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MONITORING MOTOR DEVELOPMENT TRAJECTORIES IN PRETERM INFANTS: CORRELATION WITH NEONATAL CHARACTERISTICS, CLINICAL AND NEUROCOGNITIVE OUTCOMES

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“Quante mani che sogni ti porgono
(Ora è meglio di prima)
Quante mani per prendere schiacciano
(Chiudi gli occhi e cammina)
Questa ruota è una giostra o una macina
Non sentirne le grida
Mi ricordo di quando ero piccolo
Ora è meglio di prima

E se mi fermo un momento
E guardo il cielo passare
Aprendo le mani
E nel vento la cenere scompare
E se fossimo rami
Invece di essere pietre
Che sanno solo giacere
O farsi spostare
Mi ricordo di quando ero piccolo
(E se fossimo rami)”

- LFA

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Abstract

Despite significant advances in neonatology that have increased the survival rates of Very Low Birth Weight (VLBW) and Very Low Gestational Age (VLGA) infants, the risk of long-term Neurodevelopmental Impairment (NDI) remains high. This PhD thesis aimed to improve early risk stratification and prediction models for NDI by integrating insights from functional neurological assessment and acute systemic comorbidity markers. The research was structured into two interconnected studies. In Part A, we prospectively studied 98 VLBW/VLGA infants, focusing on early, non-invasive neurological assessments. We found that the General Movements (GMs) assessment, which analyses spontaneous movement patterns, was a highly effective early warning system. Infants exhibiting anomalous GMs trajectories were up to 16 times more likely to experience motor delay as early as 6 months Corrected Age (CA). Furthermore, the observed correlation across motor, language, and cognitive domains supports the developmental cascade hypothesis, suggesting that mastering early motor skills is a key catalyst for broader cognitive and linguistic advancement. In Part B, we shifted focus to acute systemic instability, using early Acute Kidney Injury (AKI), defined according to serum creatine or urinary output criteria (UO), as an exemplar in a retrospective cohort of 339 VLBW/VLGA infants. The study identified Extremely Low Birth Weight (ELBW), non-steroidal anti-inflammatory drugs (NSAIDs) use, and inotropes as significant risk factors for early AKI. Crucially, while AKI UO was associated with poorer short-term clinical outcomes (longer hospitalization, lower growth Z-scores), its link to long-term NDI at 12-24 months CA disappeared after adjusting for critical neonatal comorbidities (periventricular leukomalacia, late onset sepsis, bronchopulmonary dysplasia). This suggests that AKI may functions as a marker of overall severe systemic vulnerability.

Our finding validates GM as a crucial, independent predictor of early motor development, providing opportunity to guide parental counselling and to optimise integrated rehabilitation programs aimed at promoting cognitive and motor neuronal plasticity. While we affirm the utility of GM, continuous refinement of systemic vulnerability markers is also necessary. Future research must therefore focus on refining

AKI definitions to optimize its detection, particularly through the adaptation of UO thresholds for preterm infants. More accurate AKI detection methods incorporating refined UO criteria may ultimately enhance the marker's discriminative performance for predicting long-term outcomes.

List of abbreviations

AAP: American Academy of Pediatrics
ADHD: Attention-Deficit/Hyperactivity Disorder
AF: abnormal Fidgety
AREDF: absent or reverse end-diastolic flow in the umbilical artery
AKI: acute kidney injury
BPD: bronchopulmonary dysplasia
BSID-III: Bayley Scales of Infant and Toddler Development, Third Edition
BW: birth weight
CA: corrected age
Ch: chaotic
CS: cramped synchronized
DV: ductus venosus
EEG: electroencephalogram
ELBW: extremely low birth weight
F-: Fidgety absent
FMs: Fidgety movements
GA: gestational age
GMA: general movements assessment
GMs: general movements
GMDS-R: Revised Griffiths Mental Development Scale
GMFCS: Gross Motor Function Classification System
GQ: General Development Quotient of infants' abilities
HC: head circumference
HINE: Hammersmith Infant Neurological Examination
HNE: Hammersmith Neurological Examination
HNNE: Hammersmith Neonatal Neurological Examination
IQR: interquartile range
IUGR: Intrauterine Growth Restriction
IVH: intra-ventricular haemorrhage
KDIGO: Kidney Disease Improving Global Outcomes
LOS: late onset sepsis
MCA: mean cerebral artery
MOM: mother's own milk
MRI: magnetic resonance imaging
NDI: neurodevelopmental impairment
NEC: necrotizing enterocolitis
NICHD: National Institute of Child Health and Human Development
NICU: Neonatal Intensive Care Unit
NSAIDs: non-steroidal anti-inflammatory drugs
PDA: patent ductus arteriosus
PMA: post menstrual age
PR: poor repertoire
PROM: prolonged rupture of membranes
PVL: periventricular leukomalacia
ROP: retinopathy of prematurity
SCr: serum creatinine
SD: standard deviation
SGA: small for gestational age
SIN: Società Italina di Neonatologia
TEA: term equivalent age
UO: urinary output
VLBW: very low birth weight
VLGA: very low gestational age
VON: Vermont Oxford Network
Zs: Z-score

1. INTRODUCTION

1.1. The Persistent Challenge of Preterm Outcomes

Prematurity (birth before 37 weeks of gestation) remains a critical global health concern, standing as a leading cause of death and disability in children under five. Globally, over 10% of all births occur prematurely, with approximately 15 million babies affected annually. Most preterm infants (around 84%) are born between 32 and 36 weeks gestational age (GA). Around 10% are delivered between 28 to 32 weeks, and approximately 5% of all births occur before 28 weeks GA. There is significant variation in the incidence of preterm birth worldwide, with the highest rates found in low and lower-middle-income countries (around 11%) and the lowest rates found in the high-income countries (6-9%)[1]. In Italy, according to the national birth register, CeDAP 2023, 6.3% of all pregnancies resulted in a preterm birth. Of these, infants born before 32 weeks GA and/or weighing less than 1500 g constitute 0.9% of all live births (around 3800 infants), while those born before 28 weeks GA represent 0.3% (around 1300 infants) [2].

The evaluation of short, medium, and long-term outcomes for preterm infants is essential, and it is based on robust epidemiological studies of large cohorts of infant born before 32 weeks GA (e.g., the French EPIPAGE-2 study) [3–5], analyses of extremely low gestational age births (e.g., the British EPICURE and the US National Institute of Child Health and Human Development [NICHD] cohorts) [6,7], meta-analyses, and international registries like the Vermont Oxford Network (VON) [8,9]. Data from the EPIPAGE-2 cohort (2205 infants born in France 2011 with 22-24 weeks GA) demonstrate significant progress in short-term survival: rates for the 22-26 weeks GA group increased from 44.1% to 51%, and for the 27-31 weeks group, from 87.7% to 94.3% compared to the EPIPAGE -1 1997 cohort. Furthermore, survival without severe morbidity at discharge also improved notably, from 15.4% to 26.3% for the 22-26 weeks GA group [3]. These improvements are largely attributed to reduced incidence of complications like necrotizing enterocolitis (NEC) and healthcare-associated infections, though the frequency of conditions such as bronchopulmonary dysplasia (BPD) remains stable [10]. Despite this progress, adverse outcomes—inversely related to the degree of

prematurity—persist, particularly for the most critical population born at less than 25 weeks GA, where improvement in short-term outcomes has been less substantial [11]. This increased survival, especially among the most fragile infants, necessitates a rigorous assessment of medium- and long-term neurodevelopmental outcomes. Cumulative follow-up data from EPIPAGE-2 at 2 and 5.5 years reveal a wide spectrum of neurodevelopmental risks, including motor, sensory, cognitive, language, and behavioural issues (Figure 1) [4,5]. Crucially, while survival rates have risen, the rates of major neurodevelopmental disabilities have remained relatively stable across different eras. For instance, the NICHD cohort (2930 infants <27 weeks GA born 2013-2016) reported a combined frequency of 50.5% for moderate and severe neurodevelopmental deficits at 22-26 months corrected age [7]. This stability in severe disability rates suggests that improved survival has extended to a higher-risk population, underscoring the enduring biological vulnerability of the preterm infant. Consequently, former preterm infants now represent a growing population of children and young adults with complex, chronic needs, demanding an escalating level of specialist care and intervention as they age.

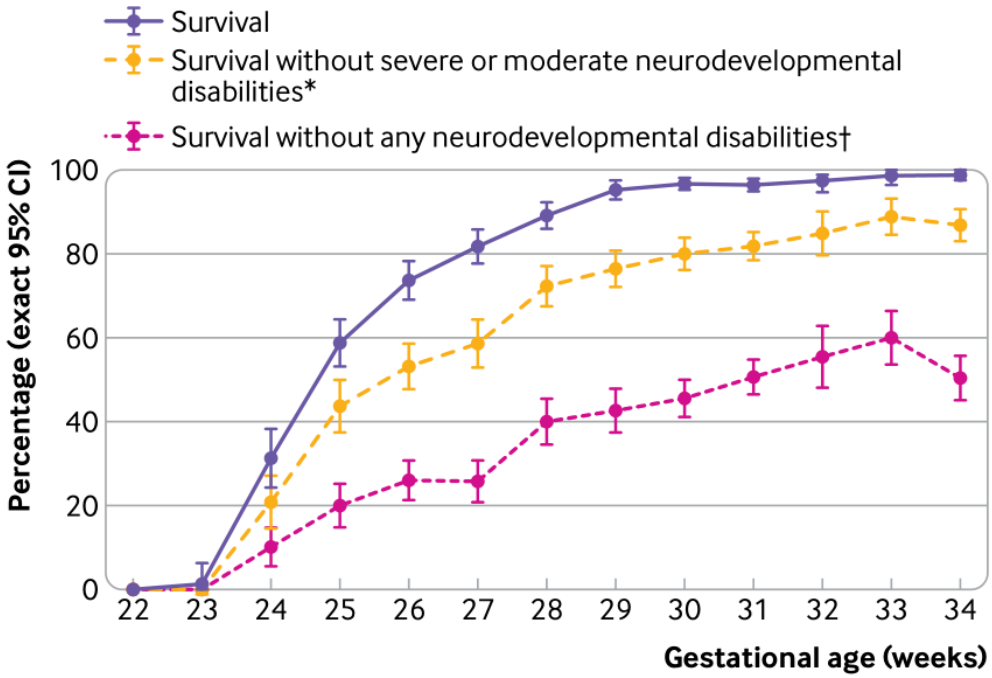

Figure 1. Survival at 5½ years, survival without severe/moderate neurodevelopmental disabilities, and survival without any neurodevelopmental disabilities by week of gestational age at birth, among preterm born children in the EPIPAGE-2 study.
Data corrected for study design and respondent selection. *severe or moderate cerebral palsy (gross Motor Function classification system level 2-5), vision (bilateral binocular visual acuity <3.2/10), hearing (unilateral-bilateral hearing loss ≥40 db not corrected or partially corrected with hearing aid), and full-scale intelligence quotient less than two standard deviations below the mean of the reference sample born at term. †includes no cerebral palsy, no vision or hearing disabilities, full scale intelligence quotient greater than or equal to one standard deviation below the mean, no developmental coordination disorder, and no behavioural difficulties. CI=confidence interval. *From Pierrat et al 2021* [5]

1.2. The Spectrum of Neurodevelopmental Impairment

The last few decades have witnessed a rapid evolution in scientific knowledge concerning child neuropsychiatric development. This progress has illuminated the intricate interplay between genetics, epigenetics, neurobiology, and environment. This foundational shift has led to significant transformations in nosography and in the models used for evaluation and intervention. Consequently, there has been a progressive unification of all neuropsychiatric conditions under the broader definition of "neurodevelopmental impairment" or "neurodevelopmental disorders" [12,13].

Preterm birth is inextricably linked to a broad and complex spectrum of neurodevelopmental impairment (NDI), the severity and frequency of which are inversely proportional to GA at birth. As established by international cohorts (e.g., the French EPIPAGE-2 study), a considerable percentage of preterm children—a proportion directly related to their lower GA at birth—still exhibit NDI at preschool age [5]. This spectrum of NDI ranges from severe disabilities like cerebral palsy to more minor, yet impactful, difficulties in behaviour, coordination, and emotional regulation [14].

Major NDI is typically defined early in life (0–3 years corrected age) and is often associated with structural cerebral lesions. These conditions represent the most severe end of the spectrum and require intensive, early intervention:

- Moderate to Severe Cerebral Palsy: Defined by a Gross Motor Function Classification System (GMFCS) score of ≥2.

- Severe Cognitive Delay: Typically, an intellectual score less than two standard deviations below the mean of the reference on standardized developmental scales (e.g., Bayley-III).

- Severe Sensory Deficits: Including bilateral hearing loss (unresponsive to aids) or severe visual impairment.

The NICHD cohort, covering children born at <27 weeks GA, reported that at a 22–26 month corrected age follow-up, 21.2% had severe NDI and 29.3% had moderate NDI [7]. This underscores the magnitude of severe impairment in the extremely preterm population.

Equally critical, yet often manifesting later (school age and beyond, 4-10 years), is the high prevalence of mild NDI or subtle functional deficits [14,15]. These issues can significantly impact quality of life, academic achievement, and social integration:

- Motor and Coordination Disorders: Such as Developmental Coordination Disorder, often termed 'clumsiness' or 'maladroit,' involving issues with gross and fine motor skills, coordination, and organization of movement.

- Cognitive and Learning Difficulties: Including deficits in executive functions (working memory, planning, organization), selective and sustained attention, and specific learning disabilities (e.g., in visual-spatial or perceptual organization).

- Behavioural and Psychological Disorders: Preterm survivors face a significantly higher risk (two to fourfold) of psychopathologies such as Attention-Deficit/Hyperactivity Disorder (ADHD), Autism Spectrum Disorder, and internalizing disorders like anxiety and depression. Some authors have even proposed a “behavioural phenotype of preterm infants” characterized by inattention, introversion, anxiety, rigidity, and a lower tendency for risk-taking [16].

While the prevalence of moderate/severe NDI is estimated at 15–25% for extremely preterm infants, the frequency of milder neurocognitive deficits is much higher, sometimes reaching 50–60%, as shown in Figure 2 [14].

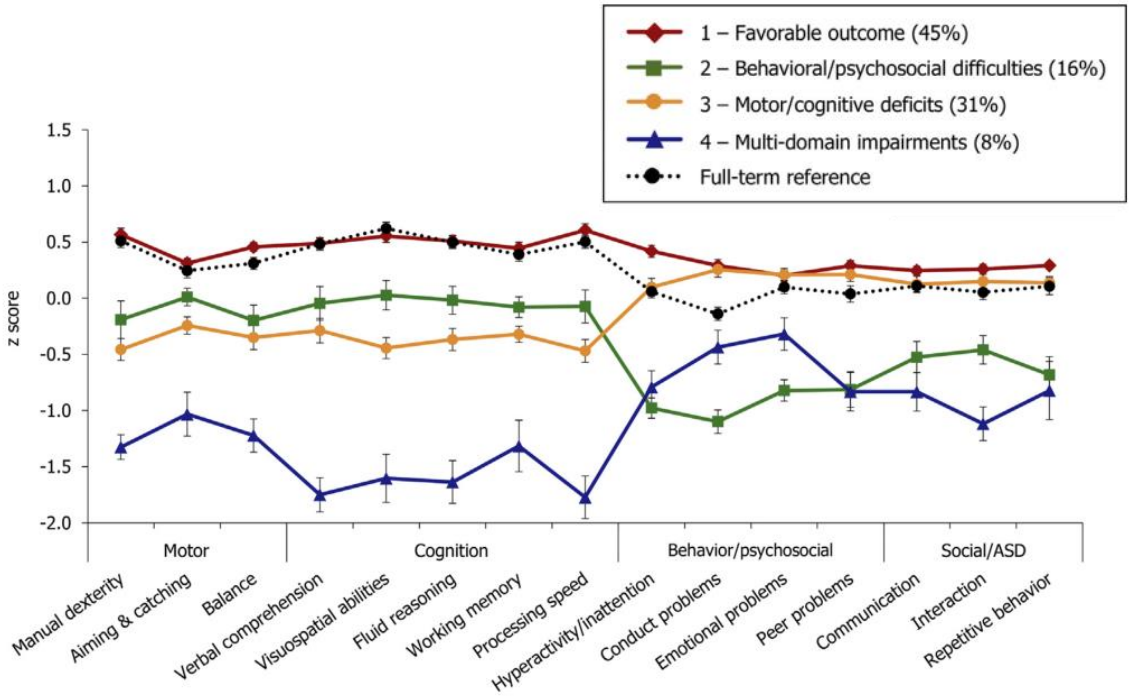

Figure 2. Profiles of Functioning in 5.5-Year-Old Very Preterm Born Children in France (EPIPAGE-2).

Two groups of profiles can be distinguished: 1 profile describing favourable outcomes across domains (45% of children) and 3 profiles describing suboptimal functioning (55% of children).The first is called the "favourable outcomes" profile, because functioning is highly similar to the full-term sample in all domains (within 0.5 SD), with significantly less symptoms of hyperactivity/ inattention, conduct, and social problems. From the profiles describing suboptimal functioning, the "behavioural/psychosocial difficulties" profile was found in 16% of the children and characterized mainly by mild behavioural and psychosocial difficulties (1 SD below full-term children). In contrast, 31% showed low-average to mildly impaired motor and cognitive functioning (1 SD below full-term children) in the absence of behavioural and psychosocial difficulties ("motor/cognitive deficits" profile). Finally, a relatively small percentage of children (8%) showed a profile with mild to moderate difficulties in all domains (1 to >2 SD below full-term children), except conduct and emotional problems. This profile is called "multi-domain impairments." Note: Functioning of full-term children is plotted as a reference (dotted line). Scores for both the very preterm and full-term sample were standardized according to the very preterm sample (i.e., z=0 corresponds to the weighted mean of the very preterm sample) and scaled so that a lower z score reflects poorer performance or an increase in difficulties for all indicators. *From Twilhaar et al 2022* [14]

The challenge lies in the fact that these deficits only become apparent as the child is expected to acquire complex skills, necessitating long-term monitoring.

1.3. The Complex Landscape of Neurodevelopmental Assessment

The assessment of the neuro-motor, sensory, cognitive, and behavioural development in the preterm infant is a task of increasing complexity. The close relationship between

nutritional, respiratory, and neurodevelopmental comorbidities in the severely premature child often makes it challenging to discern which deficits are purely neurological and which result from the interplay and interaction of other systemic factors. Furthermore, defining the long-term prognosis, the evolution of clinical signs, and the pathways of resilience for both the child and their parents is not always straightforward, particularly in infants born at the lowest gestational ages. This intricacy mandates a rigorous and standardized approach to post-discharge care. Major international follow-up networks, including the American Academy of Pediatrics (AAP), the NICHD, and the VON, all strongly advocate for a global evaluation of neurodevelopment, termed the "Comprehensive Neurodevelopmental Assessment." The Comprehensive Neurodevelopmental Assessment is an essential, multidisciplinary approach designed to provide a holistic view of the child's functional status [17]. This evaluation extends beyond a standard physical check-up to ensure that all domains of potential impairment are thoroughly examined. Specifically, this assessment is structured to include: standard neurological examination, to identify clinical signs of central nervous system dysfunction; evaluation of motor function assessing both gross and fine motor skills, which are critical early indicators of potential difficulties like cerebral palsy or developmental coordination disorder; cognitive and language development using formal testing to detect delays in intellectual function and communication skills; behavioural assessment, evaluating socio-emotional regulation, attention, and executive functions; parental questionnaires utilizing structured questionnaires to gather crucial developmental and behavioural information from the parents' perspective; instrumental examinations, Including neuroimaging (such as magnetic resonance imaging [MRI] and electroencephalogram [EEG]) and biochemical/epigenetic investigations where indicated, to supplement clinical findings.

Given the importance of long-term outcomes, this comprehensive follow-up should ideally be prolonged at least until the onset of school age (6 years) to accurately identify both the major disabilities, which are often detected early, and the more subtle minor deficits that frequently emerge when children are challenged by the academic and social demands of school.

1.4. The Dual Imperatives: Follow-up and Early Risk Stratification

The compelling evidence of long-term risk has necessitated the establishment of two critical, interconnected paradigms that delineate the future trajectory of both post-discharge clinical surveillance for the preterm infant and the contiguous research domain: establishing long term multidisciplinary neurodevelopmental follow-up and identifying early clinical and neonatal predictors.

Due to the complexity of the outcomes, a comprehensive neurodevelopmental assessment is mandated by major international networks (AAP, NICHD, VON). This requires a coordinated, multidisciplinary team comprising neonatologists or paediatricians, neuropsychiatrists, physiotherapists, and psychologists. The follow-up pathway must be long-term. While conventionally extending to 24–36 months corrected age (CA), the latest guidelines from the Italian Neonatal Society (SIN) and the literature strongly recommend an ideal duration of at least 6 years (preschool age), and potentially longer, to reliably capture all minor disabilities [17]. The primary goal is the early detection of potential impairment to facilitate precocious and customized intervention, maximizing the benefit of brain plasticity and environmental factors. Furthermore, the systematic collection of data through these follow-up programs is essential for ongoing research and defining the evolving needs of this population, which is one of the research priorities identified by the ReCAP (Research on Children and Adults born Preterm) European project [18].

A crucial step in improving long-term outcomes is empowering neonatologists and follow-up clinicians to identify early indicators of neurodevelopmental risk during the neonatal period and the first months of life. This allows for targeted, preventive, or very early intervention. While GA remains a primary factor, several clinical variables are known to significantly modify neurodevelopmental risk and therefore warrant intense monitoring. One major independent risk factor is pulmonary morbidity, specifically BPD. Evidence from the EPIPAGE-2 cohort, for instance, demonstrates that infants with BPD experience a significantly higher frequency of below-threshold scores across all developmental domains (55.3% for affected vs. 39.5% for non-affected infants),

underscoring its profound developmental impact. Similarly, perinatal or placental compromise substantially elevates risk [19]. Conditions such as Intrauterine Growth Restriction (IUGR) before 32 weeks GA, particularly when complicated by absent or reverse end-diastolic flow (AREDF) in the umbilical artery, are particularly concerning, carrying an approximately 11 times greater risk of moderate/severe neuromotor and/or sensory disability at two years CA compared to infants with normal prenatal umbilical artery doppler ultrasound parameters [20]. Finally, the presence of Infections—including perinatal infections, sepsis, and NEC—are all well-established variables known to negatively impact neurodevelopmental trajectories [21,22].

2. PROJECT OUTLINE

The persistent challenge of preterm birth as a global health issue is underscored by the observation that, despite major advances in neonatology significantly improving survival rates, the improvement in medium-to long-term neurodevelopmental outcomes has been less pronounced. International cohort data consistently show a high percentage of preterm children, inversely related to their lower GA, still suffer from NDI, ranging from severe disabilities like cerebral palsy to more subtle yet impactful difficulties in behaviour, coordination, and emotional regulation.

This persistent neurodevelopmental risk and the challenge of timely intervention necessitate a dual approach: establishing multidisciplinary follow-up pathways for continuity of care, monitoring motor and neurocognitive development throughout early childhood, and empowering neonatologists with the ability to identify early clinical indicators of neurodevelopmental risk during the neonatal period and the first months of life.

Driven by this necessity, the core objective of this PhD thesis is to improve risk stratification and prediction models for NDI in Very Low Birth Weight (VLBW)/Very Low Gestational Age (VLGA) infants. The project is thus structured around two distinct yet interconnected studies:

Part A investigates the utility of functional and neurological assessments, specifically monitoring motor development trajectories through the analysis of General Movements (GMs), to establish non-invasive, early indicators of neurodevelopmental risk (as early as six months CA);

Part B assesses the impact of systemic predictors by exploring how acute organ comorbidity markers and systemic instability - exemplified by markers such as Acute Kidney Injury (AKI) - correlate with later neurocognitive and clinical outcomes.

The ultimate goal is to synthesize these functional and systemic findings to propose a more integrated and accurate model for risk stratification in VLBW/VLGA infants.

3. METHODS

3.1. Study design and ethics

The study employed a single centre ambispective cohort design, comprising a prospective component (Part A) and a retrospective component (Part B). All procedures adhered to the ethical principles outlined in the Declaration of Helsinki. The study protocol (study code: 76/2013/U/Sper/AOUBo; EM 194–2017; EM 193–2018; EM 1229–2020) received full approval from the Ethical Board of IRCCS S. Orsola Hospital, Bologna, Italy (CE AVEC). Prior to participation, written, informed consent was secured from the parents or legal guardians of all infants, covering both involvement in the study and the subsequent publication of the data. The study was conducted in collaboration with the Department of Psychology “Renzo Canestrari” of the University of Bologna.

3.2. Study Population and Inclusion Criteria

Infants meeting the criteria of either very low gestational age (VLGA, GA < 32 weeks) and/or very low birth weight (VLBW, birth weight [BW] < 1500 g) and admitted to the Neonatal Intensive Care Unit (NICU) of the IRCCS Azienda Ospedaliero-Universitaria of Bologna, were included in the study. The cohorts were defined by their admission periods at the study centre: the prospective cohort (Part A) included infants admitted between November 2020 and May 2023, and the retrospective cohort (Part B) included infants admitted to the same NICU between January 2013 and December 2017. Infants with primary kidney disease or major congenital malformations or genetic syndromes involving the kidney were excluded from the study. As per internal clinical protocol, all included preterm infants were subsequently enrolled in a developmental follow-up program following NICU discharge, involving the periodic assessment of clinical conditions, growth, and neurodevelopment.

3.3. Data Collection

Data on maternal health, pregnancy outcomes, delivery characteristics, and neonatal health were collected from medical records, according to the VON Database guidelines [23]. Specifically, the following variables were recorded: GA, type of delivery, sex, twin pregnancy, IUGR [24], small for gestational age (SGA) defined as BW <10th centile for

GA according to Italian INeS growth charts [25], Apgar score at 5 min, antenatal administration of steroids and magnesium sulphate, pH and base excess from umbilical artery, BW, length and head circumference (HC) at birth, major congenital malformations, maternal hypertension, chorioamnionitis, duration of hospitalization, postmenstrual age (PMA) at discharge, growth parameters at discharge, culture proven early (EOS) and late onset sepsis (LOS), Bell stage ≥ II NEC [26], patent ductus arteriosus (PDA) requiring any treatment, BPD, defined as persistent oxygen need at 28 days of life and graded according to respiratory support at 36 weeks PMA [27], retinopathy of prematurity (ROP) [28], intra-ventricular haemorrhage (IVH) [29], periventricular leukomalacia (PVL), respiratory distress syndrome [30], surfactant administration, need for mechanical ventilation. BW centiles for GA were calculated electronically using the Italian INeS growth charts. The Z-scores (Zs) for weight, length, and HC at discharge and during follow-up were assessed electronically according to the Intergrowth-21st longitudinal Charts for Post-Natal Growth of Preterm Infants [31]. Extra-uterine growth retardation was defined as a loss of Zs > 1 standard deviation (SD) in weight from birth to discharge.

3.4. Neurological examination and Motor Assessment

Standard neurological examination was performed following the Hammersmith Neurological Examination (HNE). The HNE is a simple, reproducible, cost-effective, validated, and scorable approach to assess newborns and infants from birth to 24 months. Two different versions are available: the Hammersmith Neonatal Neurological Examination (HNNE), designed for babies up to 1 month of age [32,33]; and the Hammersmith Infant Neurological Examination (HINE), used for infants between 2 and 24 months of age (or CA for preterm infants). In preterm infants, the HNNE can be administered from Term Equivalent Age (TEA) and throughout the first month post-term [34]. It comprises 34 items grouped in 6 domains evaluating tone, tone pattern, reflexes, movements, abnormal signs, orientation, and behaviour. Each item is given a raw score from one to five, and the sum of the individual scores determines a raw global score. It is possible to convert raw scores (range 1–5) to optimality scores (range 0–1) based on

the distribution of raw scores in a cohort of typically developing infants born at term. For the study purpose, raw global score and raw subsection score were used.

HINE comprises 26 items grouped into 5 domains that evaluate cranial nerves, posture, movements, tone, reflexes and reactions. Each item is given a score from zero to three, and the sum of the individual scores determines a global score that can range from a minimum of 0 to a maximum score of 78.

Early assessment of motor function was also performed through qualitative analysis of the GMs, which are spontaneous movements that represent the most frequent pattern of the motor repertoire observed in the foetus and neonate. GMs are part of a complex, spontaneous movement repertoire that begins around the 9th−10th week of GA and continues until approximately 20 weeks post-term, when voluntary and anti-gravity movements become predominant. Because GMs are believed to originate in a complex neural network, possibly a Central Pattern Generator located primarily in the brainstem, their quality is considered a direct reflection of central nervous system integrity and development [35,36].

Two distinct, age-specific GMs patterns are recognized from TEA onwards. Writhing Movements are observed from the foetal period (around 9th−10th weeks GA) until approximately 7−9 weeks after birth. The main characteristics of Writhing GMs include smoothness, fluency, variability, and complexity. They increase and decrease gradually in intensity, strength, and speed, have a gradual beginning and end, and rotate along the axis of the limbs and trunk. Slight changes in the direction of movement make them fluid and create an impression of complexity and variability. They can be further divided into foetal/preterm movements (present until term age) and proper writhing movements (from term until 7−9 weeks post-term). It is common for preterm infants to exhibit poorer quality and variability of spontaneous movements and higher rates of abnormal GMs at TEA.

Fidgety Movements (FMs) begin to appear from the 6th week PMA and reach their peak between 9th and 16th weeks PMA. FMs involve all body segments. They are small,

variable in amplitude and acceleration with moderate speed, and occur in different directions. These movements are consistently noticeable when the infant is awake but disappear when the baby is crying or during visual fixation. At around 3 months of CA, alongside the "fidgety" movements, other movements become apparent, primarily directed towards the midline (such as hand-to-hand, hand-to-mouth, and hand-to-face), preceding the onset of voluntary movements.

In the late 1980s, Prechtl and associates established a systematic, qualitative analysis of GMs known as the General Movement Assessment (GMA). To evaluate GMs quality, serial video-recorded observations are carried out using a "gestaltic perception technique" based on specific criteria. GMs are categorized as either "normal" or "abnormal" based on their quality.

Abnormal Writhing Movements (observed from the foetal period to 9 weeks PMA) are categorized into three patterns: Poor Repertoire (PR), characterized by monotonous sequences lacking complexity and variability. PR GMs are often a transient abnormality that normalizes over time, giving them limited predictive value. Cramped-Synchronized (CS) movements are described as rigid and stiff, lacking typical fluidity, with simultaneous contraction and relaxation of the trunk and limbs. CS GMs always indicate severe neurological dysfunction and are often associated with later development of cerebral palsy; the earlier their appearance, the more severe the functional cerebral palsy impairment tends to be [37]. Finally, Chaotic (Ch) movements are large-amplitude movements that lack fluency or smoothness.

Abnormal Fidgety Movements (observed from 6 to 20 weeks PMA) include two patterns: Absent Fidgety (F-), where normal FMs are never observed during the typical age window, and Abnormal Fidgety (AF), where the movements look like normal FMs but their amplitude, speed, and jerkiness are moderately or greatly exaggerated. Abnormal patterns in FMs (Absent or Abnormal) are widely recognized as having the highest predictive validity for later cerebral palsy [38].

3.5. Neurocognitive Outcome Measures

Intellectual functions, defined as the complex mental and psychic faculties that enable humans to reason, comprehend reality, and cope with new situations, are examined using standardized tests. These tests provide measurements expressed as Developmental Quotients during the first 36 months of life, and Intelligence Quotients after three years of age. Developmental quotients obtained at one and two years using standardized tools (such as the Griffiths Scales or Bayley Scales) allow clinicians to assess the evolutionary trajectory across different developmental areas, including motor, communicative, emotional, and performance skills [39].

The Bayley Scales of Infant and Toddler Development, Third Edition (BSID-III) serves as a widely utilized clinical instrument to provide a detailed description of a child's global development and to compare their performance against a standardized, age-matched peer sample [40]. The BSID-III is administered to children aged 1 to 42 months. It is comprised of five principal scales. Three of these scales—the Cognitive Scale, the Language Scale (assessing both receptive and expressive skills), and the Motor Scale (assessing fine and gross motor function)—are administered through direct interaction with the child and function independently of one another. The remaining two scales, the Socio-Emotional Scale and the Adaptive Behaviour Scale, utilize information supplied by the parents via a structured questionnaire.

The Revised Griffiths Mental Development Scales, 0–2 years (GMDS-R), are a valuable tool used to assess a child's developmental profile [41]. It requires approximately 45 min to administer and evaluates five domains: Locomotor sub-scale (54 items: gross motor skills including the ability to balance and to control and coordinate movements); Personal-Social sub-scale (58 items: proficiency in the activities of daily living, level of independence and, social competences); Hearing & Language sub-scale (56 items: auditory discrimination and communicative-linguistic comprehension and production); Eye & Hand Coordination sub-scale (54 items: fine motor skills, manual dexterity, and visual motor skills); and Performance sub-scale (54 items: cognitive functions for planning and completing intentional actions and representing objects mentally). The

GMDS-R provides a General Development Quotient of infants' abilities (GQ) and five sub-scale quotients.

3.6. Statistics

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 28.0 (IBM Corp., Armonk, NY). A p-value <0.05 was considered statistically significant for all analyses.

Data distribution was assessed using the Kolmogorov-Smirnov test. Since most variables did not follow a normal distribution, non-parametric tests were used throughout the study. Continuous data are presented as median (interquartile range [IQR]). Categorical data are presented as frequency and percentage.

The primary goal of Part A was to identify variables related to developmental delay in each BSID-III composite score (motor, cognitive, and language). Variables potentially related to developmental delay were first identified using the Mann-Whitney U test for continuous variables and the chi-square test for dichotomous variables. Potential correlations between variables were tested using the Spearman correlation test. Variables that were significantly different between groups (normal development vs. developmental delay in each BSID-III composite score) were used to build a separate logistic regression model for each neurodevelopmental outcome (motor, cognitive, and language).

The analysis in Part B focused on identifying factors associated with AKI and the subsequent impact of AKI and neonatal comorbidities on neurodevelopmental outcomes at 12 and 24 months CA. Prenatal and neonatal variables potentially associated with the development of AKI (according to each proposed definition) were investigated using the Mann-Whitney U test for continuous variables and the chi-square test for dichotomous variables. Potential collinearity among independent variables intended for regression models was checked using the Pearson correlation coefficient or the point-biserial correlation coefficient, as appropriate. Correlation was defined as "strong" when coefficients were above 0.6. Variables significantly different between

infants with and without AKI were included in one logistic regression model for each proposed AKI definition. Two different linear regression models were built to assess the role of AKI and other neonatal comorbidities on neurodevelopmental outcomes at 12- and 24-months CA, after confirming association through univariate analysis.

4. PART A: GENERAL MOVEMENTS TRAJECTORIES AS EARLY PREDICTORS OF MOTOR DEVELOPMENT AT SIX MONTHS CORRECTED AGE

4.1. Introduction

Early identification of VLBW/VLGA infants at high risk for NDI is crucial for a timely intervention which would potentially improve long-term outcomes [42]. However, an accurate prediction of NDI in this vulnerable population remains a significant challenge, due to NDI multifactorial nature, related to perinatal factors, neonatal characteristics and comorbidities, genetic susceptibility, and environmental influences [31,43,44]. Research has investigated the utility of spontaneous movements assessment and comprehensive neurological and behavioural examination in the first weeks and months of life, using tools such as GMA and HNE, and suggesting that abnormal patterns of GMs alone or in combination with HNE and brain MRI might serve as reliable indicators of later motor and cognitive impairment in VLBW/VLGA infants [37,38,45–48]. However, conclusive identification of readily available and non-invasive markers that can be assessed early in life, potentially even before the emergence of overt NDI, is still lacking. The aim of our study was thus to evaluate whether antenatal, perinatal, postnatal clinical variables, and/or early tools for infant motor assessment (GMA, HNE) would predict impaired motor, cognitive, and/or language development, assessed by the BSID-III, as early as six months CA.

4.2. Specific Methods

For the study purposes maternal educational level was classified as >13 or ≤13 year [49]. In addition, collected nutritional data included: day of enteral feeding beginning, age at full enteral feeding, and mothers' own milk (MOM) feeding at TEA.

As part of the follow-up program, GMA was performed at TEA and between 10 and 15 weeks CA (approximately 3 months CA). Infants were evaluated and video-recorded during the inter-feeding time, undressed, calm, in the supine position. For the purposes of the experimental study, GMA at TEA and during Fidgety period was performed by the same advanced certified GMA clinician (EZ) and by an experienced researcher according to the standard methodological principles of Prechtl's method [35]. For questionable

cases, videos were reassessed by a second advanced certified GMA clinician (VP). At TEA, writhing movements were defined as normal (N), or abnormal, the latter including PR, Ch, or CS movements. Fidgety movements were defined as normal and further classified into continuous (F++) or intermittent (F+), and pathologic, and further classified into absent (F-) or anomalous (AF). A variable representing the GMs trajectory over time (from TEA to 3 months CA) was built, defining a normal GMs trajectory (normal GMs at both evaluations), an improving GMs trajectory (PR, CS, CH pattern at TEA, and normal FM at 3 months CA), and a persistently pathologic GMs trajectory (PR, CS, or CH pattern at TEA, and pathologic FM at 3 months CA).The GMs optimality score was not used for the study purpose, prioritising operational efficiency and practical implementation [50].

For the study purposed, HNNE was performed at TEA, while HINE was performed at 3-, and 6-months CA by the same trained physician; the items were scored separately, and the global score was calculated.

Motor, Cognitive, and Language developmental domain were evaluated at 6 months CA using the Italian version of the BSID- III [51]. The BSID-III provides norm-referenced standardised composite motor, cognitive, and language scores, each with a mean of 100 and a SD of 15. BSID-III standardised composite scores for our cohort were calculated by referring to the normative values of the original North American standardisation as normative value for the Italian population were not available for the first year of life yet [40]. For the study purposes, delay in motor, language and cognitive function was defined using the cut-off value of <80 (−1.25 SD), as proposed in the study by Johnson et al [52]. Three trained neuropsychologists, blinded to neurological and GMs evaluation, conducted the BSID-III assessment. For each assessment always two of the three trained neuropsychologists were present, one assessing the child and the other observing her/him.

4.4. Results

A total of 98 preterm infants were included (Figure 3).

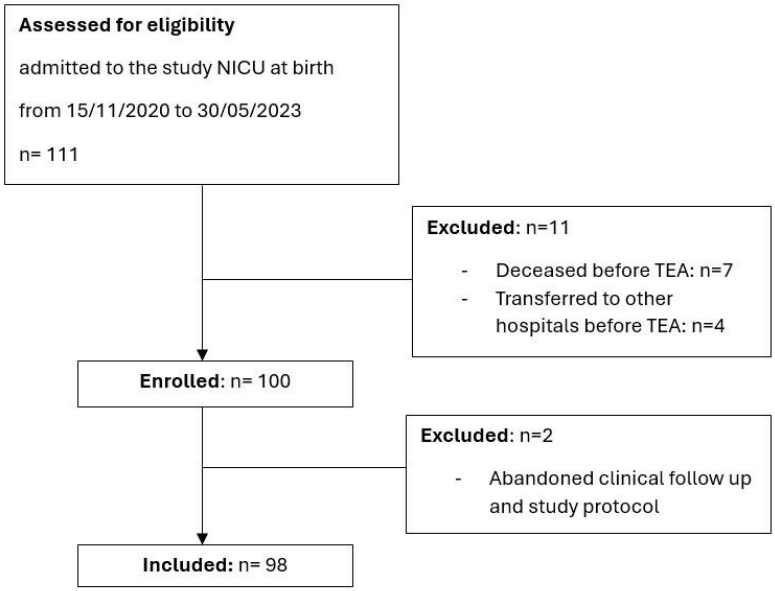

Figure 3. Flowchart of the study enrolment.

Demographic, antenatal, perinatal, and postnatal clinical characteristics of the study population are summarized in Table 1. Participants were evaluated at TEA (median [IQR]: 40.4 [40–41.6] weeks PMA), 3 months CA (median [IQR]: 13 [12–13] weeks CA), and 6 months CA (median [IQR]: 6 [5.9–6.1] months CA).

Table 1. Antenatal, perinatal, and postnatal clinical characteristics of the study population. Continuous variables are reported as median (IQR), and categorical variables as number [percentage].

Characteristics	**n = 98**
Antenatal characteristics	
Maternal education	
Educational level > 13 years	54 [60%]
Educational level ≤ 13 years	40 [41%]
Antenatal steroids prophylaxis	86 [88]
Magnesium sulphate prophylaxis	41 [42%]
Maternal hypertension	16 [16%]
Chorioamnionitis	3 [3%]
Perinatal characteristics	
Gestational age (weeks)	31.1 (27.4-31.3)
Birth weight (g)	1231 (937.8-1434)
Birth weight Z-score	-0.03 (-0.8; 0.5)
Birth weight less 1000 g	31 [3%2]
Intrauterine growth restriction	15 [15%]
SGA	16 [16%]
Length at birth	39 (35-40)
Length at birth Z-score	-0.1 (-0.8; 0.6)
Head Circumference at birth	27.3 (25-29)
HC at birth Z-score	0.05 (-0.7; 0.9)
Female sex	41 [42%]
Multiple birth	43 [44%]
Caesarean section	87 [89%]
Apgar score at 5 min	9 (8 – 9)
Postnatal characteristics	
Respiratory distress syndrome	93[95%]
Culture-proven EOS	2 [2%]
Culture-proven LOS	14 [14%]
Necrotizing enterocolitis (Bell stage ≥2)	6 [6%]
Surgical necrotizing enterocolitis	4 [4%]
PDA needing pharmacologic treatment	37 [48%]
Retinopathy of prematurity	16 [16%]
Intraventricular haemorrhage	11 [11%]
Low Grade (I-II)	5 [5%]
High Grade (III-PVHI)	6 [6%]
Post-haemorrhagic ventricular dilation requiring CSF drainage	4 [4%]
Periventricular leukomalacia	2 [2%]
Bronchopulmonary dysplasia	37 [38%]
Grade I	17 [20%]
Grade II	16 [19%]
Grade III	4 [4%]

Mechanical ventilation	38 [39%]
Surfactant administration	57 [58%]
Day of enteral feeding beginning	2 (2 – 4)
Age at full enteral feeding (days)	20 (14 – 37.3)
Duration of hospitalisation (days)	57 (39.3 – 86.5)
Post-menstrual age at discharge (weeks)	38.4 (36.7 – 40.1)
Growth	
Weight (g) at discharge	2300 (1975.5 – 2727.5)
Discharge weight Z-score	-1.5 (-2.5; 0.7)
Extrauterine growth restriction	63 [64]
Length at discharge	46 (43 – 48)
Discharge length Z-score	-1.3 (-3.1; 0.1)
Head Circumference at birth	33 (32 – 34.8)
Discharge HC Z-score	-0.7 (-1.5; 0.1)

SGA: small for gestational age, HC: head circumference, EOS: early onset sepsis, LOS: late onset sepsis, PDA: patent ductus arteriosus, PVHI: periventricular haemorrhagic infarction; CSF: cerebrospinal fluid, TEA: term equivalent age.

Follow-up for all these children is currently ongoing and is planned to be performed up to 30 months CA. Anthropometric variables collected during follow-up are shown in Table 2.

Table 2. Anthropometric variables during follow-up. Variables are expressed as median (IQR)

	TEA n=97	**3 months CA** n=98	**6 months CA** n=98
Age	40.4 (40-41.6) weeks PMA	13(12-13) weeks CA	6 (5.9-6.2) months CA
Weight (g)	2910 (2480 – 3400)	5404 (4668.8 – 6047.5)	7260 (6383.8 - 7810)
Weight Z-score	-1.2 (-2.0; -0.2)	-0.7 (-1.7; 0.3)	-0.4 (-1.3; 0.2)
Length (cm)	48.3 (47 – 50)	58 (56 – 61)	65 (63.5 – 68)
Length Z-score	-1. (-1.9; -0.2)	-0.73 (-1.8; 0.3)	-0.3 (-1.2; 0.8)
HC (cm)	35 (33.5 – 35.9)	40 (39 – 40.9)	43 (42 – 44)
HC Z-score	-0.3 (-1.3; 0.8)	0.05 (-0.6; 0.9)	0.3 (-0.4; 1.1)

TEA: term equivalent age; PMA: post menstrual age; CA: corrected age; HC: head circumference

Neurodevelopmental assessment

Ninety-one infants were assessed with the HNNE scale at TEA, 98 infants with the HINE scale at 3 months CA and 97 infants at 6 months CA. HNNE raw scores at TEA and HINE raw scores at 3- and 6-months CA are summarized in Table 3.

Table 3. Hammersmith Neonatal (HNNE) and Infant Neurological Examination (HINE) raw scores. Variables are expressed as median (IQR).

HNNE	**TEA** n=91	
Total score	32 (30 – 33)	
Tone	9 (8 – 10)	
Tone pattern	5 (4 – 5)	
Reflexes	6 (5 – 6)	
Movements	3 (3 – 3)	
Orientation and behaviour	7 (6 – 7)	
Abnormal sign	3 (3 – 3)	
HINE	**3 months CA** n=98	**6 months CA** n=97
Total score	60 (57.1 – 63)	68 (66 – 69)
Cranial nerve function	15 (13 – 15)	15 (15 – 15)
Posture	13 (11.5 – 13.9)	15 (14 – 16)
Movements	6 (5.3 – 6)	6 (6 – 6)
Tone	23 (22 – 24)	24 (24 – 24)
Reflexes and reactions	5 (4 – 6)	9 (8 – 10)

TEA: term equivalent age; CA: corrected age

Figure 4 illustrates the longitudinal trajectories of HINE scores from 3 to 6 months CA and their relationship with the final motor outcome. The diagram tracks the flow of infants across time points, stratifying them based on optimality cut-off scores derived from low-risk term infants (≥ 67 at 3 months and ≥ 70 at 6 months) [53]. The width of the bands is proportional to the number of patients, visualizing how infants transitioned between sub-optimal and optimal categories or maintained a stable classification, ultimately leading to their clinical status of 'motor delay' or 'no motor delay' at 6 months CA.

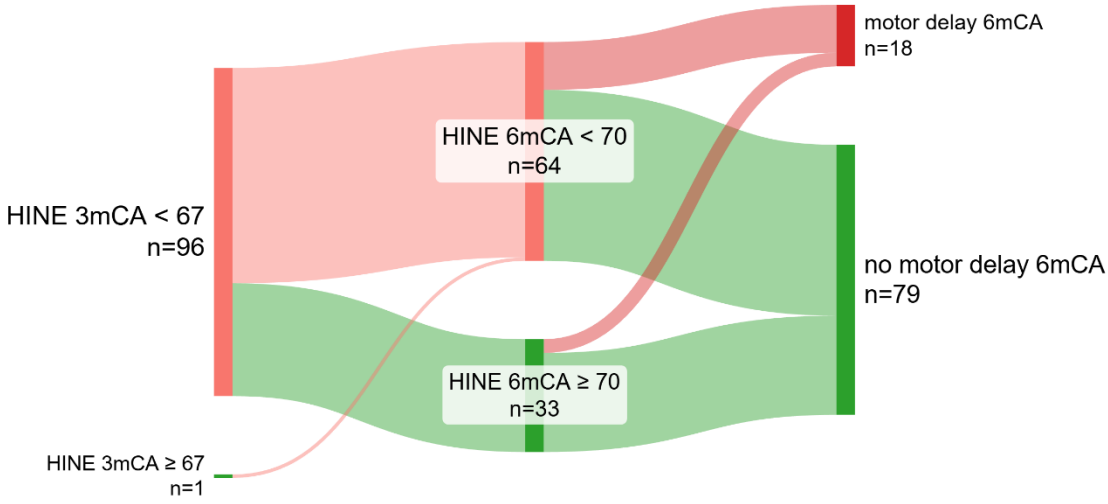

Figure 4. Sankey diagram representing subjects' developmental paths across Hammersmith Infant Neurological Examination (HINE) evaluations at 3 and 6 months CA and their relationship to motor outcome.

The Sankey diagram is a visualization technique that allows the representation of flows between different entities and their magnitudes. Several entities (nodes) are represented by vertical rectangles. The initial and central nodes represent the infants' classification based on HINE total scores, stratified using optimality cut-offs established in low-risk term infants. The nodes on the left correspond to the assessment at 3 months CA, classified as "suboptimal score" (<67) or "optimal score" (≥67). The central nodes correspond to the assessment at 6 months CA, similarly classified as "suboptimal score" (<70) or "optimal score" (≥70). The final nodes on the right depict the clinical motor status at 6 months CA, categorized as "no motor delay" and "motor delay". The size of each node reflects the number of infants within each score range and outcome group. The connecting arcs visualize the longitudinal developmental pathways of the infants. The width of each arc is proportional to the number of infants transitioning between suboptimal/optimal score categories and subsequent motor outcome groups, highlighting patterns of stability or change relative to term-equivalent standards.

At TEA, GMs were assessed in 97 infants and scored as normal in 55 (57 %) infants, while as abnormal in 42 infants (43 %). Among the latter group, 90 % of the infants showed a PR pattern, 10 % a CS pattern, and none a CH pattern. One infant did not have GMA at TEA due to sub-optimal state. At 3 months CA, Fidgety movements were normal in 87 children (89 %) and pathologic in 11 children (11 %). GMs trajectories (from TEA to Fidgety period) were built for 97 infants: GMs evaluation was normal in both evaluations in 55 (57 %) children, improved over time in 31 (32 %) children (PR or CS pattern at TEA, normal Fidgety at 3 months CA), while was persistently pathologic in 11 (11 %) children

(PR or CS pattern at TEA, pathologic Fidgety at 3 months CA). BSID-III was administered to 98 children at 6 months CA. The median (IQR) score of the cognitive scale was 100 (91.3–105), of the language scale was 91 (86.5–92.3), and of the motor scale was 94 (82.8–103). A composite score <80 (−1.25 SD) was detected in 3 (3 %) infants for the cognitive scale, in 8 (8 %) infants for the language scale, and in 18 (18 %) infants for the motor scale. Since the sample of patients was too small, it was decided not to use the cut-off of −2 SD to further distinguish infants with severe developmental delay. Moreover, according to the auditory tests performed as per internal clinical protocol, one infant was diagnosed with bilateral deafness as early as 6 months CA. The correlation between the three different developmental domains, assessed at 6 months of CA, resulted statistically significant: correlation between motor and cognitive BSID-III composite score (rho = 0.62, $p < 0.001$), correlation between the language and cognitive composite score (rho = 0.23, $p = 0.02$), and correlation between the motor and language composite score (rho = 0.29, $p = 0.003$). Similarly, the correlation between cognitive and language delay was also found to be significant (correlation coefficient = 0.54, $p < 0.001$) as well as the correlation between language and motor delay (correlation coefficient = 0.5, $p < 0.001$) and between motor and cognitive delay (correlation coefficient = 0.46, $p \leq 0.001$).

Motor delay

Eighteen infants (18 %) were diagnosed as having motor delay (motor score < 80), as assessed by the BSID-III, at 6 months CA. According to univariate analysis (Table 4), some specific items were associated with motor delay: head circumference Z-score at discharge was lower in infants with motor delay ($p = 0.02$), as well as HINE total and subscale “movements” score at 3 months CA ($p = 0.005$ and $p = 0.002$, respectively). A relationship was found between motor delay at 6 months CA and abnormal GMs assessed at TEA ($p < 0.001$), pathologic FMs ($p = 0.004$), Fidgety quality ($p = 0.001$), and GMs trajectories ($p < 0.001$). Moreover, infants not receiving MOM at TEA had a higher risk of motor delay at 6 months CA ($p = 0.02$). Variables associated with motor delay in the univariate analysis were reviewed and assessed for potential interactions one with the others. GMs at TEA and FMs proved to be correlated (rho = 0.38, $p < 0.001$).

Furthermore, since the evaluation of GMs at TEA and Fidgety quality at 3 months CA are reflected by the trajectory of GMs, which provides similar information by summarizing the evolving pattern of an infant's spontaneous movements, those two items were excluded from the final regression model. Despite a correlation was documented between FMs and HINE at 3 months CA (rho = 0.4, $p < 0.001$), HINE was included in the regression model as it represents a different assessment tool. In the final model (Table 5), only the GMs trajectory was found to be predictive of motor delay; specifically, subjects with improving or persistently pathologic GMs trajectories had a higher risk of motor delay compared to infants with a normal GMs trajectory.

Table 4. Variables associated at the univariate analysis with neurodevelopmental delay in the motor, language, or cognitive domain, assessed at 6 month corrected age by the Bayley Scales of Infant Development-III. Continuous variables are reported as median (IQR), and categorical variables as number [percentage]. A p value < 0.05 is considered statistically significant.

	Normal	**Delay**	***p***
Motor development	n=80	n=18	
HC Z-score at discharge	-0.54 (-1.08; 0.19)	-1.57 (-3.01; -.63)	.02
HINE movements score 3 mCA	6 (6;6)	5 [5;6]	.002
HINE global score 3 mCA	61 (57.88; 63.13)	58.25 [55; 60]	.005
MOM at TEA	54 [68]	7 [41]	.02
GMs at TEA normal	51[64]	2 [12]	<.001
GMs at TEA pathologic	29 [36]	15 [88]	<.001
GMs at TEA			
Normal	46 [64]	2 [13]	<.001
Poor repertoire	25 [35]	11 [68]	<.001
Cramped-synchronized	1 [1]	3 [19]	<.001
FM normal	75 [94]	12 [67]	.004
FM pathologic	5 [6]	6 [33]	.004
Fidgety quality			
F +	53 [69]	4 [24]	=.001
F ++	19 [24]	7 [41]	=.001
AF	2 [3]	2 [11]	=.001
F-	3 [4]	4 [24]	=.001
GMs normal trajectory	53 [66]	3 [16]	<.001
GMs improving trajectory	22 [28]	10 [56]	<.001
GMs pathologic trajectory	5 [6]	5 [28]	<.001
	Normal	**Delay**	***p***
Language development	n= 90	n=8	
GA, weeks	30.21 (28.57; 31.39)	26.36 (25.71; 29.98)	.04

Intubation delivery room	19 [25]	5 [63]	.04
Surfactant	50 [56]	7 [88]	.05
Need for MV	31 [34]	7 [88]	<.001
Length hospitalisation, days	52 (37.75; 79.75)	88 (53.5; 100)	.02
MOM at TEA	59 [66]	2 [25]	.03
HC Z-score at 3 mCA	0.05 (-0.63; 0.87)	-1.24 (-2.08; -0.53)	.005
GMs at TEA- normal	52 [58]	1 [1]	.04
GMs at TEA pathologic	37 [42]	7 [88]	.04
GMs at TEA			
Normal	47 [58]	1 [14]	.002
Poor repertoire	32 [40]	4 [57]	.002
Cramped-synchronized	2 [2]	2 [29]	.002
FM normal	82 [91]	5 [62]	.04
FM pathologic	8 [9]	3 [38]	.04
Fidgety quality			
F +	56 [66]	1 [14]	.02
F ++	22 [25]	3 [44]	.02
AF	3 [3]	1 [14]	.02
F-	5 [6]	2 [28]	.02
GMs normal trajectory	54 [61]	1 [12]	.007
GMs improving trajectory	28 [31]	4 [50]	.007
GMs pathologic trajectory	7 [8]	3 [38]	.007
	Normal	**Delay**	***p***
Cognitive development	n=95	9=3	
HC at TEA, cm	35 (33.95; 36)	32 (32; 32.75)	.02
HINE subscale tone at 3 mCA	23 (22;24)	20 (16;21)	.02
GMs at TEA			
Normal	54 [59]	1 [34]	<.001
Poor repertoire	36 [39]	0	<.001
Cramped-synchronized	2 [2]	2 [66]	<.001
FM normal	86 [90]	1 [34]	.03
FM pathologic	9 [10]	2 [66]	.03
Fidgety quality			
F +	57 [63]	0 [0]	<.001
F ++	25 [28]	1 [34]	<.001
AF	4 [4]	0 [0]	<.001
F-	5 [5]	2 [66]	<.001
GMs normal trajectory	54 [57]	1 [34]	.004
GMs improving trajectory	32 [34]	0 [0]	.004
GMs pathologic trajectory	8 [9]	2 [66]	.004

HC: head circumference, HINE: Hammersmith Infant Neurological Examination; mCA: months corrected age; TEA: term equivalent age; MOM: mother's own milk; GMs: general movements; FM: fidgety movements

Table 5. Logistic regression model for motor developmental delay. A p value <0.05 was considered statistically significant

Dependent variables	B (coefficient)	S.E.	*p* (sig.)	Exp (B) (OR)	95% C.I. OR
Discharge HC Z-score	-.24	.20	.229	.78	.53 – 1.17
HINE 3 mCA	-.14	.09	.135	.87	.72 – 1.04
MOM at TEA	-.70	.85	.411	.50	.09 – 2.64
GMs normal trajectory			.083		
GMs improving trajectory	2.41	1.15	*.035*	*11.16*	*1.18 – 105.31*
GMs pathologic trajectory	2.78	1.41	*.049*	*16.05*	*1.02 – 253.50*
Constant	4.90	5.74	.393	133.94	

HC: head circumference; HINE: Hammersmith Infant Neurological Examination; mCA: months corrected age; MOM: mother's own milk; TEA: term equivalent age; GMs: general movements

The Sankey diagram in Figure 5 illustrates the flow of the study population through key developmental stages, specifically examining GMs trajectories from TEA to the Fidgety period in relation to motor outcome at 6 months CA as assessed with the BSID-III.

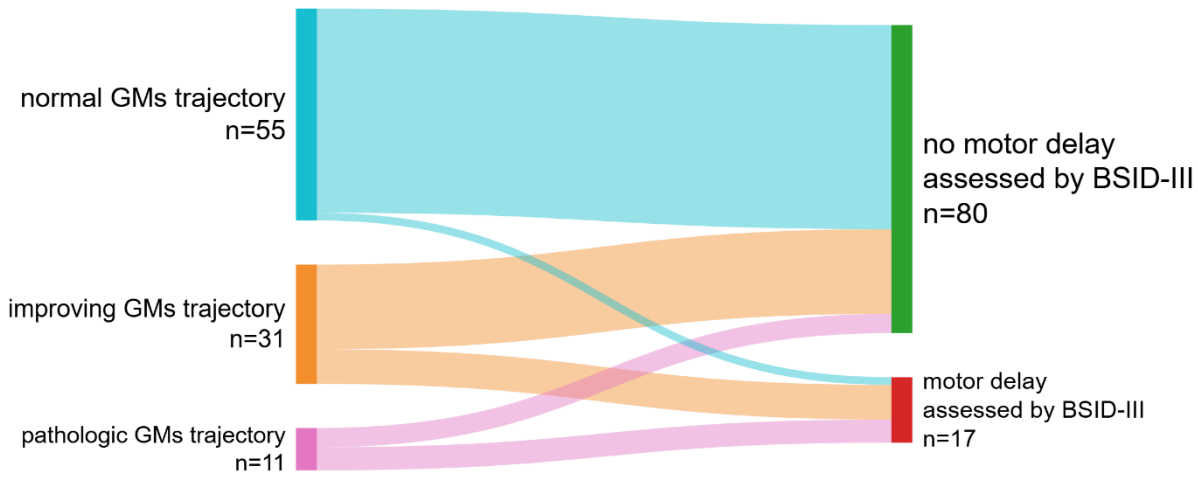

Figure 5. Prevalence (number of infants) and developmental course of General Movements (GMs) trajectories on motor outcome at 6 months CA.
Sankey diagram illustrating GMs trajectories from TEA to the Fidgety period and their association with motor outcome. The nodes on the left classify infants into three evolutionary paths:

"normal GMs trajectory" (normal at both time points), "improving GMs trajectory" (abnormal at TEA normalizing by the Fidgety period), and "pathologic GMs trajectory" (persistently abnormal). These flows extend to the final nodes on the right, which depict the clinical outcome at 6 months CA categorized as "motor delay" or "no motor delay" (evaluated via BSID-III). The dimensions of nodes and connecting arcs are proportional to the number of infants, visualizing the magnitude of transitions between GMs patterns and subsequent motor status
GMs: General Movements, TEA: term equivalent age, BSID-III: Bayley Scales of Infant and Toddler Development, Third Edition.

Language delay

Eight infants (8 %) were diagnosed as having a language delay (language score < 80), as assessed by the BSID-III, at 6 months CA. According to univariate analysis (Table 4), length of hospitalization ($p = 0.02$), GA ($p = 0.04$), Z -score of HC at 3 months CA ($p = 0.005$), intubation in the delivery room ($p = 0.04$), surfactant administration ($p = 0.05$), and need for mechanical ventilation ($p < 0.001$) were found to be correlated to language delay, assessed by BSID-III at 6 months CA. A relationship was also found between language delay at 6 months CA and abnormal GMs assessed at TEA ($p = 0.04$), pathologic FMs ($p = 0.04$), Fidgety quality ($p = 0.001$), and GMs trajectory ($p = 0.007$). Moreover, infants not receiving MOM at TEA had a higher risk of language delay at 6 months CA ($p = 0.03$). Variables associated with language delay in the univariate analysis were reviewed and assessed for potential interactions one with the others. Due to the strong association between mechanical ventilation and both intubation in the delivery room ($rho = 0.301$, $p = 0.005$) and surfactant administration ($rho = 0.664$, $p = 0.001$), only mechanical ventilation was included in the final multivariate analysis. Regarding GMs, only GM trajectory was included, as discussed above. Since a strong correlation between GA and length of hospitalization ($rho = -0.79$, $p < 0.001$), only GA was included in the final model. According to the results of the final regression model, none of the included variables was found to be predictive of language delay at 6 months CA.

Cognitive delay

Only three infants (3 %) were diagnosed as having a cognitive delay (cognitive score < 80), as assessed by the BSID-III, at 6 months CA. According to univariate analysis (Table 4), HC at TEA ($p = 0.02$) and HINE subscale tone at 3 months CA ($p = 0.02$) were found to be associated to cognitive delay, assessed by BSID-III at 6 months CA. A relationship was also found between cognitive delay at 6 months CA and abnormal GMs at TEA ($p < 0.001$), pathologic FMs ($p = 0.03$), Fidgety quality ($p < 0.001$), and GMs trajectory ($p = 0.004$). Regarding GMs, only GMs trajectory was included in the final regression model as discussed above. The results of the final regression model, however, showed no significant relationship between the selected variables and cognitive delay at 6 months CA.

4.5 Discussion

Our prospective study aimed to evaluate whether prenatal and postnatal clinical variables, and/or early tools for infant motor assessment (GMA, HNE) would predict impaired motor, cognitive, and/or language development as early as 6 months CA, assessed by the BSID-III, in a cohort of VLBW/VLGA preterm infants.

The results of our study show that preterm VLBW/VLGA infants are at increased risk of showing some degree of NDI as early as 6 months CA, most commonly in the motor (18%) and language (8%) domains. Regarding motor outcome, these findings are consistent with the available literature, that reports rates of sequelae ranging from 4% to 53%, depending on the timing of assessment and the specific evaluation tool used [46,54–56]. Of clinical importance, structural imaging alone did not fully predict these outcomes in our cohort: while 18 infants had motor delay, only 8 presented with high-risk cranial ultrasound findings (6 with IVH grade III/IV, 2 with periventricular leukomalacia) and 5 with low-risk findings (IVH grade I/II). This observation highlights the importance of carefully monitoring and performing GMA even in infants with "normal" or low-risk cranial ultrasound and suggests the utility of performing brain MRI for infants at higher risk to identify white matter injury not visible on ultrasound. Multiple studies have reported that GMA can be used as a diagnostic tool for the early

prediction of motor neurodevelopmental disorders, both for cerebral palsy (CP), and also for minor motor impairment [57–61], with the highest predictive value for CP in the Fidgety period [38]. In addition, in our study, motor delay at 6 months CA was found to be associated also with earlier GMs abnormalities, as motor delay was predicted by both the persistently pathologic GM trajectory, and also by the improving GM trajectory (abnormal GMs at TEA and normal FMs): this observation might reflect a long-lasting and independent impact of abnormal GMs at TEA on early motor outcome, thus highlighting the need of early careful monitor of these infants, regardless later Fidgety quality.

Recently, research has focused on longitudinal trajectories of GMs rather than single time points to better understand the dynamic nature of neurodevelopment and improve accuracy for the prediction of severe and milder motor outcomes [62–65]. Tescheler et al. found no significant association between GMs global character assessed at specific single timepoints, or persistence of pathological GMs, and gross and fine motor outcome, examined separately, assessed at 12 months CA using the BSID-III [64]. In contrast, Kadam et al., using the Developmental Assessment Scales for Indian Infants (DASII), found that the GMs trajectory between 35 and 40 weeks was associated with motor development assessed at 12 months CA [62]. However, at present no studies have analysed the relationship between GMs and motor development assessed as early as 6 months of CA.

As for language evaluation, several studies have documented that preterm infants are at increased risk of speech and language morbidities, with 22 to 45% of children displaying below-average language scores, across all components of language function, when assessed at preschool and school age [66–68]. In contrast, our population showed a low incidence of language delay (8% of infants), but this disparity is likely related to the early age of assessment of our population [69] . Interestingly, our analysis suggested that some neonatal factors (i.e. intubation in the delivery room, need for mechanical ventilation, surfactant administration), which are directly related to oropharyngeal health and care, might be associated with early language delay. These potential risk factors were not confirmed at the multivariate analysis, possibly due to the very low

number of subjects with pathologic outcomes. Nonetheless, these findings offer intriguing insights into the identification of early cues for focused monitoring and tailored interventions related to preterm infants' oral care.

Our data also suggest a possible association, even if not confirmed by the multiple logistic regression model, between cognitive delay at six months CA and both GM global characteristics at TEA and GMs trajectory. This finding is coherent with a recent study by Tescheler et al., reporting significant associations between both preterm GMs evaluated at 35 weeks PMA and persistent pathological GMs with cognitive outcomes at 12 months, considering both the tendencies of GMs and their global character (a given movements pattern was described by a predominant global character and in addition by intermittent improved or poorer parts, e.g., PR with a tendency towards CS) [64]. Cognitive deficits occur in 25–50% of former preterm children, and the number increases with inverse proportion to each week of shorter gestation [14]. Unfortunately, to date, no clear early indicators of cognitive impairment have been identified and early diagnosis of infants at risk of cognitive impairment is still a challenge. GMA has proven to be a reliable tool that reflects the integrity of the young nervous system, and it has been suggested that abnormal GMs may also reflect impairments of brain areas involved in cognitive development [48]. Spittle et al. reported on an association between pathological FMs and BSID-III cognitive outcome at 24 months CA, while the global character of Writhing Movements at 1 month CA showed no correlation with cognitive outcomes [70]. Other authors reported an association between pathological preterm GMs as well as pathological FMs and reduced cognitive outcome at 12, 18, and 24 months CA [59,62]. Nonetheless, our study doesn't allow to draw conclusion on this topic, firstly because of the limited sample size in the cognitive delay group (3 patients) and secondly because the assessment at 6 months of CA might be too early for the reliable detection of later cognitive impairment. One further possible limitation should be acknowledged, as we did not perform serial GMA before TEA and we did not assess GMs tendencies beyond GMs global characters, which could improve predictive value of GMA.

Our data also show a correlation between the three different developmental domains, motor, language and cognitive, assessed at 6 months of CA with BSID-III. This finding aligns with the developmental cascade hypothesis [71,72], suggesting that progress in one developmental area, such as motor skills, can influence other domains. Consequently, acquiring new motor skills can be a key driver of broad developmental advancement [73], potentially unlocking new learning experiences and facilitating the acquisition of skills across various developmental domains. A recent study [74] examined motor (gross and fine) and communication (receptive and expressive) skills in VLGA infants at six months CA, focusing on the association between sitting acquisition and early vocal production. Using BSID-III for assessment and CHILDES software for coding vocalizations during parent-infant play, correlational analyses revealed positive associations between motor composite scores and language scores, and between gross motor and expressive language scores. Interestingly, a negative association was found between supported sitting duration and vocal utterances. Furthermore, infants who were sitters exhibited significantly higher expressive language scores, and vocal utterance frequency compared to non-sitters and emerging sitters. These findings provide new evidence linking early motor and vocal development in very preterm infants, highlighting a novel research area and potential cues for enhancing clinical follow-up and promoting development in at-risk infants.

Several general considerations warrant acknowledgement. While the BSID-III is a valid, reliable, and widely employed tool in clinical and research settings for both preterm and full-term infants, this study relied on the original North American standardisation due to the lack of available normative BSID-III data for the Italian population in the first year of life. Future research incorporating a full-term Italian control group would enable the collection of such population-specific normative values. It is important to acknowledge that utilising Noth American norms in other countries may lead to over- or underestimation of developmental outcomes [75].

5. PART B: ACUTE KIDNEY INJURY, PERINATAL RISK FACTORS AND NEURODEVELOPMENTAL OUTCOME AT TWO YEARS CORRECTED AGE

5.1. Introduction

AKI is recognized as a significant and frequently encountered complication in preterm infants, particularly those with VLBW. The reported incidence of AKI in this vulnerable population during NICU hospitalization is substantial, ranging from 12% to 56% across various studies [76–78]. Neonatal AKI is associated with increased length of stay, higher mortality [76,79–81], and increased hospital costs [82]. The consequences of AKI extend beyond the neonatal period: VLBW infants who experienced AKI in the NICU are more likely to have kidney dysfunction at 3–7 years of age compared to those without AKI [83], and severe AKI is associated with elevated blood pressure at 2 years of age [84]. Emerging evidence also suggests a potential link between AKI and poorer neurodevelopmental outcomes [85,86]. The modified Kidney Disease Improving Global Outcomes (KDIGO) criteria, encompassing both elevated serum creatinine (SCr) and reduced urine output (UO), are widely used to define AKI in older populations. To note, the diagnosis of AKI in VLBW infants presents unique challenges [76,87]: repeated blood sampling for SCr measurement can exacerbate anaemia, a common problem in this population [88], and accurate UO measurement, a key component of the oliguria criterion, is often technically difficult, leading to under documentation and potentially underdiagnosis of AKI. Consequently, although several studies have investigated risk factors for AKI in very preterm infants, many have failed to incorporate UO into their AKI definition [78,89,90]. A comprehensive understanding of risk factors for AKI is crucial for improving neonatal care [91,92]. Identifying these factors can minimize unnecessary blood sampling, optimize AKI detection, and facilitate the development of targeted interventions to reduce AKI risk and mitigate its long-term consequences. This, in turn, can enable personalized follow-up care for high-risk infants.

The primary aim of our single-centre cohort study was to identify perinatal risk factors for early neonatal AKI, occurring in the first ten days of life, defined using the two most common definitions available in the literature, which are based either on SCr or UO criteria. The secondary aim was to explore the impact of early neonatal AKI, irrespective of the specific definition applied, on long-term outcomes, including growth at hospital discharge and neurodevelopment assessed during the first two years of life.

5.2. Specific Methods

Definitions and Classification of AKI

Changes in SCr and UO were used to diagnose neonatal AKI. SCr measurements collected during the first 10 days of life were analysed for study purposes. Since the retrospective nature of the study, the timing of Sc measurements analyzed for study purposes was determined by our institutional standard of care protocol that was in effect during the study period: routine SCr measurements were obtained on postnatal day 1, 2, 5 and again on day 7-10, with additional measurements collected as clinically indicated. As per standard of care, UO was measured every 3 hours via diaper weight. Neonatal AKI was diagnosed using the KDIGO criteria with neonatal modifications, and, for the study purposes, the two different definitions were used, either based on the SCr or UO criteria [76,93]. According to SCr criteria, AKI was diagnosed when the absolute SCr value was >0.5 mg/dl and then staged as follows: SCr ≥ 0.3 mg/dl above the baseline SCr or increased by 1.5–1.9 times compared to the previous lowest SCr value (Stage 1); SCr increased by 2.0–2.9 times above the baseline SCr (Stage 2); SCr increased ≥ 3 times above the baseline SCr or absolute SCr value ≥ 2.5mg/dl (Stage 3). Comparison to the lowest prior SCr value is necessary because SCr values physiologically rises during the first two days of life and then decline over the first weeks after birth such that the "baseline" SCr is constantly changing. We defined the earliest baseline SCr as the value measured on day 2 of life [93]. According to UO criteria, AKI was diagnosed when the UO was below 1 ml/kg/h and then staged as follows: UO > 0.5 and ≤ 1 mL/kg/hour (Stage1); >0.3 and ≤0.5 mL/kg/hour (Stage 2), ≤ 0.3 mL/kg/hour (Stage 3). Mean UO

(ml/kg/hour) every 12-hour period between day 2 and day 10 was calculated for study purposes.

All AKI episodes that occurred during the first 10 days of life were considered. AKI was diagnosed according to each possible AKI definition: AKISCr (defined by SCr criteria) and AKIUO (defined by UO criteria). Risk factors for AKI according to each definition were explored. Due to the nature of testing multiple AKI definitions, the definition of the control group shifted for each analysis. Specifically, for any given AKI definition being tested, the controls were defined as the infants who did not meet the criteria for that specific AKI definition. Infants meeting either AKI^{SCr} or AKI^{UO} criteria (one and/or the other) were classified as AKI^{Any}, and this latter definition was used to assess the overall impact of AKI on long term outcomes (growth and neurodevelopment).

Additional data, specific for this part of the project, were collected: prenatal Doppler ultrasound data (classified as AREDF, alterations in the blood flow in the ductus venosus [DV], alteration in the in the blood flow middle cerebral artery [MCA]), preterm prolonged rupture of membranes (pPROM). Exposure to potentially nephrotoxic drugs, such as non-steroidal anti-inflammatory drugs (NSAIDs), inotropes and specific antibiotics (i.e. aminoglycosides and vancomycin), was also recorded. Auxological assessment at discharge was carried out using the INTERGROWTH-21st charts.

As per internal clinical protocol, all VLGA and/or VLBW infants admitted to the study NICU and surviving the neonatal period attended, after discharge, a developmental follow-up including periodic assessment of clinical conditions, growth, and neurodevelopment until 24 months CA. Neurodevelopmental evaluation was performed at 12- and 24-months CA by a trained neuropsychologist using the GMDS-R 0-2 years. GMDS-R GQ was calculated using standardized score tables for the English infant population (mean ± SD, 100.5 ± 11.8), as no standardized data are available for the Italian population. For the study purposes typical development was defined as a GQ score ≥88.7, mild neurodevelopmental impairment as a GQ score ranging from 88.6 to 76.9 (-1 to -2 SD below the normative mean), moderate neurodevelopmental impairment as a GQ score ranging from 76.8 to 65.1 (-2 to -3 SD below the normative

mean) and severe neurodevelopmental impairment as a GQ score ≤65 (<-3 SD below the normative mean) [94].

5.3. Results

Data from 339 infants were collected (Table 6); 318 infants survived the neonatal period. Median GA was 30 weeks (IQR 27.7-31.4), and median BW was 1250 g (IQR 882.5 - 1440.5 g); 31.1% infants had an extremely low birth weight (ELBW, BW <1000 g) and 24.1% had IUGR. Females accounted for 46.5% of the population; most mothers (89.7%) had received antenatal steroid prophylaxis, while only a minority (36.7%) had received antenatal magnesium sulphate. Exposure to potentially nephrotoxic drugs was common. Consistent with the clinical protocol in place during the study period, all VLGA and VLBW infants received antibiotic prophylaxis including aminoglycosides, during the first days of life. Furthermore, 27.3% of infants were administered NSAIDs for PDA pharmacological treatment according to local protocol. All VLGA and VLBW infants in this cohort received caffeine (same timing and same dosage for all included infants) as per the established clinical protocol and international guidelines [30]. Moreover, 25.7% of these infants necessitated inotropic/vasoactive support. The specific inotropic/vasoactive drugs and their prevalence were: dobutamine (23.4%), dopamine (18.6%), milrinone (1.8%), epinephrine (1.2%), and norepinephrine (0.3%).

Table 6. Demographic and antenatal, perinatal, and postnatal clinical characteristics of the study population. Continuous variables are reported as median (IQR). and categorical variables as number [percentage]

Characteristics	**n = 339**
Gestational age (weeks)	30 (27.7; 31.4)
Mortality	21 [6.2%]
Inborn	295 [89.1%]
Antenatal steroids prophylaxis	262 [89.7%]
Magnesium sulphate prophylaxis	94 [36.7%]
Maternal hypertension	78 [26.7%]
Chorioamnionitis	5 [2.0%]
Birth weight (g)	1250 (882.5; 1440.5)
Birth weight Z-score	0 (-1; 0.6)
Birth weight less 1000 g	103 [31.1%]

Intrauterine growth restriction	62 [24.1%]
SGA	65 [19.8%]
Length at birth	38 (35;40)
Length at birth Z-score	0 (-1.2; 0.4)
Head Circumference at birth	28 (25-29)
HC at birth Z-score	0 (-1; 0.8)
Female sex	151 [46.5%]
Multiple birth	111 [33.5%]
Caesarean section	273 [82.5%]
pPROM	84 [31.7%]
AREDF	57 [19.6%]
DV	11 [3.8%]
MCA	23 [7.9%]
Apgar score at 5 min	9 (1 – 10)
Cord blood pH	7.0 (7.23; 7.35)
Cord blood BE	-3 (-5.73; -0.85)
Major congenital anomalies	23 [6.8%]
NSAIDs	87 [27.27%]
Inotropes 10days	87 [25.7%]
Early onset sepsis	21 [6.6%]
Late onset sepsis	57 [17.9%]
Necrotizing enterocolitis (Bell stage ≥2)	7 [2.2%]
Surgical Necrotizing enterocolitis	6 [1.9%]
PDA	149 [46.3%]
needing pharmacologic treatment	87 [27.3%]
needing surgical treatment	14 [4.4%]
Retinopathy of prematurity	36 [11.6%]
Intraventricular haemorrhage	96 [29.9%]
Low Grade (I-II)	81 [27.3%]
High Grade (III-PVHI)	14 [4.7%]
Periventricular leukomalacia	10 [3.2%]
Respiratory distress syndrome	292 [90.1%]
Bronchopulmonary dysplasia	44 [14.1%]
Grade I	14 [34.1%]
Grade II	13 [31.7%]
Grade III	14 [34.1%]
Mechanical ventilation	120 [36.5%]
Duration of hospitalisation (days)	42 (28; 63)
Post-menstrual age at discharge (days)	36 (35.3; 38.1)
Weight (g) at discharge	2020 (1820; 2345)
Discharge weight Z-score	-1 (-2.2; -0.3)
Length at discharge	44 (42; 45)
Discharge length Z-score	-2 (-2.5; -0.5)
Head Circumference at discharge	32 (30.6;33)
Discharge HC Z-score	-1 (-1.9; 0)

SGA: small for gestational age. HC: head circumference. pPROM: premature rupture of membranes. AREDF: Absent or reverse end-diastolic flow. DV: ductus venosus. MCA: middle cerebral artery. BE: base excess. PDA: patent ductus arteriosus. PVHI: periventricular haemorrhagic infarction. TEA: term equivalent age. GQ: General Quotient.

The incidence of AKI differed according to each proposed definition: AKI^{SCr} occurred in 141/339 (42%) infants and AKI^{UO} in 24/309 (7%) infants, of whom 7 had only AKI^{UO} and 17 had both AKI^{UO} and AKI^{SCr}. As for disease severity, seventy-five infants (53%) had stage 1 AKI^{SCr}, 51 (36%) stage 2 AKI^{SCr}, 15 (11%) stage 3 AKI^{SCr}; in addition, 17 infants (71%) had stage 1 AKI^{UO} and 7 (29%) had stage 3 AKI^{UO}.

Risk factors for AKI

As shown in Table 7, low GA and BW ($p < 0.001$), ELBW ($p < 0.001$), and treatment with NSAIDs and inotropes ($p < 0.001$ for both) were all risk factors for both AKI^{SCr} and AKI^{UO}. Additional risk factors differed according to AKI specific definition: newborns with AKI^{SCr} were less frequently SGA, female, exposed to maternal hypertension, and were less likely to have AREDF in utero compared to controls ($p < 0.05$ for all comparisons). Conversely, newborns with AKI^{UO} had lower cord blood pH and BE compared to controls and had more frequently a history of antenatal doppler alterations in the MCA compared to controls ($p<0.05$). Variables associated with AKI in the univariate analysis were reviewed, and potential interactions among them were evaluated. Given the strong correlations observed between GA and BW (rho = 0.7, p = 0.01), and between ELBW and both GA (rho = -0.7, p = 0.01) and BW (rho = -0.8, p = 0.01), ELBW was selected for inclusion in all subsequent multiple logistic regression models to mitigate the risk of multicollinearity. Similarly, as cord blood pH and BE proved to be correlated (rho = 0.7, p = 0.01), only cord blood pH was included.

Table 7. Characteristics for each specific AKI definition comparing AKI^{SCr} vs. No AKI^{SCr}, and AKI^{UO} vs. No AKI^{UO}.

	Participants, n (%)					
Characteristics	**AKI Scr**	**No AKI Scr**	***p* value ***	**AKI UO**	**No AKI UO**	***P* value***
	(n= 141)	(n=198)		(n=24)	(n=285)	
Gestational age, median (IQR), weeks	28.71 (3.93)	30.71 (2.72)	***<.001***	26.28 (4.57)	30.14 (3.43)	***<.001***
Apgar score at 5 min, median (IQR)	8 (1)	9 (1)	**<.001**	8 (4)	9 (1)	***<.001***
Cord blood pH, median (IQR),	7.31 (.10)	7.28 (.11)	.11	7.25 (.21)	7.30 (.11)	***.02***
Cord blood BE, median (IQR)	-2.75 (5.26)	-3.1(4.6)	.35	-8.0 (8.4)	-3.0(4.6)	***<.001***
Birth weight, median (IQR), g	1103.5 (580)	1335.0 (331)	***<.001***	734.0 (288)	1298 (464)	***<.001***
Birth weight less 1000 g	62(43.9)	31(15.7)	***<.001***	20 (83.3)	72(25.3)	***<.001***
Fetal growth restriction	23(16.3)	38(19.2)	.11	5(20.8)	56(19.6)	.78
SGA	19(11.3)	44(22.2)	***.02***	5(20.8)	58(20.4)	.79
AREDF	17(12.0)	38(19.2)	***.01***	6(25)	49(17.2)	.24
DV	7 (5.0)	3(1.5)	.19	1(4.2)	9(3.2)	.52
MCA	8 (5.7)	14(7.0)	.37	5 (20.8)	17(6.0)	***.013***
Female sex	56(39.7)	93(46.9)	***.02***	9 (37.5)	137(48.1)	.39
Multiple birth	43(30.4)	61(30.8)	.47	7 (29.2)	96(34.4)	.82
Caesarean section	111(78.7)	152(76.8)	.07	18 (75)	242(85)	.24
PROM	35 (24.8)	45(22.7)	.59	4 (16.7)	75(26.3)	.44
Antenatal steroids prophylaxis	119(84.4)	134(67.7)	.69	19(79.2)	234(82.1)	.48
Magnesium sulphate prophylaxis	47(41.2)	45(22.7)	.29	9(37.5)	83(29.1)	.31
Maternal hypertension	22(15.6)	54 (27.3)	***<.001***	6(25)	70(24.6)	.8
Chorioamnionitis	3 (2.6)	2 (1.0)	.66	0 (o)	5(1.8)	1
NSAIDs	58(50.8)	26 (13.1)	***<.001***	18 (75)	68(23.9)	***<.001***
Inotropes 10 days	59(51.8)	29(14.6)	***<.001***	16 (66.7)	67(23.5)	***<.001***

In the final model (Figure 6), only ELBW (OR 2.96, 95%CI 1.45-5.81, p=0.002), NSAIDs (OR 2.14, 95%CI 1.05-4.35, p=0.037) and inotropes/vasopressors administration (OR 2.26, 95%CI 1.10-4.66, p=0.026) were found to be predictive of a higher risk of AKI^{SCr}; conversely, maternal hypertension (OR 0.51, 95%CI 0.27-0.97, p=0.038) and female sex (OR 0.56, 95%CI 0.32-0.98, p=0.037) maintained their protective role against AKI^{SCr}. As for AKI^{UO}, ELBW (OR 6.52, 95%CI 1.71-24.87, p=0.006) and inotropes/vasopressors exposure (OR 3.60, 95%CI 1.05- 12.30, p=0.04) were the only risk factors for neonatal AKI^{UO}, after adjustment for other variables.

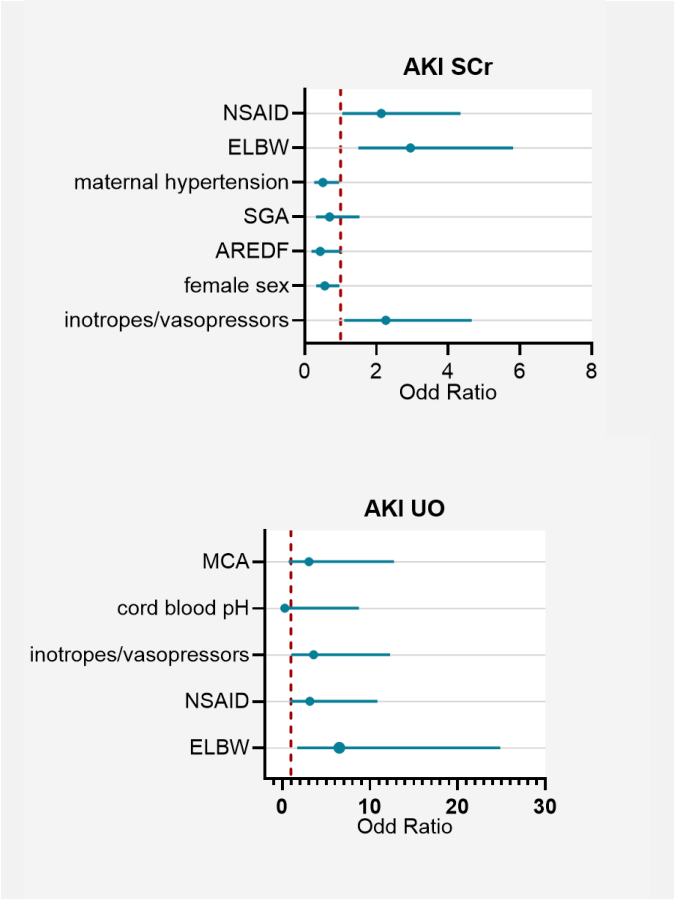

Figure 6. Multivariate analysis for risk factors associated with early acute kidney injury (AKI) according to serum creatinine criteria (AKISCr) and to urinary output criteria (AKIUO). NSAID: non-steroidal anti-inflammatory drugs, ELBW: extremely low birth weight; SGA: small for gestational age; AREDF: absent or reverse end-diastolic flow in the umbilical artery

Neonatal AKI and long-term outcomes

Both infants with AKI^{SCr} and AKI^{UO} experienced longer hospitalization compared to controls (median days 50 [IQR 36-85] vs. 36 [IQR 24-47] for AKI^{SCr} and 81 [44-116] vs. 41 [29-60] for AKI^{UO}; p <0.001 for both comparisons). At discharge, infants with AKI^{UO} had higher PMA (40 [37-42] vs. 36 [35-38] weeks, p=0.004), and lower weight (-2 [-3.2 / -1.2]

vs. -1 [-2.2 / -0.3], p=0.04), length (-4 [-4.4 / -1.7] vs. -1 [-2.4 / -0.5], p=0.002) and HC Z-scores (-2 [-3.2 / -1.5] vs. -1 [-1.7 / - 0.05], p=0.002) compared to non-AKI^{UO} infants. Growth Z-scores for infants with AKI^{SCr} at discharge were not significantly different compared to controls.

Neurodevelopmental assessment was available for 224 infants at 12 months CA and for 207 infants at 24 months CA. At 12 months CA median GQ (IQR) was 100 (91.5, 105.6); 19 (6%) infants had mild, 7 (2.2%) moderate and 4 (1.3%) severe NDI. At 24 months CA median GQ (IQR) was 95 (83; 103); 35 (11%) infants had mild, 19 (6%) moderate and 14 (4.4%) severe NDI. To assess the overall impact of AKI on neurodevelopment, a variable combining both types of AKI was built (AKI^{Any}). Early neonatal AKI, regardless of the specific definition, was found to be associated with lower GQ at both 12- and 24-months CA at the univariate analysis (p=0.014 and p=0.013, respectively).

In addition, GQ at 12 months CA was found to be associated with IVH (p<001), PVL (p=0.002), LOS, (p<0.001), BPD (p<0.001), ROP (p=0.004), PDA requiring pharmacological treatment (p=0.02), and the Z-score for weight at discharge (rho 0.33, p<0.001).

Collinearity assessment revealed no strong correlation among variables potentially associated with GQ. The variables which remained significantly associated with 12-months GQ at the regression analysis (Table 8) were IVH (p=0.010), PVL (p<0.001), BPD (p=0.006), and a low Z-score for weight at discharge (p<0.001).

As for GQ at 24 months CA, a significant association with PVL (p<0.001), LOS, (p<0.001), BPD (p<0.001), PDA requiring pharmacological treatment (p=0.01), and the Z-score for weight at discharge (rho 0.21, =0.002) was documented. The variables which remained significantly associated with GQ at 24 months CA at the regression analysis (Table 8) were PVL (<0.001), LOS (p=0.010), and a low Z-score for weight at discharge(p=0.002).

Table 8. Different models evaluating the effect of specific neonatal characteristics and acute kidney injury (AKI) on general quotient (GQ) at 12 months and 24 months corrected age. A p value < 0.05 was considered statistically significant.

Model 1: GQ at 12 months CA

Parameter	B	Standard error	Beta	B 95% confidence interval min	max	p
(Costant)	103.866	1.157		101,585	106,147	<0.001
AKI^{Any}	-1.908	1.413	-0.083	-4.692	0.876	0.178
IVH	-4.015	1.538	-0.159	-7.046	-0.985	**0.010**
PVL	-16.789	4.395	-0.232	-25.451	-8.128	**<0.001**
LOS	-0.379	2.118	-0.012	-4.553	3.795	0.858
BPD	-6.612	2.396	-0.190	-11.333	-1.890	**0.006**
ROP	0.374	2.232	0.010	-4.024	4.772	0.867
PDA^{pharm}	0.164	1.691	0.006	-3.169	3.497	0.923
Weight Z -score at discharge	2.296	0.507	0.273	1.297	3.294	**<0.001**

Model 2: GQ at 24 months CA

Parameter	B	Standard error	Beta	B 95% confidence interval min	max	p
(Costant)	97.933	1.573		94.832	101.034	<0.001
AKI^{Any}	-2.675	2.027	-0.086	-6.672	1.321	0.188
PVL	-30.778	6.298	-0.302	-43.177	-18.380	**<0.001**
LOS	-7.620	2.938	-0.179	-13.412	-1.827	**0.010**
BPD	-2.991	3.101	-0.067	-9.104	3.122	0.336
PDA^{pharm}	-1.026	2.413	-0.029	-5.782	3.731	0.671
Weight Z -score at discharge	2.186	0.722	0.195	0.785	3.587	**0.002**

GQ: general quotient, CA: corrected age, AKI^{Any}: acute kidney injury episode meeting either serum creatinine or urinary output criteria, IVH: intraventricular hemorrhage, PVL: periventricular leukomalacia, LOS: late onset sepsis, BPD: bronchopulmonary dysplasia; ROP: retinopathy of prematurity, PDA^{pharm}: patent ductus arteriosus needing pharmacologic treatment.

5.4. Discussion

This single-centre retrospective cohort study investigated the incidence, risk factors, and long-term outcomes of early AKI in VLGA and/or VLBW infants, using both SCr and UO criteria.

The study revealed discrepancy in AKI incidence depending on the diagnostic criteria used, with 42% of infants experiencing AKI based on SCr criteria and only 7% based on UO criteria. The high incidence of AKI^{SCr} aligns with previous literature regarding AKI in preterm infants [78,90]; conversely, the incidence of AKI^{UO} is lower compared to the few available studies reporting data according to this specific definition [77,89]. The lower incidence of AKI^{UO}, despite the implementation of standardized UO measurement protocols, suggests that oliguria may be less frequently observed, or more difficult to be accurately captured, in this population. Several factors could contribute to this discrepancy. Firstly, the technical difficulties associated with accurate UO measurement via diaper weighing [95], particularly in ELBW infants, may lead to underestimation. Secondly, the intensive monitoring of UO may influence physician therapeutic decisions, potentially impacting kidney function and, consequently, UO itself. Furthermore, inconsistencies in UO evaluation across studies, such as variations in measurement intervals (e.g., 2-hour vs. 3-hour) and the lack of standardized optimal UO cut-off values, contribute to the heterogeneity of findings.

In agreement with previous investigations, low GA and BW, particularly ELBW, were identified as significant risk factors for both AKI^{SCr} and AKI^{UO} [89,90]. This association highlights the inherent vulnerability of immature kidney development and function in these infants [96]. Consistent with the existing literature, the administration of NSAIDs and inotropes emerged as independent risk factors for AKI^{SCr} [97]. Notably, inotropes/vasopressors exposure was also significantly associated with AKI^{UO}. Several hypotheses may explain the observed relationship between inotropes/vasopressors exposure and AKI in our study. Firstly, certain inotropes may reduce kidney blood flow, particularly in the presence of inadequate circulating blood volume [98,99]. Consequently, inotrope use could directly contribute to AKI. Secondly, inotrope

administration may serve as a marker for hemodynamic instability, hypotension, and the severity of critical illness, potentially leading to AKI in this population. However, a limitation of our retrospective study design is the inability to disentangle the independent effects of specific agents (e.g., dopamine versus dobutamine). Irrespective of the specific mechanism, these findings underscore the necessity for meticulous kidney function monitoring in preterm infants exposed to these medications or experiencing these clinical conditions.

Intriguingly, in line with previous reports, maternal hypertension and female infant sex were identified as protective factors for AKI^{SCr} [90,100]. Reduced odds of neonatal AKI in infants exposed to maternal hypertension suggest a potential protective effect, either direct or mediated through an alternative pathway. Several hypotheses may explain this association. Firstly, maternal antihypertensive medication, and the subsequent control of maternal hypertension, could contribute to a reduction in neonatal AKI. Secondly, altered uteroplacental blood flow in hypertensive pregnancies might enhance nutrient and oxygen delivery to the foetus, potentially improving foetal kidney function; furthermore, intermittent fetoplacental ischemia could precondition the neonatal kidneys to better tolerate ischemic events after birth [101]. Thirdly, it is plausible that these factors are not directly renoprotective, but rather reflect an association with meticulous management, including complete course of antenatal corticosteroid prophylaxis and planned delivery, to mitigate risks for both mother and infant, and increased exposure to supportive care which may improve neonatal kidney haemodynamics. However, further investigation is warranted to elucidate the precise mechanisms underlying these findings.

The protective role of female sex is less clear and may reflect sex-specific differences in kidney physiology or response to injury [102]. Further research is warranted to explore this observation.

The study demonstrated that both AKI^{SCr} and AKI^{UO} were associated with prolonged hospitalization, highlighting the overall clinical impact of neonatal AKI. Moreover, AKI^{UO}, but not AKI^{SCr}, was associated with lower weight, length, and head circumference Z-

scores at discharge. This suggests that AKI defined by UO criteria may reflect a more severe or prolonged insult to kidney function, potentially impacting growth and nutritional status.

Furthermore, in univariate analysis, any episode of AKI, irrespective of the diagnostic criteria employed, was associated with poorer neurodevelopmental outcomes at 12- and 24-months CA. However, in contrast to a recent study [85], after adjusting for pertinent neonatal comorbidities in a multivariate model, only PVL, LOS, and low weight Z-scores at discharge remained significantly associated with poorer neurodevelopment at 24 months CA. The observed lack of association between BPD and neurodevelopmental outcomes at 24 months CA warrants caution; stratifying by severity might highlight prognostic gradients not detectable through dichotomous analysis alone, consistent with previous literature [103]. Consequently, AKI appears as a marker of severe systemic vulnerability, but not an independent predictor of long-term NDI compared to structural brain injury and inflammation. Discrepancies with previous studies may be attributed to differences in study population size and the incidence of AKI^{UO}, which was higher in the cohort reported by Chen et al [85]. Notably, De Mul et al. demonstrated that modified AKI definitions incorporating higher UO thresholds significantly improved the discriminative performance for predicting mortality [104]. Consequently, it could be argued that more accurate AKI definitions incorporating UO criteria and improved AKI detection methods may also enhance the discriminative performance for predicting outcomes beyond mortality. A very recent study assessing UO in VLBW infants in the first 28 days of life showed that UO is dynamic in the postnatal period and differs significantly between GA cohorts in the subgroup of extremely low gestational age neonates, suggesting the importance of an adaptation of UO thresholds for neonatal AKI in preterm infants [105].

It is important to acknowledge the limitations of this study. Firstly, the retrospective design does not allow to establish causality. Secondly, the single-centre nature of the study may limit the generalizability of the findings. Thirdly, a fully time-dependent analysis of risk factors was not performed, and the duration of AKI episodes was not

explicitly assessed, both of which could provide further insights into the relationship between AKI and outcomes [86,90].

6. CONCLUSIONS AND FUTURE DIRECTIONS

Early identification of infants at higher risk for neurodevelopmental impairment is a priority in the follow-up of VLBW/VLGA preterm infants. This is essential to guide parents' counselling and to optimize integrated rehabilitation programs aimed at promoting cognitive and motor neuronal plasticity. Our study on GMs trajectories contributes to enriching insights into the neurodevelopmental outcomes of preterm infants at 6 months CA. We found that these infants are at an increased risk of early developmental delay, especially in the motor domain, which was associated with GMs features at specific time points and GMs trajectories. Our research highlights the importance of early and regular assessment, particularly through the evaluation of GMs and their trajectories, in predicting motor outcomes as early as 6 months CA. Furthermore, our data show a correlation between the three developmental domains—motor, language, and cognitive—as assessed by the BSID-III at 6 months CA. This finding aligns with the developmental cascade hypothesis, which suggests that progress in one developmental area, such as motor skills, can positively influence other domains. This underscores the critical importance of promoting and supporting motor development in high-risk preterm infants, as the acquisition of new motor skills can be a key driver of broad developmental advancement. GMA may be a promising predictive tool for early neurodevelopmental delay, potentially extending to the cognitive and language domains, thanks to its non-invasiveness, ease of assessment, and potential integration with routine clinical evaluations. Nevertheless, long-term follow-up programs that include comprehensive motor, language, and cognitive assessments in preschool-age children remain necessary. Further research in larger cohorts is needed to develop standardized protocols for longitudinal GMA and to investigate GMs trajectories and their integration with other potential predictors of motor, cognitive, and language development impairment for optimized risk stratification.

In conclusion, Part B of this research underscores the importance of utilizing both SCr and UO criteria in defining AKI in very preterm and/or VLBW infants, revealing differences in incidence based on the diagnostic approach. While low GA, BW, and exposure to nephrotoxic medications remain key risk factors, the role of inotropes/vasopressors and the protective association of maternal hypertension and female sex against AKI SCr warrant further investigation. Notably, AKI UO appears to correlate with more pronounced growth deficits and prolonged hospitalization, suggesting a potential marker for more severe kidney compromise. Although univariate analysis revealed an initial association between AKI and poorer neurodevelopmental outcomes, this relationship did not persist in the multivariate model. This suggests that other neonatal comorbidities, reflecting the complex white matter injury in preterm infants—such LOS, BPD, PVL, IVH- and comorbidity burden, such as low Z-score weight at discharge, exert a more significant influence on long-term neurodevelopment in this cohort. Future research should focus on refining AKI definitions to optimize AKI detection in VLBW infants, exploring the underlying mechanisms of protective factors, and conducting longitudinal studies to elucidate the independent impact of AKI on later outcomes and its use as a sentinel biomarker of cumulative systemic stress.

The future direction of our research is focused on developing holistic, quantitative, and longitudinal monitoring frameworks to enhance the early identification of neurodevelopmental risk. Current projects are underway to realize this goal by advancing the integration of technology into clinical follow-up. A primary focus is the automation of movement analysis from early assessments, such as analysis of GMs videos, to provide objective, standardized, and clinically interpretable biomarkers. Furthermore, these initiatives aim to extend monitoring into later stages by analysing parameters from wearable sensors registered in formerly preterm infants from the onset of independent walking up to 24 months CA. This massive, integrated dataset—combining movement analysis, clinical data, and environmental factors—will be leveraged using advanced AI-based algorithms to establish Digital Twin frameworks. These virtual models will allow for unprecedented predictive analytics and personalized

risk stratification, ultimately leading to the implementation of more precise and timely intervention

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