley III. It is a screening tool for early identification of social-emotional deficits in subjects from birth to 42 months. It is a questionnaire to be completed by parents and it allows an evaluation of child emotional-functionality, communicative needs, interactive relationship and use of interactive emotions to social problem-solving [41]. This scale will be done by the parents at T0, T1, T2 and T3.

Parenting stress index (PSI)

This questionnaire, containing 36 items, is divided into 3 subscales and requires about 5–10 min to fill out. It detects defined features of parents, of their children and of their environment that are frequently related to parenting stress. It is a tool, widely used for early detection of parent-child dysfunction in their relationships [42]. It will be filled in by the parents at T0, T1, T2 and T3.

Analyses

Statistical Package for Social Sciences (SPSS) will be adopted for carrying out the statistical analyses. Descriptive statistics will be performed to create the record of the data for each group. Potential baseline differences and between group differences will be explored computing the p values. When necessary, Bonferroni correction will be applied. Firstly, delta changes immediately after treatment (T1, primary endpoint) vs baseline will be calculated to assess the short-term effects of CT-R training versus IM in primary and secondary outcome measures, taking account as covariates base level of motor development, type and grading of brain lesion, family compliance and dose of intervention. After, multivariate statistics will be performed.

DISCUSSION

This paper shows rationale and protocol for an RCT aimed at evaluating the efficacy of a new EI tool, called CT-R, respect to IM in brain-damaged infants. It is built on several studies carried out over the last years [15, 17–20, 43] in infants without brain lesions. According to our experience, there are some limitations in performing high-quality home-based EI that could be different from family to family because home environments are various and difficult to control. On the other hand, the advantages for families are that they do not have to go to a clinic for every intervention session and they can personally learn how to stimulate their infant in their own home. In our proposal, the abovementioned disadvantages can be overcome thanks to the option of remotely managing home-training from the clinic, in this way parent behaviour can be controlled and EI will have greater access.

CT-R system is a completely new technological tool for delivering at home personalized EI in infants. The precise study protocol, that follows the CONSORT guideline [44, 45] and the comparison with another EI, will permit to scientifically assess the effects of CT-R intervention on neurodevelopment in a sample of brain-lesioned infants.

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CHAPTER 8

Home-based early intervention with CareToy- R promotes motor development in infants with early brain injury: results from a randomized control trial

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INTRODUCTION

Cerebral Palsy (CP) is the most common motor disability in childhood.[1] Recent population-based studies have reported a prevalence of 1 to 4 per 1,000 live births with a possible higher prevalence in low- to middle-income countries.[2] CP results after a brain injury occurring during the perinatal period. In this phase, the central nervous system is particularly vulnerable to insults and, at the same time, it shows the highest neuroplastic potential for recovery.[3] For this reason, it is important to develop evidence-based rehabilitation protocols that take advantage of this window of opportunity to maximize functional outcomes and minimize complications. Recently published guidelines based on comprehensive reviews have set the cornerstones of the Early Intervention (EI) in infants at high risk of CP.[4,5] To be effective, an EI should be early, intensive, goal-directed and based on the principles of the enriched environment.[5,6] Moreover, the intervention should be multi-axial (focused on different aspects of motor, cognitive, and visual development) and family-centred.

The CareToy system (CT) is a modular and biotechnological telerehabilitation tool that embeds all the aforementioned features providing a home-based, parent-delivered and intensive EI for infants with neurodevelopmental risk. It is composed of a baby gym with mechatronic toys, a module for the promotion of visual attention and gaze, and a sensorized mat. Technical specifications and functioning have been previously described.[7–9] A training with CT can be remotely monitored and updated by rehabilitation professionals according to specific developmental needs, while parents actively play with their child.

A previous RCT study has shown that a 4-week training with CT can significantly improve visual and motor development in preterm infants without brain injury and thus considered at low risk of CP.[10,11] Based on these promising findings, the former version of CT has been updated and adapted to meet the needs of high-risk infants (CareToy-Revised or CT-R): a new postural modular system has been added, new goal-directed activities have been introduced and the video library has been updated with highly contrasted videos for the infants with visual impairment. Acceptability, usability and feasibility of the

CT-R training have been recently established in high-risk infants, paving the way for the use of this new medical device in this unique population.[12]

The primary objective of this RCT was to determine the efficacy of CT-R on the neurodevelopment of infants with perinatal brain injury, thus at high risk of CP. To ensure that every participant had the opportunity to receive an EI, we decided to compare the effect of CT-R to another widely known EI, namely the Infant Massage (IM).

METHOD

Participants and recruitment

Infants were recruited from 3 participating Neonatal Intensive Care Units (NICUs) in Tuscany, Italy: the NICUs of Santa Chiara University Hospital (Pisa, Italy), Meyer Children Hospital (Florence, Italy), and Careggi University Hospital (Florence, Italy).

To obtain the earliest and most accurate selection of infants at high risk of CP, a pre-eligibility evaluation was conducted before discharge from the Neonatal Intensive Care Units (NICUs), based on the presence of perinatal brain injury and abnormal or specific neurological signs at the neurological examination. Infants were then re-assessed for eligibility to the intervention phase at around 3 months post-term age, during a neurodevelopmental follow-up visit. Infants were then included in the intervention phase in case of: absent or abnormal fidgety movements according to the Prechtl General Movements Assessment (GMA) or presence of abnormal/specific signs according to the Hammersmith Infant Neurological Examination (HINE).[13,14] Infants were excluded if they had polymalformative syndromes of cerebral malformations or severe sensory impairments (retinopathy of prematurity stage III-IV-V or deafness). Once the eligibility was established, parental informed consent for participation in the study was obtained.

The study received ethical approval from the Tuscan Region Pediatric Ethics Committee (no. 84/2017) and it was registered on Clinical Trials.gov (NCT03234959). Comprehensive details of the study protocol and methodology have been published elsewhere.[15]

Procedures

Design

All the participants received a baseline assessment one week before the intervention (T0) and a final assessment one week after the completion of the intervention (T1). Then, follow-up visits were performed 8 weeks after the end of the intervention (T2) and at 18 months of age (T3). Each assessment was video recorded so that it could be scored offline by a blinded assessor. During the experimental procedure, any participation to other forms of intervention as required by the local health care providers was continued and recorded in a specific diary.

Randomization

As soon as each infant fulfilled the minimum motor requirements (initial head control as scored by the Ages & Stages Questionnaire),[16] a random allocation to the CT-R or the IM arm was made using an automated generation of 1:1 sets. No a priori stratification was used in terms of age, prematurity, or type of brain injury.

Intervention protocols

Each participant was allocated to receive an intensive (5 days a week) training either with CT-R or with IM with a total duration of 8 weeks.

For each participant in the CT-R group, a CT-R system was delivered at home. After the installation of the device, a dedicated therapist trained parents to use the system and interact with their infant with CT-R during the first 5 days and then once a week for the following weeks with home visits or remote video calls. CT-R daily training was structured to last around 30-45 min and was remotely supervised by clinical professionals who could manage and update the training based on the need of each infant. Further details on the CT-R trainings have been published elsewhere.[12,15,17,18]

For the infants allocated to the IM group, an experienced physical therapist provided the families with a practical course of 5 sessions of 1 hour each. During the home sessions, she instructed the parents to properly stimulate and interact with their infant according

to the sequence of movements of the IM.[19] Then, parents were asked to perform the IM at least 20 minutes a day for a minimum of 5 days a week. Information about the duration of the massage and the sequences of movements were recorded by parents on a dedicated sheet.

Primary and secondary outcome measures

The primary outcome was motor development at T1 as measured by the Infant Motor Profile (IMP), a video-based assessment of motor behaviour which is designed to evaluate motor abilities, variation, fluency and symmetry of the infant movement repertoire.[20] The IMP has been validated as a discriminative measure in infants with neurodevelopmental risk and previous studies demonstrated solid construct validity and predictive validity for CP and other neurodevelopmental disorders.[21–23] Results are expressed in raw scores. As required by the IMP protocol, the IMP Adaptability domain was not assessed in infants of less than 6 months.[20,24]

Secondary outcome measures included motor functions measured by Peabody Developmental Motor Scale 2 (PDMS-2),[25] cognitive development assessed with the cognitive scale of the Bayley Scale of Infant Development-III (BSID-III),[26] and visual acuity measured with Teller Acuity Cards (TAC).[27] IMP, PDMS-2 and TAC were assessed at every time point (T0, T1, T2, T3) while PSDI-III was administered only at T0 and T3.

All the clinical assessments were performed at the infants' home and administered by dedicated therapists, and by a child neurologist. The scoring of the assessments was performed offline at the end of the study by a single physical therapist blinded to the study group.

Covariate

The severity of brain injury is a well-known predictor of responsiveness to intervention. For this reason, brain MRI scans were independently assessed by a pediatric neurologist to develop a severity score based on the best available literature and consistent with previous similar trials.[28–31] In the case of multiple available scans, we decided to choose the series closest to term equivalent age (TEA) for preterm infants and the series closest

to day 7 for infants with hypoxic-ischemic encephalopathy. MRI severity score was coded as: 1 = unlikely to have CP; 2 = likely to have ambulant CP (e.g. focal vascular insults); and 3 = likely to have non-ambulant CP (e.g. basal ganglia/thalamus lesions or diffuse brain injury).

Sample size calculation

Sample size calculation was based on estimates obtained from a pilot study and from previously conducted RCTs including preterm infants without perinatal brain injury.[11,18,32] Considering an 80% power, a significant level of 0.05 and an effect size of at least 0.6, a minimum sample of 38 infants was required. To facilitate parental work and participation in the project, eligible twins were allocated into the same group.

Statistical analysis

Analysis was carried out using SPSS and reported according to the CONSORT statement.[33] Descriptive statistics (frequencies, means and 95% CI) were used to describe the study sample at T0. Normality of distribution was checked using the Shapiro-Wilk's test. To test baseline differences between the two groups for characteristics (gestational age, sex, age at enrollment and neuroimaging score) and baseline measures (IMP, PDMS-2, TAC and BSID-III) the t-test for unrelated samples and the non-parametric Mann-Whitney U test were used for normal and non-normal distributed data, respectively.

To compare the effect of the two interventions over time while controlling for the possible confounding variables, we performed a one-way ANCOVA analysis for repeated measures including the baseline raw scores and the neuroimaging severity score as covariates. The partial eta squared was considered as a measure of the effect size.[34] Finally, we analyzed the relationship between total therapy dose and motor outcomes on the primary measure with a linear regression analysis.

RESULTS

Participant flow

Between September 2017 and June 2020, 98 infants were screened for eligibility before discharge from the NICUs. 59 were then excluded for not meeting the inclusion criteria (57) or because families declined to participate (2). A total of 39 infants from 37 families were recruited and randomized to CT-R (n=20) or IM (n=19). One set of twins was randomized to IM. Adherence to study protocol was excellent with only one participant to the CT-R arm dropping out of the intervention after 11 days for not being able to dedicate sufficient time to the training. All the other participants completed the intervention phase and the follow-up home visits. Patient flow is shown in the CONSORT flow diagram (Fig. 1).

The baseline characteristics of the study sample are reported in Table 1 including the drop-out participant. Groups were equivalent at baseline for all infant characteristics except for a minor difference in age of enrollment where the IM group started the intervention at a slightly and non-significant earlier age than the CT-R group (p=.12). Also, the fluency domain scores of the IMP were slightly higher in the IM group than in the CT-R group. Table I describes the characteristics of the study sample.

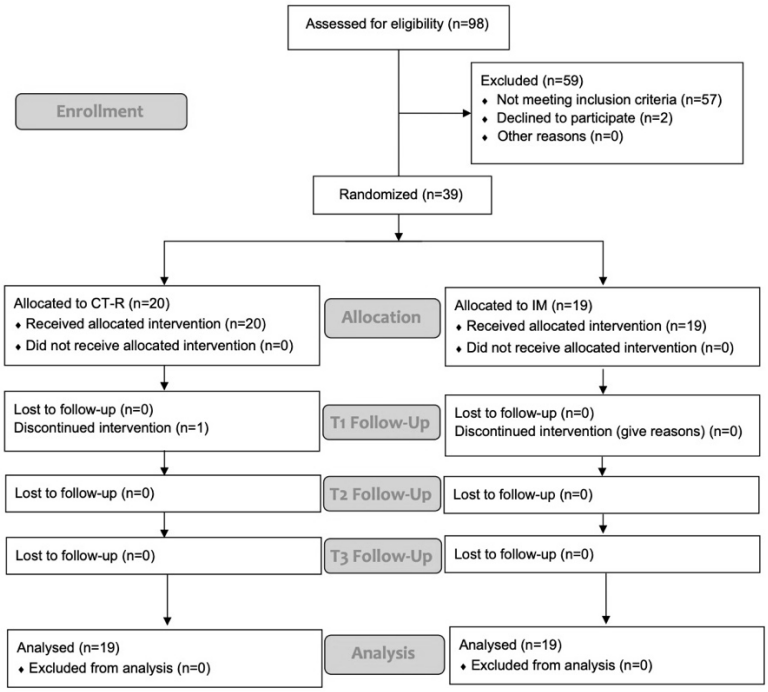

Figure 1 Flow of the participants through the trial

		CareToy n = 20	Infant Massage n = 19
AGE AT ENROLLMENT	Corrected age (months) at T0, mean (SD)	5.93 (1.99)	4.86 (1.27)
SEX	Male, n (%)	12 (60)	10 (53)
GESTATIONAL AGE	Birth gestational age (weeks), mean (SD)	35.00 (5.84)	32.37 (5.26)
	28 weeks, n (%)	4 (20)	4 (21)
	28-31 weeks, n (%)	1 (5)	6 (32)
	32-36 weeks, n (%)	4 (20)	3 (16)
	>36 weeks, n (%)	11 (55)	6 (32)
NEUROIMAGING AT TEA	Severity score		
	1 - Mild, n (%)	6 (30)	5 (26)
	2 - Moderate, n (%)	6 (30)	6 (32)
	3 - Severe, n (%)	8 (40)	8 (42)
	Unilateral, n (%)	12 (60)	7 (37)
	Bilateral, n (%)	8 (40)	12 (63)

CLINICAL MEASURES				
IMP total score	Total raw score (SD)	66.1 (6.4)	65.9 (6.0)	
IMP variation	Total raw score (SD)	63.7 (7.0)	62.3 (6.8)	
IMP symmetry	Total raw score (SD)	82.0 (13.1)	87.4 (12.8)	
IMP fluency	Total raw score (SD)	70.6 (8.0)	75.4 (1.9)	*
IMP performance	Total raw score (SD)	48.1 (8.1)	47.5 (8.3)	
PDMS total raw score	Total raw score (SD)	70.4 (25.1)	61.8 (22.6)	
TAC	Cycles/degree (SD)	4.5 (2.6)	3.0 (1.8)	
Bayley-III (cognitive)	Composite cognitive score (SD)	84.0 (18.3)	84.2 (18.9)	

Table 1 Characteristics of participants at baseline (T0). * $p < .05$

Data normality

Baseline and follow-up scores were normally distributed for the IMP (total and subscales), PDMS-2 and BSID-III assessments and therefore we used parametric statistics. The total dose of therapy was also normally distributed. TAC scores, gestational age and age at follow-up were all abnormally distributed and therefore we used non-parametric statistics.

Clinical outcomes

Primary and secondary outcomes for the CT-R and IM groups are reported in Tab. 2

Timepoints	**Measure**	**Group**		**Effect size (Partial Eta Squared)**	**p-value**
		CareToy (n=19) Mean (SD)	Infant Massage (n=19) Mean (SD)		
T1	IMP total score	73.54 (7.55)	71.11 (6.35)	**.277**	**.001**
	IMP variation	73.25 (11.10)	68.47 (7.52)	**.149**	**.02**
	IMP symmetry	86.79 (12.82)	83.11 (9.70)	.048	.20

	IMP fluency	75.22 (4.50)	75.70 (2.94)	.002	.39
	IMP performance	59.25 (9.49)	57.64 (10.26)	.040	.25
	PDMS-2 total raw score	101.63 (29.96)	98.00 (33.58)	.019	.42
	TAC	7.89 (3.53)	5.95 (2.98)	.018	.43
T2	IMP total score	76.15 (8.84)	72.24 (8.14)	**.156**	**.02**
	IMP variation	78.60 (10.47)	73.56 (9.62)	.076	.10
	IMP symmetry	88.35 (12.70)	86.60 (8.83)	.002	.82
	IMP fluency	77.41 (5.61)	76.97 (4.68)	.028	.32
	IMP performance	67.95 (12.27)	66.06 (14.79)	.004	.71
	PDMS-2 total raw score	130.95 (37.39)	120.58 (41.72)	.000	.98
	TAC	9.80 (3.93)	8.51 (3.06)	.000	.90
T3	IMP total score	82.63 (10.92)	84.53 (10.21)	.040	.24
	IMP variation	79.25 (10.67)	82.63 (11.23)	**.144**	**.02**
	IMP symmetry	89.18 (14.00)	91.05 (9.78)	.047	.21
	IMP fluency	80.22 (11.98)	81.71 (11.37)	.000	.98
	IMP performance	82.00 (18.85)	83.51 (14.99)	.017	.44
	PDMS-2 total raw score	192.79 (67.16)	191.16 (66.30)	.019	.42
	TAC	11.73 (2.87)	11.55 (2.91)	.016	.46
	BSID-III composite cognitive score	85.71 (20.65)	82.64 (19.61)	.016	.51

Primary outcome measure (T1)

After controlling for the baseline measures (IMP score at T0, gestational age and neuroimaging severity score), we found that IMP total score was significantly higher in the CT-R group after the conclusion of the intervention phase (T1). A significant difference in favour of CT-R was also found in the IMP variation domain. The effect of CT-R intervention was large [F (1, 33) = 12.64; sig 0.001] with a partial eta squared of .28 where any effect over 0.14 is considered large.[34]

Primary outcome measures (T2, T3)

A significant difference between the two groups was still evident 8 weeks after the conclusion of the intervention (T2) with higher IMP total scores in the CT-R group. At the follow-up evaluation (T3) performed at 18 months, no difference was found between the two groups for the primary outcome measure while higher scores in the Fluency domain of the IMP were found in the IM group.

Secondary outcome measure (T1, T2, T3)

At the timepoints of T1, T2 and T3, no statistically significant between-group differences were found at the PDMS-2, TAC or BSDI-III assessments.

Child diagnostic outcome

At 18 months (T3) n=20 participants (53%) received a diagnosis of CP, 10 of which (50%) had received a training with CT-R. Three additional children presented minor neurological dysfunctions (i.e. abnormal tone, abnormal reflexes, or mild asymmetries in motor behaviour). 5 subjects showed other signs of abnormal neurodevelopment (3 presented a cognitive delay, 2 a social communication impairment).

Dose of intervention

In both groups, high adherence to treatment was found with no significant difference in the total dose of intervention. For the CT-R group, infants received a mean of 22.40 (SD 5.15) hours of total intervention with a mean duration of daily activities of 30.30 (SD 4.42)

minutes. Regarding the IM group, participants received 21.04 (SD 8.49) hours of total treatment with a mean daily duration of 27.79 (DS 7.88) minutes. Details about family adherence to both treatments have been previously published.[12,35] No significant correlations were found between motor outcome at T1 and total dose of intervention (R = - 0.07).

DISCUSSION

In this clinical trial, we compared the effect of two EI approaches demonstrating that, in the medium term, an early and intensive training with CT-R significantly improves motor outcome when compared with a similar dose of IM. The effect of the CT-R intervention on motor function was strong when assessed 4 weeks after the conclusion of the training and was still evident 2 months after the conclusion of the intervention.

Consistently with previously published data, the effect of CT-R was mainly reflected by the IMP scores (primary outcome measure) and especially by the variation domain. This finding was not unexpected since CT-R was developed with the stated aim of enriching the variation of the infant motor repertoire offering widely diverse motor activities with different degrees of complexity. Variation of the infant motor behaviour is increasingly considered as a faithful expression of the complexity of brain connectivity and a solid predictor of poor motor performance in infants with brain injury.[36,37] On the other side, no significant difference was found at the PDMS-2, an assessment that is more focused on gross and fine motor achievements.

Previous studies have reported a significant effect of both IM and CT in improving the visual acuity of premature infants without brain injury.[11,38] In our study, visual acuity improved throughout the study with no significant differences between the two groups. At 18 months, both groups showed normal visual acuity for age confirming that the two interventions have a comparable effect over visual functions.[39] Since the presence of retinopathy of the premature was considered an exclusion criterium for the study, it is possible that we selected those infants whose visual capacity was already better preserved. Also, in infants with cerebral visual impairment due to early brain injury, acuity is only one of the many aspects of vision that can be affected.[40] The mere assessment of

visual acuity with TAC may have prevented us from detecting more subtle and complex impairments of visual function.

Previous data demonstrated a small positive effect of EI on cognitive outcome.[41] In our study, cognitive measures at 18 months were comparable between the two groups. This is probably explained by the relatively limited duration of the intervention period (8 weeks) which may have prevented a significant improvement of cognitive performances. Future studies will try to elucidate whether a longer duration of the intervention could lead to an improvement in cognitive skills.

The current study adds to a growing list of home-based EI programs based on the concept of the enriched environment.[29,42–44] In general, these programs are based on a parent-delivered intervention that is periodically supervised and updated by professionals. These EI are hardly standardizable since frequency, dose of the intervention and compliance of caregivers are not easily measurable. Unlike most of these studies, our intervention with CT-R was entirely performed at home and it was remotely monitored and updated by rehabilitation professionals based on the quantitative measures provided by the system.

Part of this study (February 2020 – June 2020) was conducted during the global COVID-19 pandemic. During the sanitary emergency, access to non-essential rehabilitation services was severely limited with most of the outpatient rehabilitative activities suspended throughout Europe.[45] Lock-down and social distancing measures have greatly narrowed the possibility of delivering physical rehabilitation with a negative impact especially on infants and children with special needs.[46] The use of a telerehabilitation platform as CT-R has allowed the participants to carry out the scheduled activities, without affecting the quality of the intervention and maintaining close contact with the rehabilitation professionals who telemonitored the program.

In our study, the great majority of the participants had a moderate to severe brain injury suggesting that our cohort may be more severely affected than a representative CP population sample.[47] This can be explained by the fact that one of the three participating NICUs (Meyer Children Hospital, Florence, Italy) serve as a referral centre for neonatal neurologic disorders admitting the most critical patients from all over the region. This may have affected the representativeness of the study sample. Pleasingly, we found that

a positive effect of the CT-R intervention was present in all the classes of brain injury severity with greater effect size in those with milder brain injury (partial eta squared = .47) than in those with severe brain injury (partial eta squared = .28). This finding is of particular interest since it suggests that the flexibility and modularity of the system make CT-R a suitable intervention also for those infants with the most severe forms of CP, for whom traditional forms of intervention struggle to provide significant results.

As a final limitation of the study, the video-scoring of the primary outcome measure (IMP) was performed offline by a single physical therapist. A re-assessment of the scores by an experienced blinded therapist is currently undergoing. Final data will provide more information about the inter-rater reliability of the IMP.

CONCLUSION

Our study suggests that an 8-week early intervention program with CT-R has a positive effect on the early development of motor function in infants with brain injury at high risk of CP. The effect was evident in infants with different degrees of severity of the brain injury confirming that CT-R can be a promising and effective early intervention in infants at risk. Further research is required to establish whether a longer intervention with CT-R can lead to long-lasting effects.

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CHAPTER 9

General discussion and future perspectives

Riccardo Rizzi

More than one in ten infants are considered at risk of neurodevelopmental disorders (NDDs), a group of disorders with lifelong consequences and a significant burden for families and society. Allowing prompt participation in early therapeutic interventions is critical to influence long-term outcome.[1,2] Two main challenges remain open: the identification of infants who would most benefit from an early intervention and the development of feasible and effective options for the intervention.[2,3]

The overarching aim of this project was to explore novel frontiers in the early detection and intervention in infants at risk of NDDs.

Growing evidence suggests that the earliest and most accurate prediction of NDDs can be achieved by the integration of clinical assessments with instrumental data. Among the available clinical assessments, the General Movements Assessment (GMA) is considered the strongest and most accurate predictor of outcome.[2,4] However, after 5 months post-term age GMA cannot be performed and therefore there is a need for other standardized and scalable tools which can help the clinicians to correctly predict the outcome and monitor development trajectories. In Chapter 2 we evaluated the predictive ability of a novel qualitative assessment called the Infant Motor Profile (IMP). IMP is a clinical tool based on the assessment of qualitative features of motor behaviour such as variation, fluency, and symmetry of movements. We demonstrated that the IMP at 5 months of age is strongly associated with neurodevelopmental outcome at 18 months (sensitivity 93%, specificity 81%) and that the IMP scores accurately reflect the severity of the perinatal brain injury. We also found that IMP correlates with GMA, making the IMP a valuable alternative for the early assessments of infants at risk when GMA is not available. These findings are of particular interest since they demonstrate for the first time that there is a clear

relationship between early motor development assessed with IMP and neurodevelopmental outcome, paving the way for wider use of this tool in clinical practice and experimental clinical trials.

The contribution of neurophysiology to the early detection of NDDs is barely explored. The recent diffusion of computational signal analysis and biomedical engineering applied to neurophysiology have opened new research lines in the field of developmental neurology. On this ground, we evaluated whether computational analysis of signals derived from electroencephalographic recordings (EEG) and functional infrared spectroscopy (fNIRS) could provide new potential biomarkers of NDDs suggesting a potential prognostic role of these two techniques in the early assessment of infants at risk.

In Chapter 3 we applied an automated analysis to sleep-EEG of infants between 2 and 5 months to look for potential biomarkers of risk of cerebral palsy after perinatal brain injury. We followed the hypothesis that a pathologic reorganization after focal brain injury could determine focal changes in brain activity during sleep. Studies on adults have revealed a potential role of sleep in the synaptic plasticity underlying recovery from stroke. Given the high interindividual stability of sleep structure, any changes in sleep structure could be looked at as a potential biomarker for abnormal outcome.[5] We developed an automatic algorithm for sleep spindles detection and quantified local spindle activity. Then, we developed an interhemispheric spindle power asymmetry (SPA) index and evaluated the correlation with neuromotor outcome. Our data confirmed that the magnitude of interhemispheric asymmetry in sleep spindles can indeed be a reliable non-invasive early biomarker of unilateral cerebral palsy. This finding provides a proof of concept for the future implementation of the SPA index in the early assessment and prognosis of infants at risk of NDDs.

To further elucidate the relation between neurophysiological measures and NDDs, in Chapter 4 we investigated the ability of fNIRS to detect signs of atypical brain functioning. Recent data suggest that fNIRS can be useful to explore both task-triggered activation and functional connectivity in subjects with NDDs, ultimately detecting specific patterns of atypical brain activity.[6,7] In our pilot work, we developed an innovative visual stimulation paradigm with a highly entertaining and ecological value which ensured its application also to non-compliant children. Thanks to this protocol, we found a significant correlation between the variability of the regional hemodynamic responses (HDR) in the occipital cortex and the presence of autistic traits in children. These novel findings can set the background for testing the value of fNIRS in the broad population of infants with NDDs, potentially providing new diagnostic biomarkers of atypical development.

The second main aim of this project was to explore new frontiers in the field of early intervention programs in infants with early brain injury, thus at high risk of NDDs. The topic of early intervention is, indeed, receiving increasing attention since growing evidence suggests that an early and intensive treatment, based on the concepts of the enriched environment, can improve the functional outcome in infants at risk.[8–10] In the last years, several therapeutic programs have been proposed, trying to comply with the international guidelines which recommend active, intensive, personalized and family-centred treatments in every infant who is considered at risk of NDDs.[11–14] In the attempt to meet these requirements while, at the same time, trying to provide a feasible and effective treatment, the CareToy system have been developed.[15–19] CareToy represents a breakthrough in the field of early intervention programs since it ensures an intensive and personalized rehabilitation program that takes place entirely at home with the active involvement of the family. To validate the use of

CareToy as an early intervention in infants at high neurodevelopmental risk, we developed a multicentre randomized clinical trial involving three Neonatal Intensive Care Units of Tuscany (Chapter 7). In the trial, 39 infants with perinatal brain injury were randomized to receive an early and intensive intervention with either the Infant Massage, a well-known early intervention which is traditionally applied to promote neurodevelopment in typically developing infants, or the CareToy. Before assessing the efficacy of the intervention, we wanted to investigate the rate of acceptability, usability, and feasibility of an intervention with CareToy since an effective but not feasible intervention could be hardly translated into clinical practice. The data presented in Chapter 6 are acquired from the training sessions and from the questionnaires that were administered to the caregivers. Results from the study documented a high rate of compliance to the program with an intervention that was globally perceived as useful and empowering by the participating families. We also assessed the feasibility of the Infant Massage, to determine whether this intervention, which is usually applied to preterm at low risk of cerebral palsy, could be also feasible in a home environment in infants with perinatal brain injury. Results reported in Chapter 5 show a high degree of acceptability and feasibility of this intervention also for this particularly fragile population.

As regards the efficacy of the intervention programs, in Chapter 8 we have presented the first results from the RCT study where we found that infants who received an 8-week intervention with CareToy showed a bigger improvement in motor function than those who received the intervention with the Infant Massage. The superiority of the effect was still evident 8 weeks after the conclusion of the intervention program and was most evident when we looked at the qualitative aspects of the motor behaviour, suggesting that an intervention with CT can effectively expand the size and variation of the infant motor repertoire, a pivotal feature for the

rehabilitation of infants at risk of neuromotor disabilities.[20] Moreover, we found that the benefit brought by CareToy was independent from the severity of the perinatal brain injury. This finding is of particular importance since it implies that even infants with severe impairments can take advantage of CT training, while most of the available interventions have struggled to prove solid effects in infants with severe brain injuries.

As a whole, this project confirms that newly available clinical and neurophysiological methodologies can substantially contribute to the process of early detection of infants at risk of atypical development. The contribution of telerehabilitation and technological solutions for the early intervention represents a feasible and effective option to maximize the neuroplastic potential and reduce the potential disabilities.

FINAL CONSIDERATIONS: LIMITATIONS AND IMPLICATIONS FOR FUTURE STUDIES

When considering the implementation of a possible diagnostic biomarker, a pivotal point is testing its predictivity in large cohorts and prospective clinical trials. This is particularly challenging when it comes to the population of newborns and infants with serious medical conditions. One of the aims of our work was to provide preliminary evidence of new potential measures of risk of neurodevelopmental disorders. To do so, we first needed to assess the reliability of those measures in relatively low-dimension cohorts. This might have undermined the immediate application of our findings into clinical practice. Also, the heterogeneity of the different approaches could be considered an added limit for their direct translation into clinical use.

Future studies should assess prospectively and in larger cohorts the validity of these measures of risk we presented, possibly combining different clinical and instrumental methodologies to achieve an even higher prediction power.

Despite the general interest of this project towards NDDs as a whole, in most of the studies we have mainly focused on cerebral palsy as the primary diagnostic outcome. NDDs are indeed a broad group of disorders that often share a common causative disruption of brain development and common genetic susceptibility.[21] Nevertheless, the single condition (e.g., cerebral palsy, autism spectrum disorder, intellectual disability) may variably interest motor, perceptual, cognitive or communication functions for which diverse biomarkers and treatments may be appropriate. Considering NDDs as a comprehensive diagnostic entity could be useful to maintain a global and integrated perspective toward children with different and complex needs; on the other hand, it could reduce the specificity of the diagnostic biomarkers. Future studies should try to determine the reliability of the proposed measures of risk for different neurodevelopmental conditions.

In Chapter 4 we investigated the correlation between fNIRS measurements and autistic traits in a population of healthy children suggesting that the variability of hemodynamic response to visual stimuli could be a possible biomarker of atypical brain development. Albeit preliminary and not directly translatable into clinical practice, our data represent a first and promising step towards the use of fNIRS in this population, establishing a model for future implementation of this tool in cohorts of children with a clinical diagnosis of autism spectrum disorders or other NDDs.

The CareToy clinical trial has proven that an intervention model based on telerehabilitation and ICT can be feasible and effective for the promotion of neurodevelopment in early infancy. The CareToy system has demonstrated high flexibility, meeting the needs of infants with different degrees of

severity. Despite that, some limitations related to the structure of the CareToy should be noted. Indeed, the baby-gym may be appropriate for the first stages of infant development when trunk control has not been consolidated. At this age, CareToy training may be useful to improve head movements, pivoting, reaching, grasping and eye-hand coordination. When the infants start to sit unaided and develop interest in the surrounding environment, such a confining space may be limiting. In our clinical trial, we chose to deliver the intervention as soon as the infant showed an initial head control and before the trunk control was consolidated. However, at the end of the 8-week training, many infants reached full trunk control making it difficult to deliver the same amount of daily treatment. Future studies should build upon this experience to expand the CareToy platform, developing smart and sensorized toys which could offer a wide range of rehabilitative experiences for the different phases of psychomotor development. As an additional observation, a detailed cost analysis of the CareToy intervention should be made to determine whether this therapeutic intervention could indeed contribute to a reduction of healthcare costs for the treatment of infants at risk.

Besides these practical considerations, the application of ICT to the world of early intervention looks even more promising in this time of global pandemic emergency, when the limitations imposed by the sanitary authorities have greatly jeopardized the possibility of delivering adequate interventions to infants in need. Future studies and large-scale implementation of these innovative tools will help to determine the real impact of these technologies in this critical population.

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CHAPTER 10

List of Publications

LIST OF PUBLICATIONS

Sgandurra G, Beani E, Giampietri M, **Rizzi R**, Cioni G and the CareToy-R Consortium.
Early intervention at home in infants with congenital brain lesion with CareToy revised: A RCT protocol.
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Beani E, Menici V, Cecchi A, Cioni ML, Giampietri M, **Rizzi R**, Sgandurra G, Cioni G and the CareToy-R Consortium.
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Mazziotti R, Scaffei E, Conti E, Marchi V, **Rizzi R**, Cioni G, Battini R, Baroncelli L.
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Front Pediatr. 2021;9:1–13.

Marchi V, **Rizzi R**, Nevalainen P, Melani F, Lori S, Vanhatalo S, Guzzetta A. Asymmetry in sleep spindles predicts motor outcome in infants with unilateral brain injury
Dev Med Child Neurol. 2022;00:1– 8.

Rizzi R, Sgandurra G, Cioni G et al.
Home-based early intervention with CareToy promotes motor development in infants with early brain injury: results from a randomized control trial
In preparation