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TESI DI SPECIALIZZAZIONE IN PEDIATRIA

Preliminary data to establish euglycaemia in preterm infants ≥ 26 and ≤ 31 weeks of gestation

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ABSTRACT

BACKGROUND Preterm infants are vulnerable to metabolic alterations, among which hypoglycaemia and hyperglycaemia are common occurrences. Glucose metabolism involves a third measurement called glucose variability (GV), which has attracted much interest in the recent time. Increased GV was shown as an independent risk factor for morbidity and mortality in neonatal studies. Mean Amplitude of Glycaemic Excursions (MAGE) is thought to be the most appropriate index to calculate GV.

Continuous blood glucose monitoring systems (CGM) are used successfully in patients that suffer from diabetes mellitus. Their use in the newborns identifies asymptomatic hypoglycaemia and hyperglycaemia, treating them promptly.

The main purpose of the study is to assess whether CGM in preterm allows for a longer time spent in euglycemia compared with patients treated with standard of care.

MATERIALS AND METHODS Infants with a gestational age between 26+0 and 31+0 weeks and a birth weight > 800 g were enrolled in the study. Enrolled newborn were randomly assigned and divided into a treatment group and a control group. All neonates received continuous glucose monitoring (CGM) for six days in the first week of life and in a later phase, starting from the 32nd week of postmenstrual age, through the application of the Medtronic Enlite sensor. Newborns assigned to the control group wore the CGM device with blinded monitor and no alarms.

RESULTS Neonates in the treatment group had a greater percentage of time spent in euglycemic range ($p < 0.001$). MAGE did not show any statistical difference between the two groups, although the babies in the treatment group presented better MAGE.

CONCLUSIONS Our preliminary data seem to corroborate that CGM reduces hypoglycaemic and hyperglycaemic episodes in preterm babies. Despite the definition

of euglycaemia remains improvable due to the lack of clinical and instrumental our preliminary data reinforce the need to further enrol patients.

ABBREVIATIONS

CBH Cerebellar Haemorrhage

CGM Continuous Glucose Monitoring

DTI Diffusion Tensor Imaging

DWI Diffusion Weighted Imaging

ELBW Extremely Low Birth Weight

GV Glycaemic Variability

IUGR Intrauterine Growth Restriction

IVH Intraventricular Haemorrhage

LGA Large for Gestational Age

MAG Mean Absolute Glucose

MAGE Mean Amplitude of Glycaemic Excursions

MRI Magnetic Resonance Imaging

NICU Neonatal Intensive Care Unit

PLIC Posterior Limb of the Internal Capsule

PTB Preterm birth

ST Standard Deviation

ROS Reactive Oxygen Species

RT-CGMs Real Time Continuous Glucose Monitoring

VLBW Very Low Birth Weight

INTRODUCTION

Preterm birth (PTB) is defined as any live birth occurring before 37 completed weeks of gestation; this can be subdivided into extremely preterm (<28 weeks), very preterm (28–<32 weeks), moderately preterm (32–<34 weeks) and late preterm (34–<37 weeks) birth based on the gestational age at delivery [1].

This subcategorization is important because gestational age is inversely associated with increased mortality, morbidity and the intensity of neonatal care required at birth [2].

Worldwide, 11.1% of infants are born preterm every year. PTB is the leading cause of perinatal morbidity and mortality and second most common cause of death, after pneumonia, in children under 5 years of age [3].

1 Hypoglycaemia in newborn

The definition of hypoglycaemia in the newborn infant has remained controversial because of lack of significant correlation between plasma glucose concentration, clinical symptoms, and long-term sequelae [4,5]. As we know glucose is the main fuel for central nervous system metabolism, so persistence of an inadequate blood value can lead to irreversible neurological damages [6].

Hypoglycaemia is a common metabolic problem in newborns caused by a difficulty to control glucose metabolism. Glucose is the primary source of energy for the growing foetus.

At the exact moment of cord clamping there is an immediate cessation of maternal oxygen and nutrient support. From this instant, the infant must spontaneously provide for its own glucose homeostasis [4].

In the first hour of life there is a rapid decline in glucose concentration followed by an increase that occurs within 2-3 hours of the newborn. Hepatic glycogenolysis is the fastest mechanism increasing blood glucose levels after birth. The high rate of glycogenolysis leads to depletion of hepatic glycogen stores, especially in preterm infants in which liver glycogen deposits are limited. Gluconeogenesis is not immediately effective after birth due to the initial low enzymatic activity of this pathway. Gluconeogenesis slowly starts after some hours from birth and reaches its maturation after 12 h [7]. Glucose oxidation supports about 70% of the energy requirement of the brain. Ketone bodies and lactate are important alternative fuels to reduce the glucose requirement. Hepatic ketogenesis markedly increases during the first hours from birth, to provide alternative fuels for brain metabolism in term infants. However, this metabolic pattern is severely limited in preterm infants due to a lack of fat stores in adipose tissue, which eventually results in failure of lipolysis [8] [Figure 1].

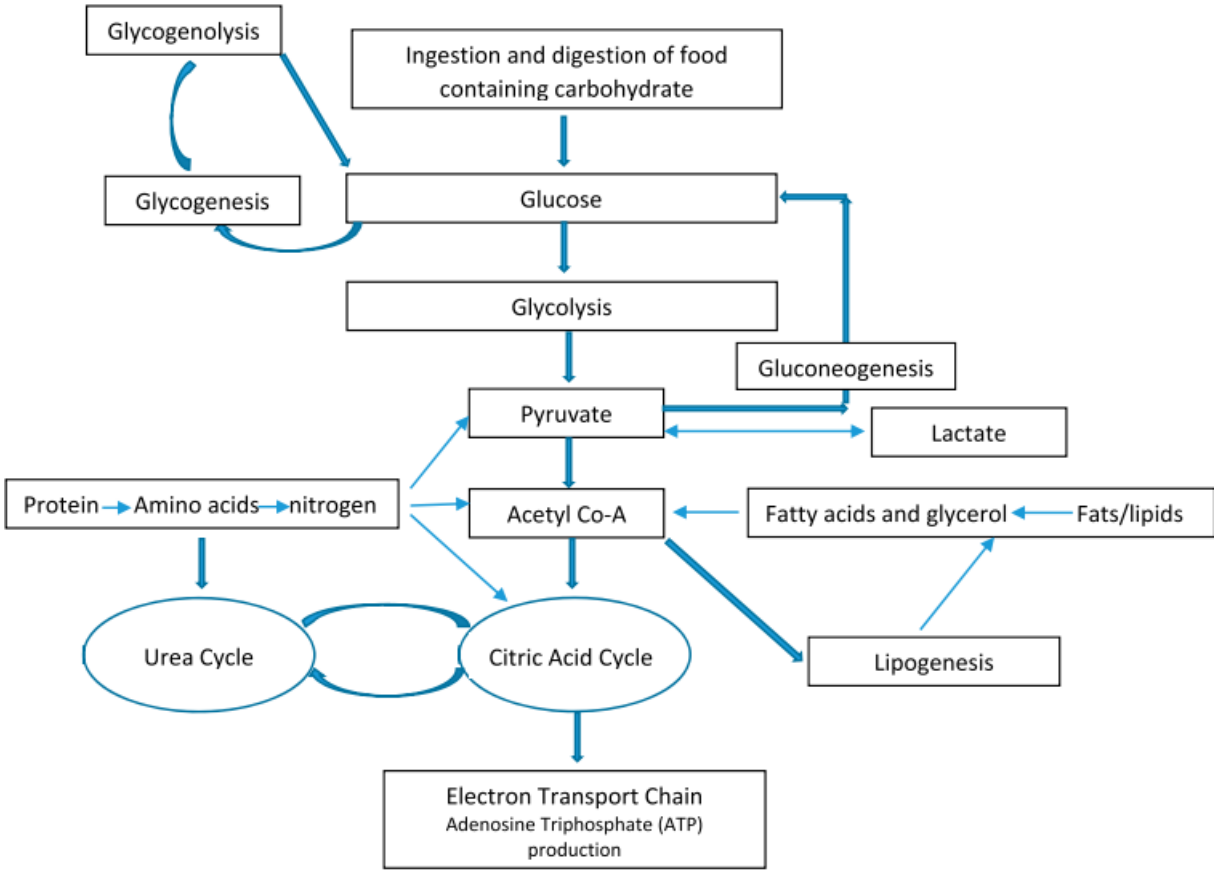

Figure 1. Glucose homeostasis

Hypoglycaemia occurs in an infant who is born with low glycogen and fat stores, with limited capacity to generate glucose via the gluconeogenesis pathway or, when, there is an excessive peripheral tissue utilization of glucose like in an infant of a mother with insulin dependent diabetes or a large for gestational age newborn (LGA) [9].

Most studies have shown that premature infants are more vulnerable than full-term infants to remain normoglycemic in the first week of life. This depends on several mechanisms. Predisposition to hypoglycaemia could be due to low glucose-6-phosphatase activity, the existence of an incompletely coordinated counter-regulatory system, increased basal metabolism of glucose, a lower capacity for production of alternative energy sources and presence of clinical conditions associated with hypoglycaemia, such as perinatal asphyxia, hypoxia, sepsis, and hypothermia[10]. Consequently, it is essential to provide exogenous glucose at birth, especially in preterm infants [11].

The definition of hypoglycaemia is controversial[12]. Neonatal hypoglycaemia is defined as a blood glucose concentration of less than 47 mg/dL (2.6 mmol/L). According to the American Academy of Paediatrics, asymptomatic blood glucose concentrations of less than 25 mg/dL (1.4 mmol/L) within 2 hours of birth in healthy term neonates do not require treatment, as this is part of a glucidic adaptation from intrauterine to extrauterine life. Intravenous glucose administration is only recommended if blood glucose levels fall below 25 mg/dL (1.4 mmol/L) within the first 4 hours, or below 35 mg/dL (2.0 mmol/L) between 4 and 24 hours after birth [13]. In term newborns, the estimated incidence of symptomatic hypoglycaemia is 1–3/1000 live births.

1.1 Hypoglycaemia and hyperglycaemia in preterm babies

Preterm infants have a threefold higher incidence of symptomatic hypoglycaemia, known to be associated with cerebral damage [6]. Preterm infant hypoglycaemia is caused by low glucose-6-phosphatase activity, insufficient glycogen stores, and a limited capacity to access alternative energy sources [7,10].

Hyperglycaemia, hypoglycaemia, and glycaemic instability are common in preterm infants and have been associated with increased risk of mortality and morbidity. Hyperglycaemia, defined as blood glucose levels greater than 10 mmol/L (180 mg/dL), is also common in extremely preterm infants [14]. In very-low-birth-weight (VLBW, <1500 g) infants, especially those born small for gestational age, the incidence of hyperglycaemia is high (20 to 86%). The pathogenesis of hyperglycaemia in VLBW infants is complex: intracellular glucose deprivation, a consequence of low postnatal insulin levels, may initiate counterregulatory responses and catabolism, leading to hyperglycaemia [15].

Hyperglycaemia is indirectly related to birth weight and gestational age and is directly related to illness, to treatment with corticosteroids, and to intravenous glucose infusions given at rates exceeding normal infant glucose turnover rates. Hyperglycaemia in very sick patients is most likely an effect of stress and thus increased levels of catecholamines, which are known to stimulate glucose metabolism. However, the glucose intolerance observed in healthy, premature infants receiving glucose infusions at rates exceeding their normal glucose turnover rate is specifically related to their immaturity and might be a result of absolute or relative insulin insufficiency, hepatic and peripheral insulin resistance, inadequate responsiveness to insulin and/or glucose, and the small mass of insulin-dependent tissue (muscle and fat) [16].

Hyperglycaemia can lead to acute problems of osmotic diuresis and metabolic acidosis and has been associated with increased risk of intraventricular haemorrhage, patent ductus arteriosus, retinopathy of prematurity, necrotising enterocolitis, and a reduction in white matter maturation [16]. Attempts to reduce risks associated with hyperglycaemia can increase the risk of hypoglycaemia, which has been associated with poor developmental outcomes.

Consequently, therapeutic changes are extremely important in the first days of life, as on the one hand the infant needs energy to grow adequately, but on the other hand sudden changes in insulin sensitivity put him at risk of both hypoglycaemia and hyperglycaemia [17].

1.2 Clinical manifestations in infancy

A rapid decline in blood glucose concentration activates an autonomic response and causes neuroglycopenia, which may lead to neurological dysfunction. In newborns, the signs of hypoglycaemia are more subtle and too often asymptomatic [18]

If present, the symptoms in newborns include cyanosis, pallor, apnoeic episodes, tachypnoea, hypothermia, hypotonia, poor feeding, abnormal cry (weak or high- pitched), irritability, diaphoresis, tremors, jitteriness, exaggerated Moro reflex, lethargy and seizures. Most of these signs are not specific to hypoglycaemia and can also be manifestations of other neonatal disorders (septicaemia, congenital heart disease, ventricular haemorrhage and respiratory distress syndrome).

Neonatal hyperglycaemia is also mostly asymptomatic and possible signs are also indicative of other pathological processes [19]. The symptoms include dehydration due to osmotic diuresis, weight loss, failure to thrive, fever, glucosuria, ketosis and metabolic

acidosis. In the newborn, especially if preterm, signs and symptoms of glucose imbalance are infrequent and when present are indicative of important alterations [Figure 2].

Neurogenic (Autonomic activation of the sympathetic nervous system)	Neuroglycopenic (Central nervous system deprivation of glucose supply)
Pallor	Weak or high-pitched cry
Jitteriness	Apnea
Tremors	Hypotonia
Tachypnea	Seizures
Sweating	Lethargy
Irritability	Coma
Tremulousness	
Vomiting	
Temperature instability	
Tachycardia	
Exaggerated Moro reflex	

Figure 2. Symptoms of hypoglycaemia in newborn

1.3 Neonatal hypoglycaemia and brain vulnerability

Hypoglycaemia causes neuronal depolarization, resulting in a significant increase in brain glutamate concentrations. Impaired glutamate reuptake results in aspartate accumulation in brain tissues. Consequently, increased levels of aspartate may activate some glutamate receptors subtypes, contributing to excitotoxicity. The sustained glutamate receptor activation is the first step of the process leading to neuronal cell death [20].

The oxidative DNA damage is the second major player in cellular death: mitochondria taken from a hypoglycaemic brain show a great capacity to generate reactive oxygen species (ROS) in response to glutamate excitotoxicity. However, ROS can also be generated by several other sources. NADPH oxidase is an enzyme which synthesizes superoxides. It has been observed that superoxide production in neuron cells affected by hypoglycaemia occurs mainly during glucose reperfusion [21].

Zinc (as Zn2+) is a neuromodulator stored within vesicles in presynaptic terminals, and it is massively released from into the extracellular space during pathological conditions such as ischemia, seizure, brain trauma, and hypoglycaemia. Zinc significantly affects the activity of many receptors, and it has been associated with the promotion of neuronal death. It induces mitochondrial dysfunction triggering mitochondrial depolarization and leading to PARP-1 activation, because of increased production of reactive oxygen species (ROS) [22].

PARP-1 facilitates the DNA repair process. During oxidative stress, reactive species damage all cellular components, including nucleic acids. PARP-1 activation helps the DNA repair machinery. While adequate PARP activation facilitates DNA repair, extensive PARP activation, caused by extensive damage from a sustained action of glutamate, induces mitochondrial permeability transition and mitochondrial damage that culminates in cell death [6,23].

The selective vulnerability of brain regions to hypoglycaemia has been demonstrated. The II and III superficial layers of the cerebral cortex, the dentate gyrus, the subiculum, the CA1 regions in the hippocampus, and the caudate - putamen, are the main susceptible areas to hypoglycaemic insult. Although the physiopathology underlying this different susceptibility is still unclear, oxidative stress seems to play a central role [24].

Another controversial topic is whether the extent of lesions found on Magnetic Resonance Imaging (MRI) and long-term outcome can be correlated. Occipital lobe damage is the predominant one. As we can observe from the images below, the occipital lobe injury can be evident in the acute phase on the diffusion-weighted imaging (DWI) [Figure 3].

Occipital cortex damage is associated with visual deficits and epilepsy of occipital origin. The outcomes depend greatly on the duration of the hypoglycaemic event and its severity.

MRI that was performed promptly after insult could be of help in predicting visual outcomes, although, as mentioned earlier, it is not always easy to correlate the extent of damage with long-term outcomes [25].

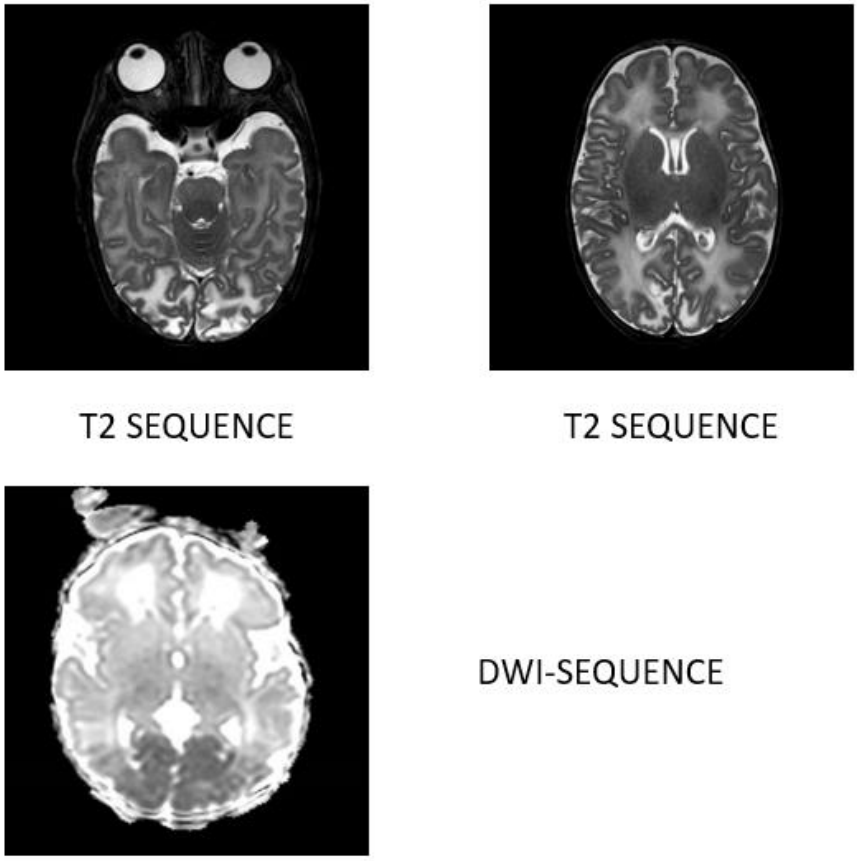

Figure 3. MRI images in a case of hypoglycemia brain damage in a newborn

1.4 The brain vulnerability of the preterm infant and the role of MRI in predicting brain maturation

Brain vulnerability changes with gestational age and depends on the characteristics and stages of brain development [26].

The brain of an extremely preterm infant is characterised by significant developmental changes that are especially noticeable in the cerebellum and in the periventricular zone, both of which host evolving germinal matrix, which is a common site of injury in the preterm.

It is vulnerable to rupture and haemorrhage due to the poor vascular integrity of involuting immature vessels and inadequate connective tissue support with a deficiency in mesenchymal and glial elements. These intrinsic characteristics of the germinative matrix increase its vulnerability and the risk of bleeding. Impaired cerebrovascular autoregulation with frequent fluctuations in carbon oxide, disturbances in coagulation, large patent ductus arteriosus and other parameters further exacerbate this danger [27].

The vulnerability of the brain between 26 and 34 weeks of postmenstrual age is mainly connected to the white matter development, especially in the periventricular zones [28]

This vulnerability appears to be multifactorial, relating to vessel anatomy with regions of relatively poor perfusion, low baseline blood flow, impaired regulation of blood flow and the inherent susceptibility of pre-oligodendrocytes to damage [29]

The usefulness of MRI at term equivalent age is well known. It can give information about the asymmetry of the PLIC (posterior limb of the internal capsule), a predictor of hemiplegia. A bilateral abnormal signal intensity of the PLIC could be an early predictor of spastic diplegia or quadriplegia [30].

Assessment of myelination at the level of the PLIC is considered the simplest and most useful way to assess the normal development of myelination in term babies and in preterm babies at term corrected age [26].

Together with brain maturation assessed via morphological analysis of brain lesions, a further method to analyse brain maturation is via Diffusion Tensor Imaging (DTI) studies. These studies allow to identify those microstructural abnormalities in the developing white matter aiming to predict and follow the changing neurological problems of ex-preterm babies, not anymore characterised by severe disabling handicaps, but more focused on psychological and behavioural problems [31].

1.5 Continuous glucose monitoring (CGM)

Strict monitoring of glucose levels is necessary to prevent and treat glucose imbalances. Although serum glucose measurement is considered the gold standard in infants, glucose monitoring depends on intermittent heel pricks and frequently repeated sampling.

This method has some limitations: low glucose concentration might not be detected by intermittent blood glucose monitoring and conversely blood glucose concentration may be assessed when the infant is only temporary hypoglycaemic, leading to unnecessary treatment. Further, serial blood glucose monitoring by heel lancing is invasive, with potential adverse effects on neurodevelopment [32].

Continuous glucose monitoring (CGM) has the advantage of tracking glucose levels continuously and thus providing a reliable glycaemic profile of patients [33]. While CGM is well established in the management of diabetes mellitus, its role in neonatal glycaemic control is more controversial.

CGM offers the possibility of adjusting treatment in real time to account for individual metabolic requirements while reducing the number of blood glucose measurements and

potentially improving long-term outcomes [34]. The clinical interpretation of CGM is challenging and in the absence of well-established guidelines, there is a risk that CGM could lead to unnecessary or even harmful intervention [35]. Finally, CGM is less accurate than heel pricks because it needs to be interpreted as a dynamic and integrated value than as a static number as in the case of glucometers.

There are several types of CGM devices, interstitial, subcutaneous or transdermal. Among the subcutaneous, there are microdialysis fibres and amperometric needle electrodes; the latter are the commercial devices currently used in newborn.

These consist of a fine needle sensor connected to a non-implantable transmitter that powers the sensor and sends raw data to a monitor, either by cable or Bluetooth. The system displays the resulting output in "real time" on the monitor. Amperometric sensors measure current flowing from an oxidation (electron producing) reaction at a working electrode to a reduction (electron consuming) reaction at a counter electrode. The working electrode is coated with glucose oxidase which catalyses the oxidation of glucose when a voltage is applied, resulting in transfer of electrons to a chemical mediator, usually hydrogen peroxide. A reference electrode is used to ensure a stable voltage applied to the working electrode, but reference and counter electrodes are often combined. In addition, subcutaneous sensors require a barrier membrane to limit glucose access to the sensor because of the deficiency of oxygen in the subcutaneous environment relative to glucose supply. Each manufacturer has their own proprietary method for combining these elements within the needle sensor [36].

Raw signal data from the electrode is generated approximately every 10 seconds and is averaged and processed to give a glucose reading every 5 minutes, thus providing near continuous output [36]. Blood glucose concentration is estimated from this signal using proprietary algorithms based on regular calibration to blood glucose measurements

(minimum 12 hourly). One limitation is that sensors require a “calibrating phase” at the beginning, which is typically around two hours, for stabilization of signal output. This results in 2 hours wait to obtain the first glucose value.

Despite some limitations, real-time CGM may help to achieve greater glucose stability in newborns, particularly in preterm babies.

1.6 Glycaemic variability (GV)

Glucose metabolism is influenced by many factors and, in common with other physiological processes, is characterized by constant fluctuations. In addition to hypoglycaemia and hyperglycaemia, the global picture of glucose metabolism involves a third measurement called glucose variability (GV), which has attracted much interest in recent years. In adults with type 2 diabetes, it was demonstrated that rapid blood glucose fluctuations have more specific triggering effects on oxidative stress than chronic sustained hyperglycaemia alone [37]. On the other hands, oxidative stress has been shown to be significant in the pathogenesis of numerous neonatal diseases, including periventricular leukomalacia, bronchopulmonary dysplasia, and retinopathy of prematurity [38].

It has been shown that increased glycaemic variability relates to oxidative stress, which results in direct cellular damage and apoptosis.

The relationship between GV and mortality was reported in VLBW infants and term neonates, but not always CGM was used [39].

Glycaemic variability is a complex phenomenon that includes both intraday and interday variability. This concept is mostly concerning the diabetic subjects, both adults and

children, and literature is dedicated to this population. There are only few studies about GV in neonates and mostly have been carried out among preterm infants [40,41].

Standard Deviation (SD) around a mean glucose value measured over a 24 h period using the CGM is probably one of the most appropriate tools for assessing intraday glycaemic variability [42].

The intraday component corresponds to the within-day vertical glycaemic fluctuations, while the interday component is defined as day-to-day glucose variations, i.e., glycaemic variability along a time-dependent horizontal axis. Such a method integrates both minor and major fluctuations but does not permit differentiation of the major from the minor ones. The other methods developed for estimating the intraday glycaemic variability are based on the determination of differences between maximum and minimum glucose levels. In particular, the Mean Amplitude of Glycaemic Excursions (MAGE) [Figure 4] index is probably more appropriate for selecting the major glucose swings that are calculated as the arithmetic mean of differences between consecutive peaks and nadirs, provided that the differences be greater than the SD around the mean values. Calculating the MAGE index requires continuous glucose monitoring, which has the advantage to detect all isolated upward and downward acute glucose fluctuations. The MAGE remains certainly the most comprehensive index for assessing the intraday glycaemic variability, it has two main advantages: first, this parameter is not dependent on the mean glucose value, and second, it is designed to quantitate major glucose swings and exclude minor ones.

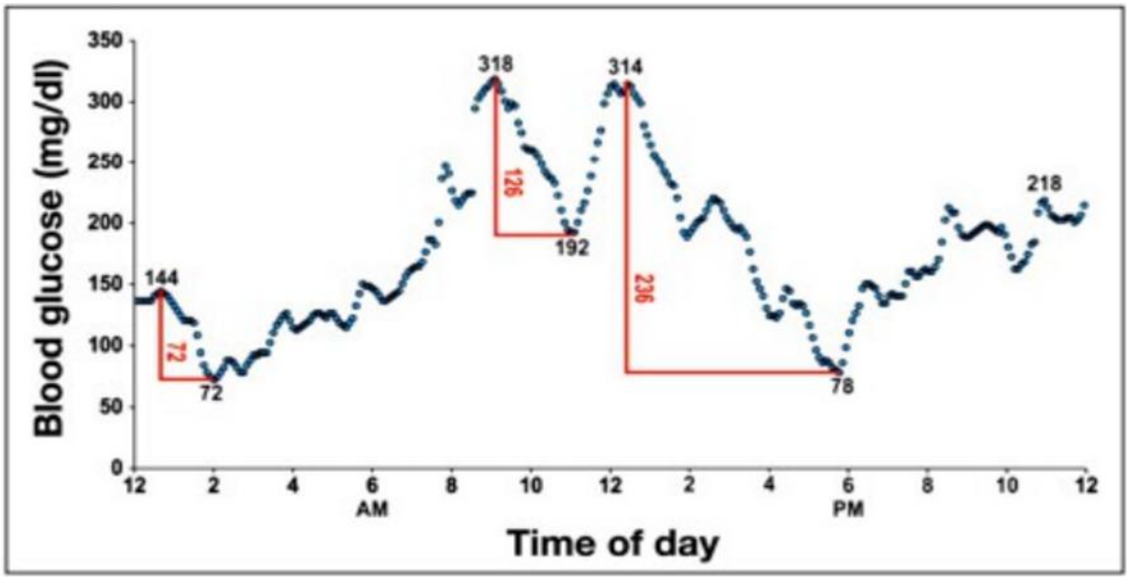

Figure 4. Example of MAGE calculation, cited by Characterizing Blood Glucose Variability Using New Metrics with Continuous Glucose Monitoring Data, Journal of Diabetes Science and Technology, 2011

The Mean Absolute Glucose (MAG) index is the summed differences between sequential 7-point self-measured blood glucose profiles per 24 h divided by the time in hours between the first and last blood glucose measurement. A limitation to MAG is that two excursions of identical extent but of different duration have different values [Figure 5].

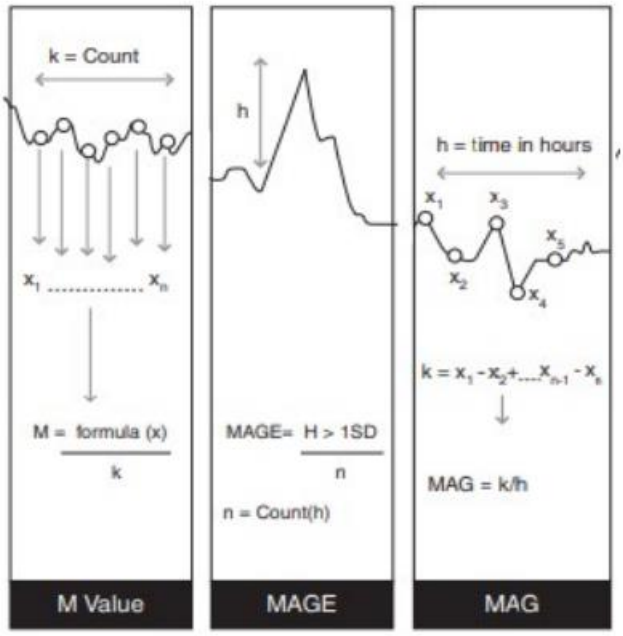

Figure 5. Graphical rapresentation of variability index

PURPOSE OF THE STUDY

Primary objective:

The main objective of the study is to assess whether continuous glycaemic monitoring in preterm babies allows for a longer time spent in euglycemia compared with patients treated with standard of care.

Secondary objectives:

1. To compare the two groups in terms of glycaemic variability, investigating whether continuous glycaemic monitoring can result in reduced glycaemic variability and better outcomes.
2. To investigate if maintenance of euglycemia results in better outcomes in the two patient groups.
3. To compare any differences on brain MRI in the two study groups at 33 weeks' postmenstrual age and at term-equivalent age, specifically by assessing developmental maturation in white matter during the first weeks of preterm life, through number of lesions, morphological abnormalities and DTI studies.

MATERIALS AND METHODS

1. Patients

This is a single-centre, prospective, pilot, and interventional study performed in the level-3 neonatal intensive care unit (NICU) of Giannina Gaslini Institute (Genoa) from January to September 2022.

These results represent preliminary data from a study that plans to include about 100 patients.

Premature infants with a gestational age between 26+0 and 31+0 weeks and a birth weight > 800 g were enrolled in the study. Recruitment was among patients born at the Obstetrics Department of the Giannina Gaslini Institute.

During the informational interview with the Family, the purpose of the study and the procedures involved in case of child's participation were described.

After informed consent was obtained, each infant was assigned an identification number and put into a database to collect the patient's general information at birth and during hospitalization.

All preterm infants enrolled were treated according to the standard of care appropriate for gestational age and birth weight.

Specifically, an umbilical venous catheter was placed within the first hour of life to ensure proper glucose supply that could not be administered enterally or through peripheral venous access. The initial glucose infusion rate was defined by the physician in accordance with the ESPGHAN 2018 guidelines [11]. The glycaemic rate is often between 4 and 8 mg/kg/min and then increased or decreased according to the infant's glucose need.

2. Eligibility criteria

a) Inclusion criteria

- Newborn with gestational age between 26+0 and 31+0.
- Patients born at the Obstetrics Department of the Giannina Gaslini Institute.
- Informed consent dated and signed by parents.
- Adult parents

b) Exclusion criteria

- Newborn with genetic diseases or chromosomal abnormalities.
- Newborn whose parents have not given consent for participation in the study.

3. Randomization

Patients were randomized into two groups with an allocation ratio 1:1. They were randomly assigned by using a software created by a researcher not involved in the study. The list generated by the algorithm was unavailable to authors.

Opaque envelopes containing the allocation group were sealed and sequentially numbered according to an electronically generated randomization list.

Data were electronically anonymized by using an individual alphanumeric code and analysed by investigators not involved in patient enrolment or data collection.

The trial was approved by the Institutional Ethics Committee of the University Hospital of Genoa on 9th April 2021.

After obtaining informed consent, eligible infants were randomly assigned to 1 of 2 study arms within 6 hours of birth: a treatment group or a control group.

4. Continuous glucose monitoring

Real-time Medtronic MiniMed MMT-7820WE Guardian Connect (Northridge, CA 91325 USA) professional continuous glucose monitors with Enlite sensors (Medtronic of Canada Ltd, Brampton, Ontario) were placed as early as possible after study enrolment, preferably within 6 hours of birth. The sensors were inserted in the lateral thigh and secured with clear adhesive dressing after adequate disinfection of the site [Figure 6].

The sensor (Enlite sensor) is minimally invasive, soft, flexible, and disposable. This device is about 1 mm-wide and 10 mm-long, and it is mounted through a hollow needle. Continuous measurements were recorded for the first 6 days of life.

Two minutes before the procedure, 0.5 mL of glucose 10% was administered to the patient to minimize pain associated with sensor insertion.

The CGM was calibrated using capillary blood glucose values measured by FreeStyle Optium Neo H glucometer (Abbott Healthcare, Massachusetts, USA). Calibrations were performed at least three times per day as per the manufacturer's instructions.

Every 5 min, 24 h a day, the sensor detects interstitial fluid blood glucose and stores it in the device, providing 288 interstitial glucose values per day.

Glycaemic monitoring was performed at two different periods of preterm life:

- the first week of life;
- on the 32nd week of corrected age.

After completing 6 days of CGM, the neonatologist removed the sensor. The data were downloaded onto a Windows-based notebook computer running Medtronic Diabetes software.

In case of detachment or malfunction, the device was replaced no more than once. The system was removed if the patient needed to be transferred to another unit or hospital. The sensors were well tolerated in our babies without any complication.

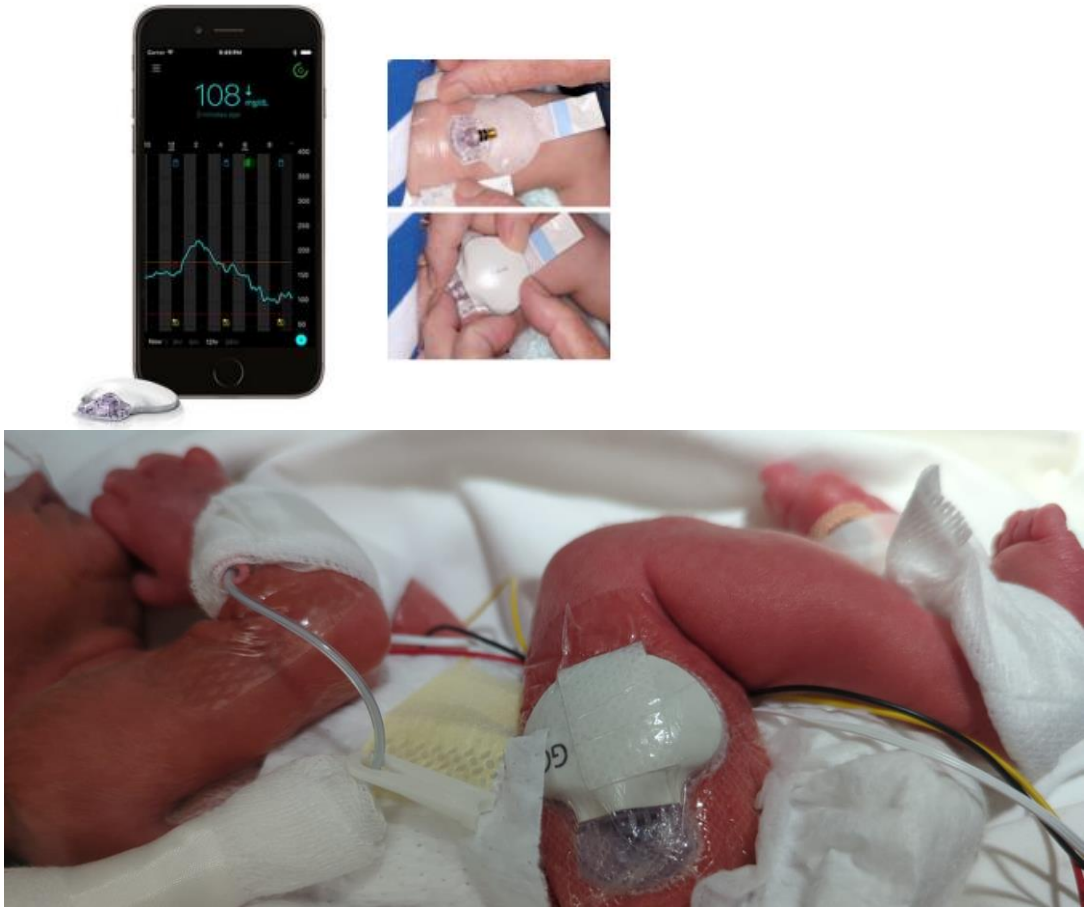

Figure 6. Insertion of a continuous glucose sensor and attachment of transmitter in the lateral thigh of a newborn infant.

5. Treatment group (Real time group)

This monitoring system gives clinicians “real time” glycaemic measurements.

Newborns assigned to the treatment group wore the CGM device with active alarms for hypoglycaemia (< 47 mg/dL) and hyperglycaemia (>180 mg/dL).

In case of hypoglycaemia or hyperglycaemia, the glucose infusion rate was changed by the physician. The possibility of administering a bolus of 10% glucose solution was evaluated based on the ESPGHAN guidelines [11]

6. Control group

Newborns assigned to the control group wore the CGM device with blinded monitor and no alarms. The monitor was placed inside a sealed envelope and connected to headphones that allowed not to hear the alarms.

Glucose infusion rate was adjusted based on blood glucose measurements performed every 3 hours in first day of life and, at minimum, every 8 hours the next days as is standard care at our department.

7. Brain Magnetic Resonance Imaging

Brain MRI was performed for all babies of the study at 33 weeks' postmenstrual age and at term-equivalent age, compatibly with the patient's condition.

At 33 weeks of corrected age, it focused on lesions typical of prematurity such as intraventricular haemorrhage (IVH), cerebellar haemorrhages (CBH) and other atypical injures (for example thalamic stroke and venous thrombosis).

At term-equivalent age, it analysed the regular appearance of PLICs and the prevalence of punctate lesions of the white matter.

All MRI scans were acquired using a 3.0 Tesla (Philips Achieva XR D-Stream).

8. Statistical analysis

Descriptive statistics were generated for the whole cohort. Data were expressed as mean and standard deviation, as median and range for continuous variables and as absolute or relative frequencies for categorical variables. The distribution of the data was analysed using the Kolmogorov–Smirnov test. Non-parametric statistics were considered as appropriate. Differences between groups were evaluated using the Mann–Whitney U-test for continuous variables and the $\chi 2$ or Fisher exact test for categorical variables. A *P*-value of <0.05 was considered statistically significant; all *P*-values were based on two-tailed tests. Statistical analysis was performed using the Statistical Package for the Social Sciences for Windows (SPSS Inc. Chicago, IL).

Glycaemic variability was measured by means of EasyGV software, available free for academic or noncommercial use at the www.phc.ox.ac.uk/research/technology-outputs/easygv website. EasyGV calculates several measures of glycaemic variability

from CGM data: we reported Standard Deviation (SD), Mean Amplitude of Glycaemic Excursions (MAGE) and Mean Absolute Glucose (MAG).

In the first week of monitoring, glycaemic variability was calculated for each day of life. In the 32nd week of postmenstrual age, glycaemic variability was calculated by evaluating all glycaemic values recorded in that week for each patient.

RESULTS

Table 1: Baseline characteristics	Real time group n=9	Standard care group (control group) n=13	p value
Gestational age, weeks Mean (SD) CI 95% mean	 28.82 ± 1.32 28-30	 28.00 ± 1.34 27-29	0.15
Birthweight, g (SD)	1210.91 ± 285.91	1106.54 ± 201.36	0.42
APGAR 1' (median, mode) APGAR 5' (median, mode)	6.36 (7; 8) 8.09 (8; 8)	6.23 (7; 8) 8.15 (8; 8)	0.78 0.69
Sex Male (%)	 2 (25%)	 9 (56.2%)	0.21
Received antenatal steroids more than 24 h before	8 (72.7%)	12 (92.3%)	0.30
Delivery mode Spontaneous vaginal delivery (%) Caesarean section (%)	 4 (36.4%) 7 (63.6%)	 2 (15.4%) 11 (84.6%)	0.36
Number of infants delivered Multiple (%)	 2 (18.2%)	 3 (23.1%)	1.0
Maternal chorioamnionitis (%)	3 (27.3%)	2 (15.4%)	0.63
IUGR	1 (11.1%)	0	0.43

Table 1. Clinical characteristics of the study patients
p Values were calculated to assess differences between newborns of the two groups (real time and control group)

Table 2: patient complications	Real time group n=9	Standard care group (control group) n=13	p value
Death	0	0	/
Sepsis (%)	2 (18.2)	3 (23.1)	1.0
Intraventricular hemorrhage (%)	1 (9.1)	1 (7.69)	0.46
Treatment for arteriosus ductus Paracetamol (%) Surgery (%)	 3 (27.3) 1 (9.1)	 7 (53.8) 0	0.23
Necrotizing enterocolitis (%)	0	1 (7.69)	0.48
Retinopathy of prematurity (%)	2 (20)	3 (23.1)	1.0
Bronchopulmonary dysplasia (%)	2 (18.2)	3 (23.1)	1.0

Table 2. Complications of the study patients
p Values were calculated to assess differences between newborns of the two groups (real time and control group)

Table 1 and Table 2 provide the characteristics and complications of the newborns of two groups. There were no statistically significant differences between the two groups about sex, gestational age, birth weight, type of delivery, Apgar score, maternal chorioamnionitis, sepsis, intraventricular haemorrhage, necrotizing enterocolitis, retinopathy of prematurity, bronchopulmonary dysplasia and death.

Twenty-five newborns met eligibility criteria for the study, but for two of them CGM data were not available. The parents of one patient did not consent to participate in the study. Thus, a total of 22 infants were included [Figure 5]. Nine of them were included in the treatment group (Real time group) while 13 were included in the control group.

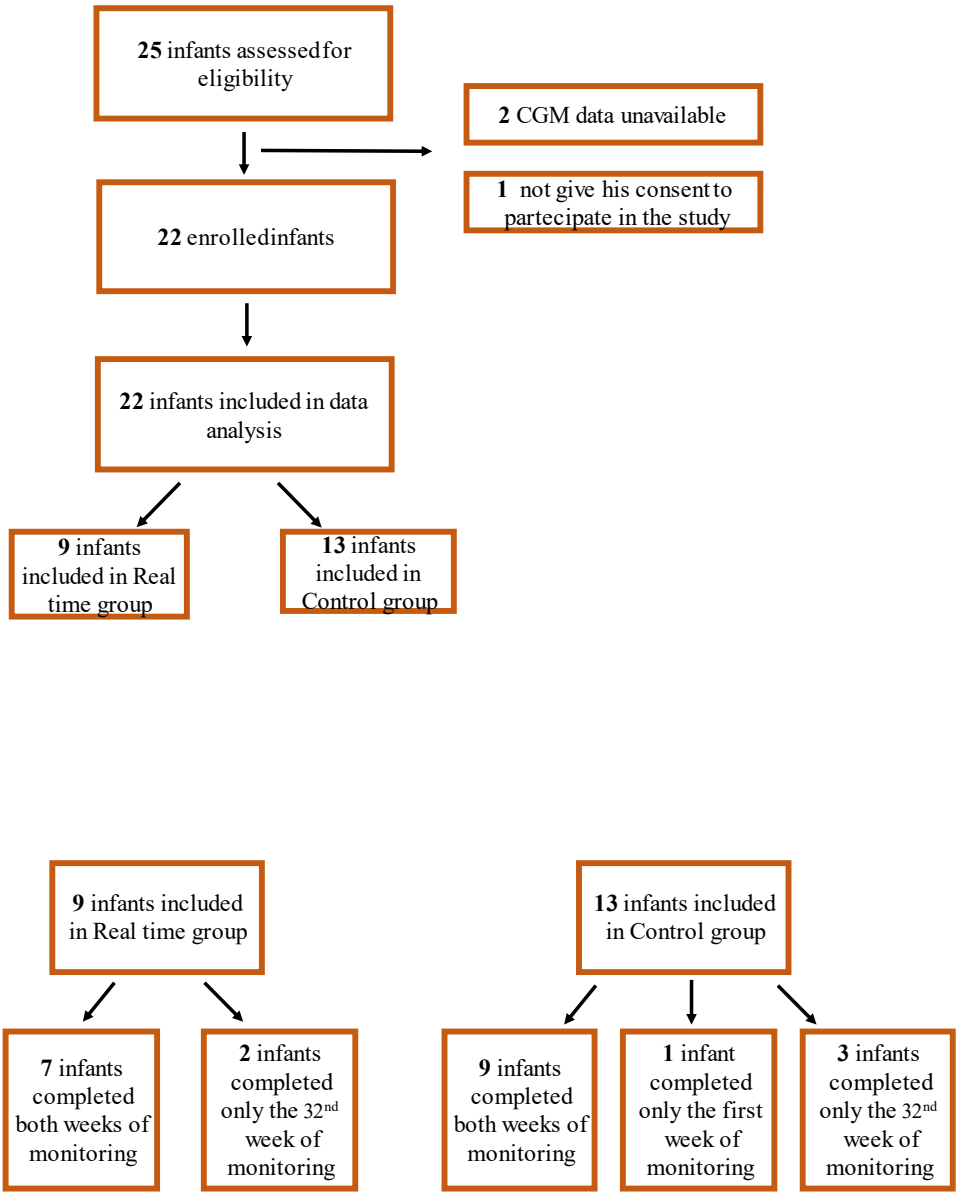

Figure 5. Trials profile and patients' enrolment in the study

As shown in table 3, the percentage of time in which euglycemia values were recorded is higher in the real time group than in the standard care group (p value < 0.005).

At the same time, we recorded a higher percentage of hypoglycaemia and hyperglycaemia events in the standard care group than in the real time group (p value < 0.005).

Table 3: primary efficacy outcomes	Real time group n=9	Standard care group (control group) n=13	p value
Proportion of time sensor shows an euglycemic glucose concentration (47-180 mg/dl)	92.3%	91.4%	0.0001
Proportion of time sensor shows an hypoglycaemic glucose concentration (< 47 mg/dl)	7.1%	7.6%	0.0001
Proportion of time sensor shows an hyperglycaemic glucose concentration (> 180 mg/dl)	0.6%	1.0%	0.0001

Table 3. Proportion of time sensor shows euglycemic, hypoglycaemic and hyperglycaemic values in the two groups.

To calculate the glycaemic variability between the two groups, we used MAGE index, which is probably the most appropriate for selecting the major glucose swings that are calculated as the arithmetic mean of differences between consecutive peaks and nadirs.

As shown in Table 4, MAGE was calculated for each day in the first week of monitoring and as a single value in the second week of monitoring.

Table 4: Index of glycemic variability	MAGE		p value
	Real time group n=9	Standard care group n=13	
Day 1	12.20 ± 11.80 (mg/dl)	11.72 ± 9.60 (mg/dl)	1.0
Day 2	7.67 ± 7.87 (mg/dl)	22.82 ± 24.86 (mg/dl)	0.15
Day 3	4.17 ± 5.60 (mg/dl)	10.56 ± 11.08 (mg/dl)	0.27
Day 4	6.67 ± 10.33 (mg/dl)	3.06 ± 6.07 (mg/dl)	0.61
Day 5	10.50 ± 6.16 (mg/dl)	13.56 ± 6.61 (mg/dl)	0.44
Day 6	13.57 ± 2.38 (mg/dl)	14.87 ± 8.25 (mg/dl)	0.90
32nd week of gestational age	15.43 ± 3.02 (mg/dl)	17.54 ± 4.92 (mg/dl)	0.08

Table 4. MAGE index to calculate glycaemic variability in the two groups.

The graphs below [Figure 6, Figure 7a and Figure 7b] report the characteristics of the brain lesions found in the infants enrolled in the study at 33 weeks of corrected age and at term-equivalent age.

At 33 weeks of corrected age, it focuses on lesions typical of prematurity such as intraventricular haemorrhage (IVH), cerebellar haemorrhages (CBH) and other atypical injures (thalamic stroke, venous thrombosis, for example).

At term-equivalent age, it analyses the regular appearance of PLICs and the prevalence of punctate lesions of the white matter.

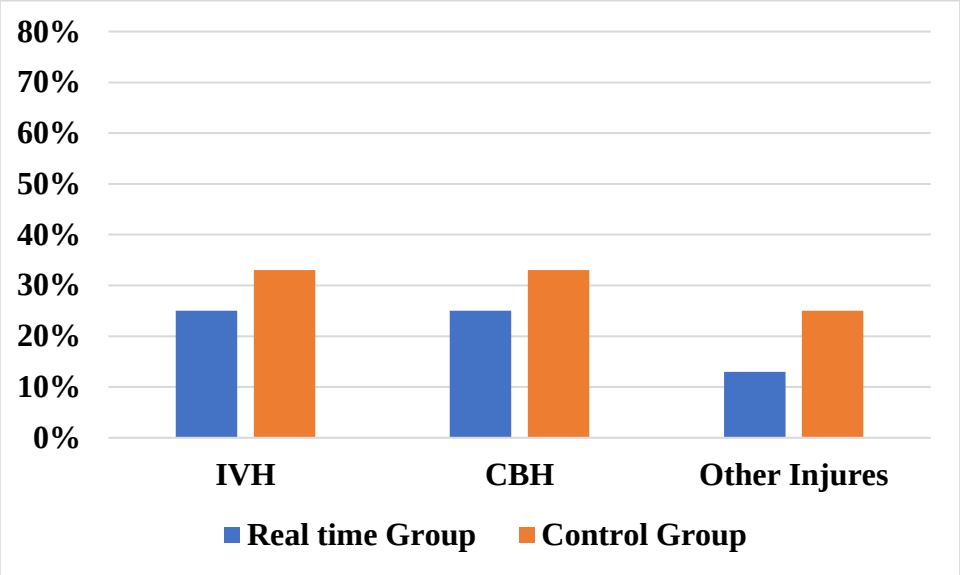

Figure 6. Characteristics and prevalence of brain lesions in the two groups at 33 weeks' postmenstrual age evidenced by the execution of 3.0 Tesla

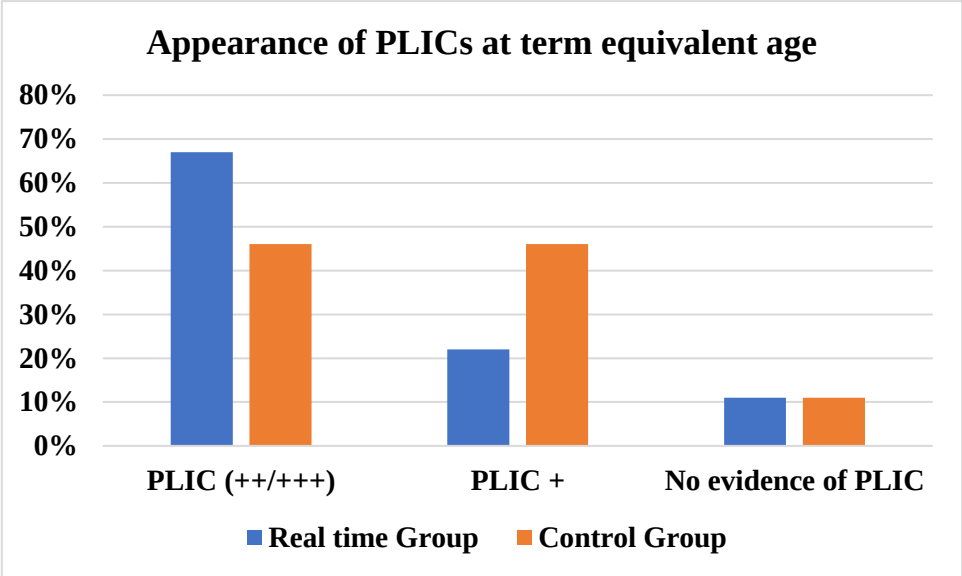

Figure 7a. Appearance of PLICs in the two groups at term-equivalent age evidenced by the execution of 3.0 Tesla

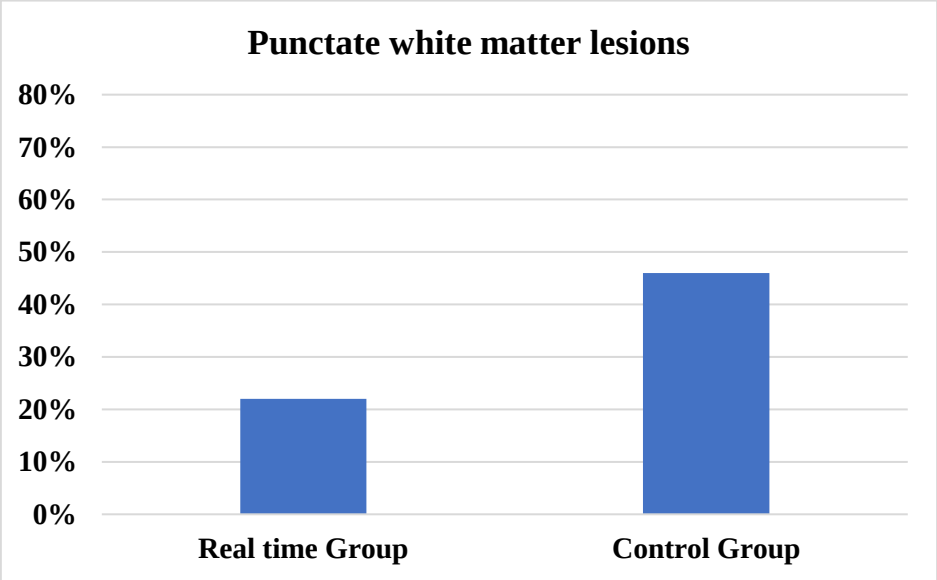

Figure 7b. Characteristics and prevalence of punctate lesions of white matter in the two groups at term-equivalent age evidenced by the execution of 3.0 Tesla

DISCUSSION

This paper aims to present preliminary data from a single-centre, prospective, pilot, and interventional study.

Preterm infants are vulnerable to metabolic alterations, among which hypoglycaemia is a common occurrence. This predisposition is due to the low activity of glucose-6-phosphatase, to their immature regulatory system, to insufficient glycogen stores, to the basal increase in glucose metabolism and to a limited capacity to access alternative energy sources[7,8,10]. Detection of hypoglycaemia is important because it is associated with increased morbidity and can result in seizures, impaired motor function and visual disturbances, among other effects[6].

Our data seem to corroborate, as assessed in previous studies, that CGM monitoring can improve glucose control in preterm infants [17,34,41].

Previous studies in which CGM monitoring has been used have shown a higher rate of hypoglycaemia detection, confirming that hypoglycaemia is more frequent, and most of the time asymptomatic, in VLBW babies [43]. Furthermore, the use of CGMs in a real-time mode provided new possibilities into the management of hypoglycaemia, allowing them to be treated earlier and to reduce their duration [34].

The risk of hypoglycaemia was not predictable, considering that these patients with increased episodes of detectable hypoglycaemia during their first days of life could not be differentiated from other VLBW neonates by their demographic, perinatal characteristics and levels of intrauterine growth restriction (IUGR) [Table 1]. Thus, the use of RT-CGMs enabled the identification of these neonates and provided the opportunity for an individual management of the glucose supply, closest to their needs.

Reducing the duration of glucose concentrations < 47 mg/dl could be protective for VLBW infants, from a neurological point of view. However, as we know, the definition

of hypoglycaemia is probably one of the most confused and controversial issues in neonatology.

A blood glucose level higher than 47 mg/dl was considered safe, according to several studies [44].

Aiming to develop an intensive care behaviour attaining euglycemia is a difficult task on very unstable babies like premature having different metabolic behaviours and needs at different gestational ages and during the first most challenging days of their life. For above mentioned reasons reducing the risks of hypoglycaemia must also include attention to reduce hyperglycaemia and high variability of glucose levels.

Studies that have assessed the correlation between hypoglycaemia in preterm infants and neurological damage show contrasting results. A randomized controlled longitudinal intervention study published in 2016 stratified preterm infants into 4 groups by glucose level. By using standardized cognitive, academic, and behavioural assessments performed at 3, 8, and 18 years of age, they compared groups. No significant differences were observed in cognitive or academic skills between the control and effected groups at any age. Participants with more severe neonatal hypoglycaemia reported fewer problem behaviours at age 18 than those without hypoglycaemia [45].

In contrast, a different study showed that early transient newborn hypoglycaemia was associated with lower achievement test scores at age 10 years [46].

On the other hand, it should not be forgotten that VLBW also presents an important risk of hyperglycaemia. Hyperglycaemia is not risk-free, nor is hypoglycaemia.

Hyperglycaemia is associated with increased morbidity and mortality, especially in extremely low-birth-weight babies (ELBW) [16].

Regarding the treatment of hyperglycaemia in the newborn, the use of insulin is controversial. While insulin may reduce the risk of hyperglycaemia when glycaemic rate

is not changed, insulin seems to increase the risk of asymptomatic hypoglycaemic episodes [47].

Continuous interstitial glucose monitoring was initially developed for the management of diabetes mellitus, resulting in metabolic control improvement [48].

To date, several studies have emphasised the efficacy and safety of CGMs. Galderisi et al. demonstrated the efficacy of glycaemic monitoring in determining a longer time spent in the euglycemic range compared to the control group [41]. A recent international, open-label, parallel-group, randomised controlled trial in 2021 reached the same result [17]. In both cases, the safety of the method was demonstrated.

Neonates in the RT-CGM group had a greater percentage of time spent in euglycemic range ($P < .001$) and decreased time spent in hypoglycaemia and in hyperglycaemia compared with control group ($P < .001$).

In our study, in addition to the above mentioned, we sought to examine glycaemic variability, which has shown increasing attractiveness and research in recent years, particularly in relation to the world of diabetes mellitus.

Increased glycaemic variability was shown as an independent risk factor for morbidity and mortality in adult and paediatric intensive care patients [49].

It has been shown that increased glycaemic variability relates to oxidative stress, which results in direct cellular damage and apoptosis. Oxidative stress has been shown to be significant in the pathogenesis of numerous neonatal diseases, including, bronchopulmonary dysplasia, retinopathy of prematurity and white matter disease so important for neurological follow up [38,50,51].

There are only few studies about GV in neonates and mostly have been carried out among preterm infants [40,41]

This year, our group published a study with the aim of assessing glycaemic variability in relation to enteral feeding strategy. Even though bolus feeding has been associated with metabolic instability in preterm infants, intermittent feeding provided lower GV values than continuous feeding in our cohort [5].

In our study, we used MAGE as index of glycaemic variability. The MAGE remains certainly the most comprehensive index for assessing the intraday glycaemic variability, because it's not dependent on the mean glucose value and it is designed to quantitate major glucose swings and exclude minor ones.

In this experience, we found an important numerical difference on the second and third day of the newborn's life in the two groups, although low simple size did not achieve a statistically significant difference.

In the second week of glycaemic monitoring, we obtained a p value closer to statistical significance, probably because the sample size was larger.

In our experience, the families accepted the placement of the device without any problems. Additionally, as has previously been pointed out in other studies, the system is safe [41]. During the trial, we recorded no adverse events.

Of course, the routine use of CGM monitoring can sometimes be complicated for staff. The nurses must remember to calibrate the sensor twice a day, and in addition, it may happen that the system stops working, leading to the need to place the sensor again and wait two hours for the next calibration.

In some cases when we downloaded the data, we realised that the system had not been recording for not short periods of time. All this can lead to the loss of data.

A larger samples size is needed to assess long-term clinical outcomes related to this form of glucose management in very preterm infants [41,45,46]

Another limitation was that it was not possible to mask the clinical or research teams to the study intervention, but compliance with masking of the CGM in the control group of the study was good.

CONCLUSIONS

The data collected show that CGM reduces hypoglycaemic and hyperglycaemic episodes in VLBW, resulting in more prolonged euglycemic values.

Our preliminary data seem to corroborate that real time CGM reduces hypoglycaemic and hyperglycaemic episodes in preterm babies. Despite the insufficient number of babies enrolled there is an interesting signal, not reaching statistical significance, showing that an early better control of glucose levels is maintained also later, from the 32nd week of gestation.

We were unable to show any statistical different between the distribution of brain lesions although a potential neuroprotective may start to appear.

Glucose control has never been clearly included in the risk factors for developing IVH although it is a problem occurring in the first difficult 3-4 days of life.

At a later stage in the life of a premature infant, the greatest risk is represented by white matter injures, particularly punctate lesions, known to be a minor form of periventricular leukomalacia[28].

White matter lesions appear to be related to white matter inflammation. Maturation and visualization of PLICs are in line with white matter maturation and probably representing a gross estimation of proper myelination at term corrected age. A higher level of glucose variability in the blood is likely to promote that oxidative stress known be one of the most common problems of the developing brain [50].

We are aware that improving glycaemic stability in VLBW is an important goal to reduce not only hypoglycaemic episodes but also glycaemic variability, which is potentially harmful to this extremely vulnerable population.

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