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"EPINEO" Monocentric Retrospective Study on Neonatal Seizures:

Incidence, ILAE Seizure Type, Epileptic Syndrome, EEG and Etiology

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Summary of the study

Purpose: In the last decade important updates occurred in the management of neonatal seizures (NS) that may have changed NS epidemiology. The International League Against Epilepsy (ILAE) has published a new classification for neonatal seizures. The aim of our study was to determine the current incidence of NS and the correlation between ILAE seizure type, epileptic syndrome, EEG and etiology.

Materials and methods: This is a retrospective single-center cohort study on consecutive neonates with neurophysiological confirmation of NS from 2009 to 2022 performed in a tertiary neonatal center. Clinical information including medical history, neurological examination, EEG/aEEG, neuroimaging, laboratory tests were inserted on a specifically designed Redcap database. Seizure type and epileptic syndromes were classified according to the new ILAE classification and EEG/aEEG with INNESCO score.

Results and conclusions: 145 neonates presented with NS: 101 term (69.7%) and 44 preterm (30.3). Incidence in the overall population at our center was 1.59/1000, in the inborn population 1.11/1000, increasing with earlier gestational age up to 17 times. In comparison with previous studies, we found a reduction in HIE-related NS and a higher contribution of genetic etiology to NS mediated by different mechanisms: functional epilepsy, metabolic epilepsy, structural epilepsy and acute provoked seizures (metabolic or vascular etiology) having a genetic etiology as primary cause triggering the cascade of events finally leading to seizures. Our study confirms the usefulness of the new ILAE classification for neonates to address etiology, confirming the association previously found between seizure type and etiology. A problematic issue is represented by the high risk of inter-operator variability regarding the use of the "sequential seizure" term. Specific types of sequential seizures with tonic-onset or tonic-clonic sequence patterns, often with alternating side onset within the same seizure or different seizures, are highly related to epileptic channelopathies.

Acronyms

GA: Gestational Age

DOL: Day of life

EEG: Electroencephalography

aEEG: amplitude-integrated EEG

EB: EEG background Activity

ESP: EEG ictal seizure pattern

NS: neonatal seizures

Neurological examination: NE

ILAE: International League Against Epilepsy

INNESCO: Italian Neonatal Seizure Collaborative Network

HIE: hypoxic ischemic encephalopathy

CNS: central nervous system

CDH: congenital diaphragmatic hernia

IEM = inborn error of metabolism

SeLNE: Self-Limited Neonatal Epilepsy

SeLFNE: Self-Limited Familiar Neonatal Epilepsy

SeLNIE: Self-Limited Neonatal-Infantile Epilepsy

DEE: Developmental Epileptic Encephalopathy

EIMFS: Epilepsy of Infancy with Migrating Focal Seizures

IESS: Infantile Epileptic Spasms Syndrome

Introduction

In neonates brain disorders often manifest with epileptic seizures and are associated with a high risk of unfavorable outcomes and epilepsy (Volpe J, Inder T, Darras B, de Vries LS, du Plessis A, Neil J, Perlman J., n.d.). Incidence of neonatal seizures (NS) is not well defined and is variable among studies, with most studies reporting an incidence between 0.95 and 3.5 for 1000 live newborns (Eriksson and Zetterström, 2008; Glass et al., 2009; Lanska et al., 1995; Lanska and Lanska, 1996; Pisani et al., 2018a; Ronen et al., 1999; Saliba et al., 1999). Differences in NS incidence depends on many factors as specific features of the studied population or country (high-income versus low-income country), type of study (population-based versus clinic-based), type of diagnosis (clinical versus EEG or aEEG diagnosis), local practices and protocols on EEG or aEEG monitoring of high-risk neonates (EEG evaluation on clinical suspicion of seizures versus systematic continuous EEG monitoring of high-risk neonates). It is known that NS incidence is inversely associated with gestational age and birth weight, with a very high risk incidence in the preterm population (Glass et al., 2017; Pisani et al., 2018a). NS are mostly provoked by an acute illness or brain insult with an underlying etiology either documented or suspected (the so called “acute provoked seizures”, previously called “acute symptomatic seizures”), whereas a minority of NS represents the manifestation of an epilepsy or epileptic syndrome with early onset (Pressler et al., 2021; Shellhaas, 2021; Shellhaas et al., 2017; Zuberi et al., 2022). Although many causes can give rise to neonatal seizures, a relatively small number accounts for most seizures (Pressler et al., 2021). Most neonatal provoked acute seizures are related to perinatal HIE, ischemic stroke, hemorrhage, infections, and acute metabolic disorders. Neonatal epilepsies and epileptic syndromes are mostly related to genetic disorders that, according to the disease mechanisms, may be schematically distinguished in functional genetic defects (as channelopathies, synaptic transmission disorders, cell signaling disorders), genetically determined structural brain disorder (as disorders of cortical malformation or disorders of neuronal migration) and genetically-determined metabolic epileptic disorders as Inborn Error of Metabolism (IEM) with epilepsy as predominant manifestation like Pyridoxine-dependent

epilepsy (Cornet and Cilio, 2019). Neonatal epilepsy as early manifestation of an acquired remote fetal brain structural injury is also possible, but it is in fact rare. Neonatal epilepsy mostly manifest with epileptic syndromes. According to the reports of the International League Against Epilepsy (ILAE) Task Force for Classification and Terminology (Fisher et al., 2017b, 2017a; Scheffer et al., 2017) and their adaptations for neonates recently produced by the ILAE Neonatal Seizure Task Force on Neonatal seizures and epileptic syndromes (Pressler et al., 2021; Zuberi et al., 2022) neonatal epileptic syndromes are distinguished in Self-Limited Neonatal Epilepsy (SeLNE) and Developmental and Epileptic Encephalopathy (DEE), depending on the favorable or unfavorable epileptic and neurodevelopmental outcome. The SeLNE are further subclassified in Self-Limited Familiar Neonatal Epilepsy (SeLFNE) when there is a familiar presentation, although features are usually similar both in familiar and sporadic presentations, and Self-Limited Infantile-Neonatal Epilepsy (SeLNIE) when in the family some patients present with neonatal onset and some patients with a later infantile onset (1-23 months). The DEE with neonatal presentation may be distinguished in Early Infantile DEE (EIDEE), Epilepsy of Infancy with Migrating Focal Seizures (EIFMS) and Infantile Epileptic Spasms Syndrome (IESS). Among the EIDEE the old terms Ohtahara Syndrome (EEG burst suppression and tonic spasm semeiology) and Early Myoclonic Encephalopathy (with or without EEG burst suppression and multifocal and myoclonic seizures in addiction to spasms) are not endorsed anymore (Zuberi et al., 2022).

An important aspect to consider in neonates in comparison with older patients, it is the very high proportion of seizures and status epilepticus with poor or no clinical manifestations, especially in critical-ill neonates as HIE and preterm neonates (Clancy et al., 1988; Murray et al., 2008; Nash et al., 2011). Without EEG monitoring in neonates with high neurological risk, most seizures cannot be detected, so continuous neurophysiological monitoring by conventional EEG (cEEG), the gold standard, or amplitude-integrated EEG (aEEG) is recommended (Dilena et al., 2021; Shellhaas et al., 2011). At the same time the correct diagnosis of the epileptic nature of paroxysmal movements is more difficult in neonates than in older patients. Given the video-EEG diagnosis of neonatal

seizures is the gold standard, it has been proved that only half of clinical seizures are correctly classified by inspection only both by doctors (independently from the specialty) or other healthcare professionals as NICU nurses (Malone et al., 2009). Without neurophysiological confirmation, many neonatal non-epileptic paroxysmal phenomena are identified as possible epileptic seizures, so in clinical practice antiseizure medication may be inappropriately prescribed in many cases. The risk of uncorrected diagnosis of seizures with consequent overtreatment and undertreatment is consistent (Rennie et al., 2019). In addition, EEG and aEEG are also precious outcome predictors in newborns with seizures: the EEG or aEEG severely abnormal background activity is associated with increased risk of neurodevelopmental outcome and chronic epilepsy both in preterm and term infants (Pisani and Spagnoli, 2015; Spagnoli et al., 2018) (Pisani et al., 2015, Pisani et al., 2020, Pisani and Spagnoli, 2016b).

Informed by the body of scientific evidence of the last years, the ILAE Neonatal Seizure Task Force on NS has worked on a new classification and framework for seizures in line with the general 2017 ILAE classification of seizures (Fisher et al., 2017b, 2017a) to promote better diagnosis and treatment. The key points of their work published in 2021 (Pressler et al., 2021) are the following: electroencephalography (EEG) for the diagnosis of neonatal seizures is crucial; neonatal seizures are considered focal at onset by definition although may have bilateral presentation; seizures can occur as electrographic-only seizures or seizures with clinical manifestations, that are distinguished according to the dominant clinical sign, not always corresponding to the onset seizure sign. The non-electrographic-only neonatal seizures, according to the dominant clinical sign are distinguished in motor seizures (automatisms, tonic, clonic, spasms, myoclonic, sequential seizures) and non-motor seizures (autonomic or behavior arrest seizures). The newest semiology motor category is the category of sequential seizures. Sequential refers to several seizure manifestations occurring in a peculiar sequence in each seizure. Typical examples for sequential seizure are seen in neonates with self-limited neonatal epilepsy or KCNQ2/SCN2A encephalopathy, which have been described as stereotyped sequence in the same seizure of a variety of manifestations including tonic, clonic,

automatisms, and autonomic features (including apnea), which often show varying lateralization (Pressler et al., 2021). As EEG is fundamental in neonatal seizure diagnosis, guidelines on ideal conventional EEG diagnosis and monitoring for neonates have been produced (Shellhaas et al., 2011), but there are significant barriers to their implementation in many centers around the world due to limited resources regarding the availability of equipment and technical and interpretive round-the-clock (twenty-four hours a day) personnel. On the other hand, despite its limitations, amplitude-integrated EEG (aEEG) (previously called Cerebral Function Monitor [CFM]) is a common alternative used in Neonatal Intensive Care Units (NICUs). In 2021 the Italian Neonatal Seizure Collaborative Network (INNESCO), on the basis of a systematic literature review and interdisciplinary discussions among neonatologists, neurologists and neurophysiologists, has proposed a consensus statement on a flexible complementary use of videoEEG (vEEG) and aEEG for the principal neonatal indications as neonatal seizures, applicable to the NICUs with different levels of complexity according to local resources and specific patient features (Dilena et al., 2021). The ILAE Neonatal Task Force has introduced an algorithm to determine degrees of diagnostic certainties for neonatal seizures (Pressler et al., 2021). According to the proposed algorithm cEEG confirmation is the gold standard and maximum level of certainty, where aEEG confirmation is considered a probable diagnosis. The expert clinical observation of focal clonic or tonic seizures may be considered a probable diagnosis too, whereas the clinical-only observation of other seizure-types different than clonic or tonic seizures may be a possible diagnosis. Seizures without EEG correlate are no more considered epileptic seizures.

cEEG and aEEG not only provide confirmation of neonatal seizures, but also irreplaceable clues toward specific etiologies especially in the case of genetic epilepsy (Carapancea et al., 2023; Cornet and Cilio, 2019; Pressler et al., 2021). The key contribution provided by EEG or aEEG assists clinicians in a promoting a targeted therapy or precision medicine approach (Cornet et al., 2018; Dilena et al., 2019, 2018, 2016; Ziobro et al., 2021), as specific EEG or aEEG patterns are associated with specific diagnosis, as for example in pyridoxine deficiency (Pearl, 2016) or

channelopathies such as KCNQ2 and SCN2A mutations (Dilena et al., 2022, 2017; Pisano et al., 2015; Sands et al., 2016; Vilan et al., 2017; Wolff et al., 2017) and this techniques also assist in monitoring therapy efficacy.

The relationship between ictal electroclinical features and etiology has been explored by a systematic review published in 2019 by the ILAE task Force (Nunes et al., 2019). In this work data were collected for every individual patient described in articles published by other groups on neonatal seizures between 2004 and 2016 containing individual electroclinical description of seizures. A genetic etiology was mostly frequently described among the cases included in this study (51%), followed by vascular (21.9%), and metabolic/vitamin-related disorders (12.6%), whereas HIE (in the clinical practice the most common cause of NS) accounted for only 4%. This is likely due to a reporting bias, as many case report of genetic syndromes have appeared in literature in the last years and maybe also because some etiologies as hypocalcemia or other electrolytes imbalance have decreased due to improved neonatal care. The availability of genetic testing has also led most authors to focus their publication on detailed description of genetic syndromes, although they account for a minority of NS (in the USA National Seizures Registry genetic syndromes accounted for 13% of 611 collected newborns) (Shellhaas et al., 2017). In this systematic review semiology individually reported by authors has been reclassified according to the ILAE new classification (Pressler et al., 2021) that at that time was published online on the ILAE site to collect comments before being published on the Epilepsia journal. The authors (Nunes et al., 2019) found significant associations between etiology and seizure types: hemorrhage with autonomic seizures, centrale nervous system (CNS) infection and stroke with clonic seizures, metabolic/inborn errors of metabolism with myoclonic seizures. Significant associations have been found also between some EEG pattern and certain etiologies: vascular disorder and electrolyte imbalance with focal ictal discharges, vitamin-related disorders with multifocal, and all categories of genetic disorders with burst-suppression. Clonic and autonomic seizures were more frequently present with focal EEG abnormalities. Tonic and myoclonic seizures with burst-suppression.

In the literature there are still few monocentric retrospective and no prospective studies that have evaluated the use of the new ILAE classification for neonatal seizures and epileptic syndromes (de Corrêa et al., 2022).

This type of studies can be useful to the have complete clinical data of the neonates (including sex and gestational age) and EEG data classified according to a standardized classification of seizure type and etiology as promoted by the ILAE Task Force on neonatal seizures. Waiting the results coming from the prospective application of the new ILAE classification, we decided to perform a monocentric retrospective observational study on the neonatal crises managed in almost 14 years at our center (Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy).

The primary aim of the study was to determine the overall incidence of neonatal seizures and the prevalence of ILAE seizure types, epileptic syndromes, EEG background and ictal findings, and etiologies, after the big changes occurring in neonatal care in the last years. The second aim of the study was to verify the correlation between ILAE seizure type, epileptic syndrome, background EEG, ictal EEG and etiology.

Material and methods

Type of study: this is a monocentric retrospective study, that was approved by the local Ethical Committee of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy. Informed consensus was provided by parents or legal guardians for patients still performing clinical follow up and for the other patients waived due to the retrospective nature of database and medical record data collection. The study was in accordance with the ethical standards required at our center and the 1964 Helsinki declaration and its later amendments.

Aims of study: the primary aim of study was to determine the prevalence in a large cohort of neonates of neonatal seizures and the prevalence of ILAE seizure types, epileptic syndromes, EEG background and ictal features and etiologies, as defined by the new ILAE classifications for neonatal seizures and epileptic syndromes (Pressler 2021, Zuberi 2022). The secondary aim was to determine the correlation between seizure type, epileptic syndrome, EEG features and etiology.

Population

Patients with neonatal onset-seizures, confirmed by EEG or aEEG, admitted at Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico between the year 2009 and 2022.

Search methods of subjects

The search for patients suitable for retrospective recruitment was performed on the electronic health record system of the Neonatology and Neonatal Intensive Care at Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico. This is a comprehensive electronic health record system were all the clinical, instrumental, and therapeutic data of the patients are recorded during the patient care. There is also a structured list of the clinical problems diagnosed in the patient by the caring physicians during the admission, as well all details related to therapies used. We search in the electronic health record system for all the patients hospitalized from 1 January 2009 to 31 October

2022 who had the diagnosis of “seizures” in their list of clinical problems or that have been treated with an antiseizure medication (as phenobarbital, phenytoin, levetiracetam) to be sure that all possible patients to consider for recruitment were extracted. In our organization neonates discharged after birth and admitted for an acute condition in the first month of life are admitted in the Neonatological department, so our data can be considered a good estimation of the epidemiological situation of neonatal seizures in our setting.

Inclusion criteria

Patients who meet all the following criteria have been included:

- Patients with neonatal seizure onset

- Infants born term or preterm;

- Postmenstrual age (PMA) at seizure onset ≥24 weeks (+0 days) and < 45 weeks;

- Epileptic nature of seizures confirmed by aEEG or cEEG.

Exclusion criteria

Patients who presented at least one of the following criteria have been excluded:

- Seizures with clinical-only diagnosis, without aEEG or cEEG confirmation of the epileptic nature of seizure

- Infants with onset of epileptic seizures at PMA ≥ 45 weeks.

The database for data collection

We build a special database for the purpose of the study called “EPINEO”, using Redcap, a known workflow methodology and software solution designed for rapid development and deployment of electronic data capture tools to support clinical and translational research and used by many research institution around the world (Harris et al., 2019, 2009).

Variables

Clinical, instrumental and therapeutic information extracted for the patients fulfilling all the inclusion criteria and without exclusion criteria have been evaluated and inserted on the structured Redcap database organized in the following sections:

- Demographics: gestational age (GA), gender, ethnicity, parental consanguinity, inborn/outborn, reason for admission, day of life at discharge or day of death - if patient died before discharge –;
- Perinatal history: use of assisted reproductive technology, maternal age, complications during pregnancy, drugs during pregnancy, fetal ultrasound features, delivery type, delivery complications, weight at birth, normality of weight for gestational age, Apgar score at 1'- 5'- 10', need of resuscitation, umbilical-cord blood gas analysis, TORCH infection;
- List of significant Neonatal comorbidities
- Neonatal Seizure Classification: seizure type according to ILAE neonatal seizure Classification (Pressler et al., 2021); non epileptic paroxysmal movements if present; Epileptic syndrome according to new ILAE classification for epileptic syndromes of the neonatal and infantile age (Zuberi et al., 2022) when appliable (only for neonatal epilepsy cases); first-onset seizure sign classified with the ILAE seizure-type classification with the exception of sequential seizures (electrographic was indicated only if no clinical sign associated). Seizure type, seizure onset sign at onset, and epileptic syndromes were classified by an experienced pediatric neurologist (RD) according to the semiology, aEEG and cEEG information contained in the medical reports;
- Neonatal Seizure history: exact time from birth at seizure onset, time from seizure remission, anti-seizure medication (ASM), ASM doses, exact time from birth at ASM start, clinical judgment of ASM efficacy, ASM duration;
- Neurophysiology section (cEEG and aEEG): type of neurophysiological instrument used for seizure diagnosis (aEEG and/or cEEG), reason for applying aEEG or EEG (seizure

suspicion, monitoring of high-risk neurological condition); EEG background activity classification and aEEG background activity classification (according to INNESCO EEG/aEEG classification) (Dilena et al., 2021); EEG seizure pattern activity at onset (ESP) (focal, multifocal, asymmetric bilateral, symmetric bilateral), presence of status epilepticus. All aEEG and cEEG information were extracted from medical records and classified by an expert pediatric neurologist (RD) in the frame of EPINEO redcap database. In all the cases where it was possible the original EEG traces were reviewed by RD (patients recorded after 2020).

- Etiology of seizures: as we recognized that the etiology of seizures is a complex matter sometimes multifactorial, we decided to classify the provoked acute seizures versus not provoked seizures (o epilepsy). Then, after deep analysis of each case we chose the etiology category or categories involved for each patients among a predefined checklist of etiology groups (similar to the terms used by the ILAE Task Force on neonatal seizure (Pressler et al., 2021)): HIE, Vascular, Infection, Inborn Error of Metabolism, Genetic, Vitamin-related disorders, Brain Malformation, Acute metabolic, Toxic, Other specific etiology, Unknown (multiple choice possible). In some neonates more than the one etiology was involved in causing seizures. This could occur both because more than one cause could act in brain disfunction processes that generate seizures (as for example in case of serious head-related trauma and HIE) or because the same specific cause could be classified in different categories (as for example in the case of tuberous sclerosis that may be classified both in the brain malformation and genetic group).. In the patient with more than one etiology we then chose the “major etiology” as the etiology we considered more relevant in the genesis of neonatal seizures. In case a primary or initial cause classifiable in a different category than the major etiology identified, we assigned the patient also a primary cause. We then subclassified each etiology when appliable: for example, in genetic etiology, we specified

the gene involved or in vascular etiology we subclassified in ischemic stroke, venous thrombosis, hemorrhage, and so on.

- Neuroimaging: brain ultrasound and brain MRI findings (with use of an abnormality severity score)
- Significant Laboratory findings
- Seizure history: days of life at seizures onset, treatment, efficacy of treatment, treatment withdrawal (database designed, data not collected yet)
- Long-term epilepsy outcome section (database designed, data not collected yet)
- Neurodevelopmental section (database designed, data not collected yet).

Statistical Analysis

The excel datasheets downloaded from our Redcap database EPINEO were processed using R Core Team (2021) and Stata 17 (StataCorp. 2021).

Position and distribution of continuous variables were expressed using mean and standard deviation if normally distributed and using median, range and percentiles if variables were not normally distributed.

We performed the comparison of the categorial variables between groups, using chi square tests. The data were analyzed looking at combinations of clinical semiology of the seizures, etiology, and EEG patterns.

Kruskal–Wallis test was used to study relationship of categorial variables (as seizure type or etiology) and quantitative measures (as day of life at seizure onset) in different groups.

Wilcoxon test was used to study the difference in quantitative measures between groups as the day of life at seizures onset between term and preterm neonates.

Results

The total number of neonates fulfilling all the criteria for inclusion in period from 01/01/2009 to 31/10/2022 was 145.

Gender count and frequency was the following: Female 62/145 (42.8%), Male (83/145, 57.2%).

Neonates born at term (>= 37 weeks at birth) were 101 (69.7%) and born at preterm (<=36.6 weeks at birth) were 44 (30.3%). Below descriptive statistical parameters of gestation age (GA) are reported.

Total Count (N)	Missing*	Unique	Min	Max	Mean	StDev	Sum	Percentile						
								0.05	0.10	0.25	0.50 Median	0.75	0.90	0.95
145	0 (0.0%)	17	24	41	36.75	4.13	5329	27	30.40	35	38	40	40	41

Parental consanguinity was reported in 5/145 (3.5%) and was related in 2 twin brothers with leucinosis (IEM) with recessive inheritance pattern, in 1 patient it was related to Pyridoxine-Dependent Epilepsy with recessive inheritance pattern, whereas in 2/5 patients (40%) parental consanguinity was not related to neonatal seizures (one patient with vascular etiology, one de novo STAG2 mutation-holoprosencephaly).

Positive familiar anamnesis for epilepsy was reported in 10/145 (6.8%) and the type of epilepsy reported was the following: Familiar Neonatal Epilepsy (7, 70%), Familiar Infantile Epilepsy (1, 10 %), Adolescent-Adult Epilepsy (1, 10 %). All the positive familiar epilepsy history was collected in term neonates (chi square $p < 0.05$).

Ethnicity was reported: White (105, 72.4%), Asian (13, 9.0%), Black /African / Caribbean (4, 2.8%), Arab (17, 11.7%), other ethnic group (8, 5.5%), Mixed ethnicity (1, 0.7%).

The count/total (rate) of preterm neonates with seizures was in white neonates 27/105 (25.7%) and in not-white neonates 17/40 (42.5%) (Pearson chi square test = 3.8612 $p = 0.049$).

Count of inborn/outborn infants were: Inborn (96, 66.2%), Outborn (49, 33.8%).

Reason for admission in the Neonatological Pathology /Intensive Care Department (NICU) was: Neonatal seizure suspicion for 45/145 (31.3%), other neonatal pathology 99/145 (68.8%).

Death before discharge occurred in 27/145 (18.8%), being significantly higher in preterm (15/44, 34.09%) than in term (12/101, 11.88%); p= 0.002.

	VARIABLE OPTIONS	PATIENTS (N = 145) (%)
GENDER	Female	62 (42.8)
	Male	83 (57.2)
GESTATIONAL AGE	Born at term (≥ 37 weeks)	101 (69.7)
	Born pre term (< 37 weeks)	44 (30.3)
INBORN - OUTBORN	Inborn	96 (66.2)
	Outborn	49 (33.8)

Incidence of neonatal seizures

In the calculation of incidence, we consider the number of neonates with seizures divided for the total number of neonates admitted at our hospital at the Department of Neonatology at Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico (Inborn and Outborn together) between 2009 and 2022. We also calculated the incidence according to gender (male/female), gestational age and weight subgroups. Results are reported in the table below and compared to the population-based incidence study on EEG-confirmed neonatal seizures of Pisani 2018 referred to a population born between 2002 and 2014 in the province of Parma (Italy).

ALL NEONATES (both inborn and outborn)		**Neonates with Seizures**	**Total number of neonates**	**Incidence – Our study [/1000] (CI 95%)**	**Incidence (Pisani 2018) [/1000] (CI 95%)**
All	All patients	145	91236	1.59 (1.34 - 1.87)	2.29 (1.87 – 2.72)
Gender	Female	62 (42.8)	44328 (48.6)	1.4 (1.07 - 1.79)	2.20 (1.61 – 2.81)
	Male	83 (57.2)	46908 (51.4)	1.77 (1.41 - 2.19)	2.38 (1.78 – 2.99)
Age	Term	101 (69.7)	78926 (86.5)	1.28 (1.04 - 1.55)	1.10 (0.80 – 1.42)
	Preterm	44 (30.3)	12310 (13.5)	3.57 (2.6 - 4.8)	14.28 (10.78 – 17.79)
EG (weeks)	< 28 weeks	9 (6.2)	666 (0.7)	13.51 (6.18 - 25.65)	85.6 /1000

	28-32 weeks	14 (9.7)	2232 (2.4)	6.27 (3.43 - 10.52)	54.9/1000 (28-30 weeks)
	33-36 weeks	21 (14.5)	9412 (10.3)	2.23 (1.38 - 3.41)	5.01 /1000 (31-36 weeks)
	≥ 37 weeks	101 (69.7)	78926 (86.5)	1.28 (1.04 - 1.55)	1.10 (0.80 – 1.42)
Weight (g)	<1000 g	10 (6.9)	905 (1.0)	11.05 (5.3 - 20.32)	127.57/1000
	1000-1499 g	9 (6.2)	1462 (1.6)	6.16 (2.81 - 11.69)	34.03/1000
	1500-2499 g	27 (18.6)	8836 (9.7)	3.06 (2.01 - 4.45)	4.8/1000
	≥ 2500 g	99 (68.3)	80033 (87.7)	1.24 (1.01 - 1.51)	1.19/1000

We decided to calculate the incidence of inborn neonates with seizures on the live births at our birth point in the same period, as we think it may better reflect the incidence in the population. Results are presented below.

VARIABLE		**Neonates with seizures N (%)**	**Live births N (%)**	**Incidence [/1000] (CI 95%)**	**Incidence (Pisani 2018)**
All	All patients	96	86255	1.11 (0.9 - 1.36)	2.29 (1.87 – 2.72)
Gender	Female	40 (41.7)	42104 (48.8)	0.95 (0.68 - 1.29)	2.20 (1.61 – 2.81)
	Male	56 (58.3)	44151 (51.2)	1.27 (0.96 - 1.65)	2.38 (1.78 – 2.99)
Age	Born at term	63 (65.6)	75582 (87.6)	0.83 (0.64 - 1.07)	1.10 (0.80 – 1.42)
	Born preterm	33 (34.4)	10673 (12.4)	3.09 (2.13 - 4.34)	14.28 (10.78 – 17.79)
EG (weeks)	< 28 weeks	8 (8.3)	453 (0.5)	17.66 (7.62 - 34.8)	85.6 /1000
	28-32 weeks	9 (9.4)	1612 (1.9)	5.58 (2.55 - 10.6)	54.9/1000 (28-30 weeks)
	33-36 weeks	16 (16.7)	8608 (10)	1.86 (1.06 - 3.02)	5.01 /1000 (31-36 weeks)
	≥ 37 weeks	63 (65.6)	75582 (87.6)	0.83 (0.64 - 1.07)	1.10 (0.80 – 1.42)
Weight (g)	<1000 g	9 (9.4)	600 (0.7)	15 (6.86 - 28.47)	127.57/1000
	1000-1499 g	6 (6.2)	1034 (1.2)	5.8 (2.13 - 12.63)	34.03/1000
	1500-2499 g	18 (18.8)	8093 (9.4)	2.22 (1.32 - 3.52)	4.8/1000
	≥ 2500 g	63 (65.6)	76528 (88.7)	0.82 (0.63 - 1.05)	1.19/1000

Day of life (DOL) at seizure onset

Regarding the day of life (DOL) at seizure onset there was a marked significant difference between term and preterm neonates (Wilcoxon test, $p< 0.001$): DOL at seizure onset mean (±SD) for term were 3.61 (±5.75) days and for preterm was 17.07 (±21.19) days, whereas median was for term 2

days and for preterm 9.5 days. The distribution was not parametric with a variance very different between the two populations, with a much higher dispersion in preterm, so data are better presented in the table with percentiles and figure below.

	DOL at seizure onset	
	Term	**Preterm**
Mean	3.61	17.07
SD	5.75	21.19
Min	0	0
5°	**0**	**1**
10°	0	1.3
25°	1	2
50° (Median)	**2**	**9.5**
75°	4	22.5
90°	9	41.1
95°	**17**	**53.2**
Max	34	96

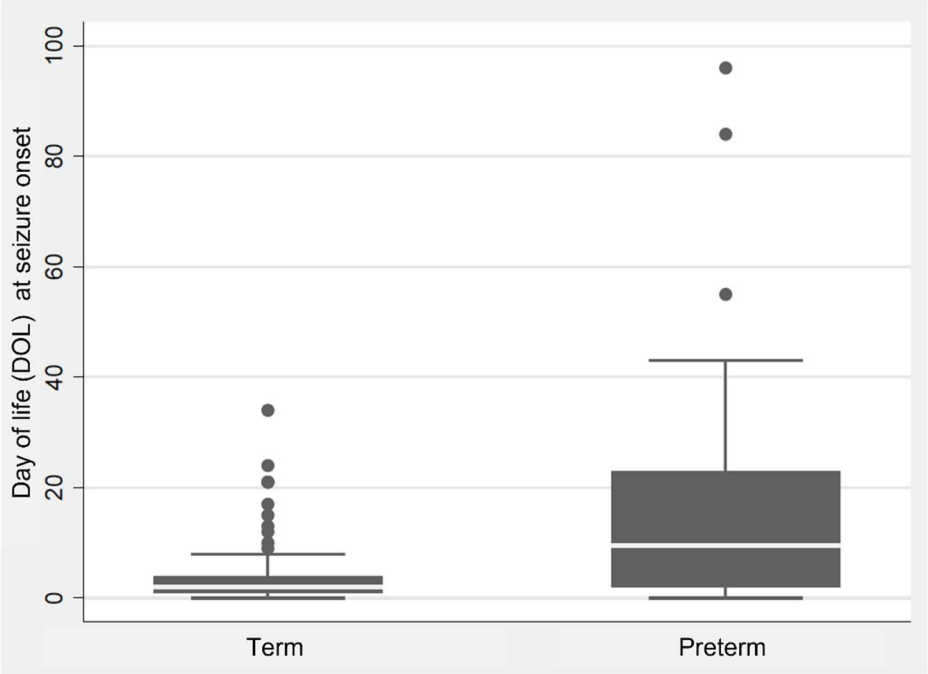

Figure: day of life (DOL) at seizure onset in term and preterm.

Similar significant difference was found in variables not independent from the gestational age (GA) variable as pathological pregnancy or multiple pregnancy or acidotic cord blood gas analysis and

related to acute brain injury risk factors for NS. Neonates born after a pathological pregnancy had significantly higher DOL at seizure onset: after physiological pregnancy DOL at seizure onset median 2 days (25° p: 1 day, 75° p: 4 days), after pathological pregnancy median 4 days (25° p: 1 day, 75° p: 17 days); Kruskal-Wallis test, p=0.043. Similar strong difference in DOL at seizure onset are found in twin neonates in comparison with non-twin neonates: in twin DOL median was 14 days, in non-twin was 2 days, Kruskal-Wallis test P=0.009. Neonates at birth with acidotic umbilical cord blood gas analysis had higher DOL at seizure onset (median: 3 days, 25°p: 1 day, 75°p: 11.5 days) than non-acidotic neonates (median: 1, 25°p: 0 days, 75°p: 3 days), Kruskal-Wallis test, p=0.0001.

We observed a tendency to a significant difference in the DOL at seizure onset in neonates with different seizure types. Neonates with electrographic seizures had a significant higher DOL at seizure onset median (median: 4, 25° p: 1 day, 75° p: 13.4 days) than neonates with motor seizures (median:1 day, 25° p: 1 day, 75° p: 5 days) or non-motor seizures (median: 0.5 days, 25° p: 1 day, 75° p: 4.5 days); Kruskal-Wallis test, p=0.08.

Regarding the neonates with epilepsy, we did not find significant differences in DOL at seizure onset between SeLNE (median: 2.5 days, 25°p: 2 days, 75°p: 3 days, min 1 day, max 5 days) and all DEE types (median: 2, 25°p: 1 days, 75°p: 5 days), Kruskal-Wallis test, p=0.3663. If we only considered SCN2A-KCNQ2 DEE cases (4 patients) the median was 1.5 days (25°p: 0.75 days, 75°p: 2.5 days, min 0.15 days, max 3.7 days), not so different in comparison with the 10 SeLNE cases above indicated.

We found a significant difference in DOL at seizure onset, being higher and more disperse in neonates with only one seizure type (median: 3 days, 25°p: 1 day, 75°p 12 days) and neonates with more than one seizure type (median: 2 days, 25°p: 1 day, 75°p: 4.5 days). Kruskal-Wallis test p= 0.0125.

We found a marked significantly difference in DOL at seizure onset according to the major etiology (Kruskal-Wallis test $p < 0.001$). Infection had the higher DOL at seizure onset (median: 17 days, 25°p: 9.5 day, 75°p: 24.5 days), whereas HIE had a DOL at seizure onset low as clinically expected (median: 1 day, 25°p: 0 days, 75°p: 2 days), vascular etiology a little superior to HIE (median: 2 days, 25°p: 1 day, 75°p: 3 days), followed by genetic etiology (median: 2 days, 25°p: 2 day, 75°p: 4 days) and metabolic etiology (median: 4.5 days, 25°p: 3 day, 75°p: 15 days). Among the less frequent etiologies brain malformation had a low DOL at seizure onset (median: 1 days, 25°p: 0.5 day, 75°p: 3.5 days) in comparison with post-natal cerebral anoxia (median: 5 days, 25°p: 2 day, 75°p: 14 days). The difference in DOL at seizure onset between acute provoked seizures and epilepsy was not significant ($p= 0.5$), as it could be expected given the high variability in DOL first of all among the provoked seizures itself, depending on the the specific acute injury.

In-hospital mortality

Neonates with seizures that died before discharge were 27/145 (18.62%).

No significant difference regarding the gender was found: female 16.13%, male 17% ($p=0.50$).

Conversely, a significant difference was observed in mortality between term 12/101 (11.9%) and preterm 15/44 (34.1%).

Regarding ethnicity a tendency to higher mortality was found in non-Caucasian (11/40, 27.5%) than Caucasian neonates 16/105 (15.24%), $p = 0.090$.

Mortality according to reason for admission in the Pathology Neonatal Unit or NICU was markedly different: 0/45 (0%) for neonates admitted for seizures and 27/99 (27.3%) for neonates admitted for other pathology.

Mortality in neonates born after pathological pregnancy was 16/50 (32%) and after physiological pregnancy was 10/94 (10.6%) for; $p = 0.002$.

Mortality according to seizure type tended to be lower for motor (14.1%) and non-motor seizure (18.7%) than for electrographic seizures (27.3%), although the difference did not reach statistical significance (p = 0.191). Among motor seizures, it was significantly lower for clonic seizures (11.1%) and higher for tonic seizures (42.9%), p = 0.067.

Mortality was higher for brain malformation (2/4, 50%), post-natal cerebral anoxia (3/6, 50%), metabolic etiology 4/8 (50% and HIE 8/34 (23.5%), whereas it was low for genetic etiology (1/21, 4.5%) and unknown etiology (0%), in the average range for vascular (16.2%) and infection (12.5%), p= 0.073.

Mortality was significantly higher for acute metabolic seizures of all types (not only IEM): 5/12 (41.67%) in comparison with other etiologies 22/133 (16.5%), p = 0.032.

Mortality of severe HIE with seizures was 8/14 (57.1%), whereas mortality of moderate HIE was 0/21 (0%), p = 0.000.

ILAE seizure type classification for neonates

Seizure type at presentation was classified as Electrographic in 44 (30.3%), Motor in 85 (58.6%), Non motor in 16 (11.0%).

ILAE SEIZURE TYPE	COUNTS (%)
ELECTROGRAPHIC ONLY	44 (30.3)
MOTOR	85 (58.6)
NON MOTOR	16 (11.0)
TOTAL	145 (100%)

Motor seizure types were subclassified in: Automatisms in 5/145 (3.4%), Clonic in 45 (31.0%), Myoclonic in 1 (0.7%), Sequential in 18 (12.4%), Spasms in 2 (1.4%) and Tonic in 14 (9.7%). Non motor seizure subtypes were subclassified as Autonomic in 14/145 (9.7%), behavior arrest in 2 (1.4%).

ILAE SPECIFIC SEIZURE TYPE	COUNTS (%)

ELECTROGRAPHIC	44 (30.3)
AUTOMATISMS	5 (3.4)
CLONIC	45 (31.0)
MYOCLONIC	1 (0.7)
SEQUENTIAL	18 (12.4)
SPASMS	2 (1.4)
TONIC	14 (9.7)
AUTONOMIC	14 (9.7)
BEHAVIORAL ARREST	2 (1.4)
TOTAL	145 (100%)

In 76 patients of 145 (52.4%) were reported other seizure types in addition to the presentation seizure type above specified with the following count/frequency: Electrographic (59, 78.7%), Automatisms (15, 20.0%), Clonic (12, 16.0%), Myoclonic (7, 9.3%), Sequential (3, 4.0%), Spasms (0, 0.0%), Tonic (11, 14.7%), Autonomic (12, 16.0%), Behaviour arrest (4, 5.3%).

We also recorded the first ictal onset sign not always correspondent to the most prominent sign that define the seizure type according to ILAE classification on NS (Pressler et al., 2021): Electrographic (44, 30.3%), Automatisms (9, 6.2%), Clonic (43, 29.7%), Myoclonic (1, 0.7%), Spasms (2, 1.4%), Tonic (27, 18.6%), Autonomic (13, 9.0%), Behavior arrest (6, 4.1%). In this case sequential was not part of the terms used as sequential described that seizure, where the prominent feature is the sequence of different signs itself and there is no single sign that could be indicated as predominant.

We evaluate the clinical seizure localization evolution: Focal – same side from onset (61, 45.2%), Focal to bilateral (29, 21.5%), Alternating-side-Focal-to-Bilateral; (13, 9.6%), Independent multifocal (12, 8.9%), Bilateral symmetric (8, 5.9%), Bilateral asymmetric (12, 8.9%).

In addition to epileptic seizures, in 27 of the 145 patients (18.6%) also non-epileptic paroxysmal movements of the following types were indicated: jitterness (16/27, 59.3% of all non-epileptic paroxysmal movements), non-epileptic myoclonus/clonus in 13, non-specific non-seizure movements in 5.

Gestational age (GA)

Unlike the gender (no significant difference between males and females has been found for all NS variables), gestational age had relevant impact on NS. We found a significant difference in seizure types between term and preterm neonates (Pearson chi square = 29.5, $p < 0.001$). A much higher frequency of electrographic seizures was found in preterm (52.3%) than term neonates (20.8%). Preterm neonates had more than triple tonic seizures than term neonates (18.2 versus 5.9%). Term neonates had more than double clonic (36.6 vs 18.2%) and autonomic (11.9 vs 4.5%) seizures than preterm neonates. We found sequential seizures only in term neonates, in whom they represented 17.8% of seizures. Infections and acute metabolic etiology had higher frequency in preterm than term neonates. The overall vascular etiology had a similar frequency in term and preterm neonates, but a different proportion of specific types of cerebrovascular disease was identified, as intraventricular hemorrhage (IVH) accounted for 22.7% of preterm NS and for only 4% of term NS, whereas arterial ischemic stroke accounted from 12.9% of term and 0% of preterm NS. Genetic etiology (without structural or inborn error of metabolism) was 21.78% in term neonates, but 0% in preterm neonates. Head trauma occurred in 3 term patients (2.97%) and 0 preterm neonates.

Variable	Variable options	Term (n = 101) N (%)	Preterm (n = 44) N (%)	P-value
Term_preterm	Born at term (≥ 37 weeks)	42 (41.6)	20 (45.5)	0.802
	Born pre term (≤ 36.6 weeks)	59 (58.4)	24 (54.5)	
Inborn_outborn	Inborn	63 (62.4)	33 (75.0)	0.198
	Outborn	38 (37.6)	11 (25.0)	
Seizure classification (ILAE)	Electrographic only	21 (20.8)	23 (52.3)	0.001*
	Motor	67 (66.3)	18 (40.9)	
	Non motor	13 (12.9)	3 (6.8)	
Seizure specific classification (ILAE)	Electrographic	21 (20.8)	23 (52.3)	<0.001*
	Automatisms	3 (3.0)	2 (4.5)	
	Clonic	37 (36.6)	8 (18.2)	
	Myoclonic	1 (1.0)	0 (0.0)	
	Sequential	18 (17.8)	0 (0.0)	
	Spasms	2 (2.0)	0 (0.0)	
	Tonic	6 (5.9)	8 (18.2)	
	Autonomic	12 (11.9)	2 (4.5)	
	Behavioral arrest	1 (1.0)	1 (2.3)	
Etiology (single major cause)	HIE (neonatal asphyxia)	25 (24.75)	9 (20.45)	<0.001*
	Vascular	27 (26.73)	10 (22.73)	
	Infectious	10 (9.9)	14 (31.82)	
	CNS infection	1 (1.0)	6 (13.6)	
	Sepsis only	0 (0.0)	3 (6.8)	
	Metabolic	6 (5.94)	6 (13.64)	
	Genetic	22 (21.78)	0 (0.0)	
	Brain malformation	3 (2.97)	1 (2.27)	
	Toxic	0 (0.0)	0 (0.0)	
	Post-natal cerebral anoxia	2 (2.00)	4 (9.09)	
	Head trauma	3 (2.97)	0 (0.0)	
	Unknown	3 (2.97)	0 (0.0)	
Checklist of all Etiologies				
HIE	Yes	26 (25.7)	9 (20.5)	0.494
	No	75 (74.3)	35 (79.5)	
Vascular	Yes	31 (30.69)	11 (25.0)	0.62
	No	70 (69.31)	33 (75.0)	
Infection	Yes	10 (9.9)	15 (31.8)	0.003*
	No	91 (90.1)	29 (68.2)	
All Metabolic	Yes	10 (9.9)	11 (25.0)	0.034*
	No	91 (90.1)	33 (75.0)	
Inborn error of metabolism (IEM)	Yes	5 (5.0)	3 (6.8)	0.699
	No	96 (95.0)	41 (93.2)	
Vitamin-related disorders	Yes	1 (1.0)	0 (0.0)	1

	No	100 (99.0)	44 (100.0)	
Acute metabolic	Yes	4 (4.0)	8 (18.2)	0.008*
	No	97 (96.0)	36 (81.8)	
Genetic	Yes	32 (31.7)	6 (13.6)	0.039*
	No	69 (68.3)	38 (86.4)	
Brain malformation	Yes	5 (5.0)	3 (6.8)	0.699
	No	96 (95.0)	41 (93.2)	
Toxic	Yes	0 (0.0)	0 (0.0)	1
	No	101 (100.0)	44 (100.0)	
Other specific etiology	Yes	5 (5.0)	4 (9.1)	0.455
	No	96 (95.0)	40 (90.9)	
	Post-natal cerebral anoxia	1 (20.0)	4 (100.0)	0.087
	Head trauma	3 (60.0)	0 (0.0)	
	Brain hypoxia in C.D.H.	1 (20.0)	0 (0.0)	
Unknown	Yes	3 (3.0)	0 (0.0)	0.553
	No	98 (97.0)	44 (100.0)	
Etiology	Acute Provoked Seizures	74 (73.3)	43 (97.7)	<0.001*
	Epilepsy	27 (26.7)	1 (2.3)	

HIE = hypoxic ischemic encephalopathy; CNS = central nervous system; C.D.H. = congenital diaphragmatic hernia; IEM = inborn error of metabolism; *p<0.05.

Etiology

Acute Provoked Seizures and Epilepsy

Neonatal seizures were diagnosed as acute provoked seizures (also previously defined acute symptomatic when manifestation of an acute structural or metabolic brain injury) in 117/145 patients (80.7%) and non-acute provoked seizures (or neonatal-onset epilepsy) in 28/145 (19.3%).

PROVOKED SEIZURES VS EPILEPSY	**COUNTS (%)**
ACUTE PROVOKED SEIZURES	117 (80.7)
UNPROVOKED SEIZURES (EPILEPSY)	28 (19.3)
TOTAL	145 (100%)

Regarding epilepsy frequency there was a significant difference among term and preterm neonates (Pearson chi square test = 11.7682, p = 0.001). All preterm neonates had acute provoked seizures except one (2,3% of preterm neonates with seizures): he was a neonate with TSC2-related tuberous sclerosis, presenting with electrographic seizures on the EEG, performed as screening test for his condition of high-risk neonate. In term neonates 27/101 (26,7%) had epilepsy, the rest had provoked seizures. The gender did not have significant influence on epilepsy.

Variable	**Term (n = 101)**	**Preterm (n = 44)**	**P-value**
Acute symptomatic	74 (63.2)	43 (36.8)	<0.001*
Epilepsy	27 (96.4)	1 (3.6)	

Variable	**Female (n = 62)**	**Male (n = 83)**	**P-value**
Acute symptomatic	49 (41.9)	68 (58.1)	0.822
Epilepsy	13 (46.4)	15 (53.6)	

Seizure type distribution was significantly different between acute provoked seizures and epilepsy ($p < 0.001$). Electrographic seizures are much more common among acute provoked seizures, whereas sequential seizures are much more common among epilepsy patients. Clonic seizures are common in both acute provoked seizures and epilepsy.

	Electrographic	Automatisms	Clonic	Myoclonic	Sequential	Spasms	Tonic	Autonomic	Behavioral arrest	P-value
Acute Provoked	42 (35.9)	5 (4.3)	39 (33.3)	1 (0.9)	3 (2.6)	0 (0.0)	13 (11.1)	12 (10.3)	2 (1.7)	<0.001*
Epilepsy	2 (7.1)	0 (0.0)	6 (21.4)	0 (0.0)	15 (53.6)	2 (7.1)	1 (3.6)	2 (7.1)	0 (0.0)	

Etiology classification

Etiology checklist

Regarding etiology of neonatal seizures, for each patient we first fulfilled an etiology checklist multiple choice field on the base of a list of a predefined etiology categories. Most patients have

been classified in only one etiological category, but some patients need to be classified in more than one etiological category. This occurred in two situations: when a unique specific etiology could be classified in more than one etiological category (as for example in the case of a patient with *ALDH7A1*-Pyridoxine Dependent Epilepsy, that need to be classified in three different categories: Genetic, Inborn Error of Metabolism, Vitamin-related disorder) or when more than one etiology was considered responsible for the neonatal seizures (as for example seizures occurring in a neonate with HIE and brain contusion due to skull fractures for a birth-related head trauma).

The results of the etiology category checklist assigned to each patient are presented in the following table. As consequence, the sum of the percentages presented in the table is superior to 100%.

ALL ETIOLOGY CHECKLIST	COUNT (%)
PERINATAL HIE	35 (24.1)
POST-NATAL CEREBRAL ANOXIA/HYPOXIA	6 (4.1)
VASCULAR	42 (29.0)
INFECTION	24 (16.6)
INBORN ERROR OF METABOLISM	8 (5.5)
VITAMIN-RELATED DISORDER	1 (0.7)
ACUTE METABOLIC	12 (8.3)
GENETIC	38 (26.2)
BRAIN MALFORMATION	8 (5.5)
TOXIC	0 (0.0)
UNKNOWN	3 (2.1)

Single Major Etiology

In addition to the above indicated etiology checklist, for each patient we evaluated the major etiology defined as the etiology that according to the integrated evaluation of all clinical and instrumental features of the case was considered the most relevant in the process of seizures generation in that patient. The results of the single major etiology (coming from a dedicated single

answer field in our database) are presented in the table below. In this table the sum of the major etiology percentages is 100%.

SINGLE MAJOR ETIOLOGY	COUNTS (%)
PERINATAL HIE	34 (23.4)
POST-NATAL CEREBRAL ANOXIA/HYPOXIA	6 (4.1)
ALL VASCULAR	37 (25.5)
INFECTIOUS	24 (16.6)
METABOLIC	12 (8.3)
GENETIC	22 (15.2)
BRAIN MALFORMATION	4 (2.8)
TOXIC	0 (0.0)
HEAD TRAUMA	3 (2)
UNKNOWN	3 (2.1)

Primary Etiology different from the major etiology

In each patient we also evaluated if there was a primary important etiology underlying the major etiology above indicated. We identified a primary etiology different from the major etiology in 20/145 (13.8%) and it was genetic in most of them (16/20, 80%) and due to other causes in 4/20 (20%).

Here we summarize the features of the 16 neonates in whom a genetic etiology was the underlying primary cause that initiated the pathological process that in the end led to neonatal seizures:

- Epilepsy in cortical brain malformation, 4 patients: 1 Alobar Holoprosencephaly, 1 Tuberous sclerosis, 1 Lissencephaly with Subcortical band heterotopia, 1 Frontotemporal pachygyria;
- Epilepsy (or unprovoked seizures) related to Inborn Errors of Metabolism (IEM), 2 patients: 1 Pyridoxine dependent epilepsy, 1 Non-ketotic hyperglicinemia;
- Acute metabolic etiology (provoked neonatal seizures, 8 patients: 6 patients with IEM presenting with acute metabolic derangement (as hyperammoniemia, dyselectrolitemia, see

them in the IEM section), 1 neonate with hypocalcemia due hypoparathyroidism to DiGeorge syndrome (22 deletion) with polymicrogyria; 1 patient with hypoglycemia due a congenital panhypopituitarism due to Pituitary stalk interruption syndrome.

- Vascular etiology (provoked seizures) in 2 patients: 1 neonate with brain vascular complication due to 21 trisomy severe cardiac malformation; 1 neonate with brain vascular complication in the context of complex brain malformation with CIC mutation (a VUS with high pathogenic potential).

The other 4 not genetic primary causes were: 1 myelomeningocele causing central nervous system infection, 1 congenital diaphragmatic hernia causing a post-natal cerebral hypoxia in severe lung disease, 1 complicated necrotizing enterocolitis with shock that led to post-natal cerebral anoxia, 1 cardiac arrhythmia that led to post-natal cerebral anoxia.

In conclusion we observed that a deep etiological evaluation showed that a genetic direct or indirect etiology could be recognized in 38/145 (26.2), with 22 (15.2%) as direct major etiology and 16 (11%) as indirect primary etiology, so a genetic contribution to neonatal seizures surpassed the contribution of perinatal HIE (34/145) or vascular (37/145) etiologies.

Specific Etiology

We further specified the etiology category indicated for the patient with a specific etiology subtype where more homogenous features could be presumed within the same etiology category.

Perinatal HIE

Among 35 perinatal HIE (35/145, 24.1%) indicated in the etiology checklist as principal or concomitant cause, female were 16/35 (45.7%) and male 19 (54.3%), Term (>= 37 weeks at birth) 26/35 (74.3%) and Preterm (<=36.6 weeks at birth) 9/35 (25.7%). As major etiology of seizures HIE was indicated in 34 (as in one patient HIE was considered comorbidity of head trauma).

According to severity, we classified perinatal HIE in mild, moderate and severe encephalopathy (Sarnat 1,2,3). In neonates with seizures, we had found moderate encephalopathy (Sarnat 2) in 21/35 (60%) and severe encephalopathy (Sarnat 3) in 14/35 (40.0%). Hypothermia treatment was performed in 28/35 (82.4%).

We calculated the incidence of moderate-severe HIE in our overall population of neonates recorded in the hospital (inborn and outborn) and also the inborn population only: moderate-severe HIE incidence in all population was 108/91236 corresponding to 1.18 for 1000 live births and in the inborn population only 70/86255 corresponding to 0.81 for 1000 live births.

We calculated the percentage of moderate-severe HIE with seizures on our total number of moderate-severe HIE: we found 34/108 (31.5%) neonates with moderate HIE had seizures for all the population of neonates admitted at our center and 19/70 (27,1%) of inborn neonates with HIE had seizures among the population of inborn moderate HIE. This means that almost one third of the moderate-severe HIE manifest seizures and around 52% of them were electrographic seizures. The association between moderate-severe HIE and electrographic seizures was significant (Pearson chi square test = 9.7411 p = 0.008).

Vascular etiology

42 patients (42/145, 29%) had the vascular etiology involved in seizure generation as principal or concomitant cause: ischemic stroke (13/42, 30.2%), venous thrombosis (5/42, 11.6%), hemorrhage (26/42, 62.8%). Vascular etiology as the principal etiology was indicated in 37/145 patients (25.5%): hemorrhage in 24/145 patients (16.6%, in three patients together with venous thrombosis) and ischemic stroke in 13/145 (9%). In 5 patients vascular etiology was a concomitant cause: in one patient venous thrombosis, in 4 patients hemorrhage (in 1 one patient together with venous thrombosis).

Vascular Etiology	Count	% of 145
Hemorrhage	24	16,6

Ischemic Stroke	13	9,0
Total vascular	37	25,5

Vascular (n = 42)	Counts (% of 42)
Ischemic stroke	13 (30.2)
Venous thrombosis	5 (11.6)
Hemorrhage	27 (62.8)

In the 26 patients with hemorrhage, the following hemorrhage subtypes have been reported IVH 2° grade (3/26, 11.5%), IVH 3° grade (5/26, 19.2%), IVH 4° grade (6/26, 23.1%), subdural haemorrhage (5/26, 19.2%), subaracnoideal haemorrhage (4/26, 15.4%), epidural haemorrhage (1/26, 3.8%), parenchymal haemorrhage (13/26, 50%). In 24 patient hemorrhage was the principal etiology of seizures, whereas in two patients was a concomitant cause.

In the 13 ischemic stroke neonates the medial cerebral artery territory was involved in 9/13 (70%) and all of them was the principal etiology.

In the 5 patients with venous thrombosis, dural (sagittal prominently) venous sinus was involved in 3/5 (60.0%), superficial cerebral veins in 2/5 (40.0%), internal cerebral veins in 2/5 (40.0%), medullary cerebral veins in 2 (40.0%). In all venous thrombosis cases in whom vascular etiology was the principal cause, hemorrhage was also present.

Arterial ischemic stroke (AIS) was strongly associated with term age (all 13 occurred in term neonates, Pearson chi test = 6.2211, p= 0.013).

Vascular etiology was significantly associated with clonic seizures in 23/42 (54,8%); Pearson chi square test= 25.5684, p = 0.001.

Among the cerebrovascular etiology subtypes there were significant differences as all 13 ischemic strokes presented with clonic seizures, whereas hemorrhage presented in 29% of patients with

electrographic seizures, in 25% of patients with autonomic seizures (apnoic seizures) and in 16% of patients with tonic seizures. Pearson chi square = 51.39, p = 0.005.

Vascular etiology is significantly associated with more than one seizure type in 28/42 (66.7%) in comparison with all the other etiologies 48/103 (48%). Pearson chi square test = 4.8157, p = 0.028. As the cerebrovascular population is heterogenous, we evaluated also separately arterial ischemic stroke, venous thrombosis and hemorrhage, finding that 80% of hemorrhage (with or without venous thrombosis) had more than one seizure type (often also electrographic), whereas in ischemic stroke only 38.4% had other types in addition to clonic seizures, mostly electrographic seizures (a percentage inferior to the non-vascular population that had 48%), Pearson chi square test = 11.34 p = 0.023.

Although extra-cerebral hemorrhages or parenchymal hemorrhages manifested with electrographic, motor or non-motor seizures, they tend to manifest with non-motor seizures (apnea) more frequently than other etiologies: 4/16 (30.5%) versus 12/132 (9%); Pearson chi square = 5.67, p= 0.059. At the same time brain hemorrhage significantly more frequently than all other etiologies manifested more than one seizure type (mostly electrographic seizure was the additional seizure type), Pearson chi square = 9.12 p = 0.003.

Infectious etiology

Infection etiology was indicated in 24/145 (16.6%). The specific microbic agent identified in our population of infectious neonatal seizures is shown in the table below.

MICROBE AGENT	COUNT (%)
STREPTOCOCCUS AGALACTIAE	6 (25.0)
ESCHERICHIA COLI	5 (20.8)
PROTEUS MIRABILIS	2 (8.3)
STREPTOCOCCUS PNEUMONIAE	1 (4.2)

KLEPSIELLA PNEUMONIAE	1 (4.2)
SEERATIA MARCESCENS	1 (4.2)
CITROBACTER KOSERI	1 (4.2)
HHV6	2 (8.3)
PARECHOVIRUS	1 (4.2)
NOT AVAILABLE	4 (16.7)
TOTAL	24 (100%)

Among 24 infections, only 3 had sepsis only, whereas 22 (91.7%) had central nervous system infections, with (7 patients) or without sepsis (15 patients). Infections were also classified according to the infection site, that could be indicated as multiple choice for each patient (so the sum is more than the total), as indicated in the table below:

Infection site	Counts (%)
Meningitis	19 (79.2)
Encephalitis	13 (54.2)
Brain abscesses	2 (8.3)
Sepsis	10 (41.7)

Infections as etiology of seizures were three times more frequent in preterm than in term neonates: 14/44 preterm (31.82%) versus term 10/101 (9,9%); Pearson chi square = 10.66, p = 0.001.

Infections presenting with different seizure types, mostly electrographic (8/24, 33.3%), clonic 6/24 (25%) or tonic 5/24 (20.8%).

Inborn Error of Metabolism (IEM)

Inborn Error of Metabolism was reported in 8/145 (5.5%) and further specified in: Leucinosis (Maple Syrup Urine Disease) (2/8, 25.0%), Methylmalonic acidemia (1/8, 12.5%), GLDC-related Non-ketotic hyperglycinemia (1/8, 12.5%), ALDH7A1-Pyridoxine-dependent epilepsy (PDE) (1/8, 12.5%), Urea cycle disorder (3/8, 37.5%). Among 3 urea cycle disorders, 1 patient had CPS1-

related Carbamoyl phosphate synthetase I deficiency, 1 patient had ASS1-related Citrullinemia, and for 1 patient the information about the specific urea cycle disorder was not available (early dead patient, not further studies available). 6 of the 8 neonates IEM (80%) were admitted for acute metabolic decompensation and during clinical and neurophysiological monitoring (aEEG /EEG) seizures have been diagnoses. IEM patients with acute provoked seizure presentation account for half of the patients presenting with acute metabolic provoked seizures discussed in the next paragraph dedicated to acute metabolic seizures.

Only two patients with IEM were admitted for neonatal seizures as prominent manifestation (and ware classified as "neonatal epilepsy" for the unprovoked seizure type presentation): a patient with ALDH7A1-Pyridoxine-dependent epilepsy (PDE) and a patient with a mild form of Non-ketotic hyperglycinemia.

Acute metabolic etiology

The acute metabolic cause was reported in 12/145 (8.3%). Most of patients had more than one metabolic alteration, so the information was collected with a multiple-choice field. The most frequent acute metabolic alterations were hypoglycemia in 5 (41.7%), dyselectrolytemia (hyponatremia, hypocalcemia, hyperpotassemia) in 6 (50.0%), hyperammonemia in 4 (33.3%), acute renal failure in 4 (33.3%), metabolic acidosis / hyperlacticaemia in 1 (8.3%).

Half of the patients with acute metabolic alterations causing seizures had also an IEM, the other half was due to other etiology, with the principal cause being acute renal failure, dyselectrolytemia and hypoglicemia. Acute metabolic etiology was significantly associated with preterm age: preterm 8/44 (18.18%), term neonates 4/101 (3.96%); Pearson chi square test = 8.1657, p = 0.004.

Genetic etiology

In 38/145 (26.2%) patients a genetic cause was directly or indirectly related to seizures: 28/145 (19.6%) had an epilepsy with neonatal onset, whereas 10/145 (6.8%) had provoked seizures caused

by another etiology linked to the primary genetic cause (structural as brain malformation, or metabolic - inborn errors of metabolism or acute metabolic -, or vascular etiology). The genetic contribution to neonatal seizures was present significantly more on term than preterm neonates with seizures: term 32/101 (31.68%), preterm 6/44 (13.64%); Pearson chi-square test = 5.16, p= 0.023.

Considering the overall genetic neonatal seizure population (both the cases manifesting with epilepsy and the cases manifesting with provoked seizures) the involved genes were the following: KCNQ2 (9/38, 23.7%), KCNT1 (2/38, 5.3%), SCN2A (3/38, 7.9%), STXBP1 (1/38, 2.6%), BRAT1 (1/38, 2.6%), 22q Deletion (1/38, 2.6%), STAG2 (1/38, 2.6%), ALDH7A1 (1/38, 2.6%), CACNA1G (1(38, 2.6%), TSC2 (1/38, 2.6%), QARS (1/38, 2.6%), CIC (1/38, 2.6%), ASS1 (1/38, 2.6%), CPS1 (1/38, 2.6%), GLDC (1/38, 2.6%), Trisomy 21 (1/38, 2.6%), Unknown genetic cause (11/38, 28.9%).

The genetic epilepsy cases were 28/145 (19.3%) and they were composed by: 22/145 (15.2%) functional epilepsy (channelopathies, synaptic transmission disorders, cell signaling disorders), 4/145 (2.7%) genetically determined cortical malformations, 2/145 (1.4%) inborn error of metabolism presenting with epilepsy as main symptom. The most common specific neonatal genetic etiology was KCNQ2-epilepsy, diagnosed in 9 of 28 neonatal epilepsy patients, so representing 32.1% of our genetic epilepsy population.

However, if we consider only the inborn neonatal epilepsy population, as we think it better reflects the general population incidence of KCNQ2 neonatal epilepsy, we found KCNQ2 epilepsy in 8 of 19 inborn genetic epilepsies (42%). Only 1 of those 8 KCNQ2 inborn neonatal epilepsy (12.5%) presented with DEE, whereas the remaining KCNQ2-epilepsies had a SeLNE phenotype. In the inborn population a definitive genetic diagnosis (with the identification of the causing gene mutation) was found in 14/19 (73.7%). The other genes involved were: KCNT1 in 1 (5.3%), BRAT in 1, CACNA1G in 1, TSC2 in 1, QARS in 1, genetic diagnosis unknown in 5 (26.3%).

On the contrary, if we considered the overall neonatal population, both inborn and outborn neonates, reflecting the different epidemiology of a tertiary center with an expected relevant selection bias toward a concentration of more severe diseases transferred by other peripheral hospital we found: KCNQ2 9 (32.1%), KCNT1 2 (7.1%), SCN2A 3 (10.7%), STXBP1 1 (3.6%), BRAT 1 (1, 3.6%), STAG2 1 (1, 3.6%), ALDH7A1 1 (3.6%), CACNA1G 1 (3.6%), TSC2 1 (3.6%), QARS 1 (3.6%), GLDC 1 (3.6%), Unknown 6 (21.4%). The genetic diagnostic rate is increased to almost four-fifths (78.6%) in all our genetic neonatal epilepsy population (inborn and outborn) with a greater rate of DEE than the inborn population alone. After KCNQ2, the most common genes were SCN2A (3 patients, 10.7%, all of them were outborn neonatal DEE) and KCNT1 (2 EIMFS patients, 7.1% of all population, 1 inborn patient and 1 outborn). All the other DEE genes were very rare, accounting for only 1 patient for each gene identified.

Regarding the epileptic syndrome classification in the inborn neonatal epilepsy population, we diagnosed SeLNE in 9 (47.3%). One SeLNIE phenotype with a history of early benign infantile epilepsy in the mother remained genetically undefined after negative NGS epilepsy panel study and KCNQ2-SCN2A MPLA investigations. All the other 8 SeLNE had KCNQ2 mutations. DEE was diagnosed in 10 among the 19 inborn neonatal-onset epilepsy cases (52.6%), whereas DEE was over-represented in all the population (inborn + outborn) with 18 /28 (64.3%), compatible with the expected concentration of severe cases transferred from peripheral centers to tertiary neonatal care centers.

The specific SeLNE syndromes identified in our overall neonatal genetic epilepsy population (inborn and outoborn) were Self-limited Neonatal Epilepsy (without previous known familiar history) (SeLNE) in 3/10 (30%, 2 KCNQ2, 1 unidentified gene), KCNQ2-Self-limited Familiar Neonatal epilepsy (SeLFNE) (6/10, 60 %), Self-limited Neonatal-Infantile Epilepsy (SeLNIE) (1/10, 10%, gene unidentified). Among the 18 DEE identified in our overall population, Early Infantile Developmental and Epileptic Encephalopathy (EIDEE) was diagnosed in 15 (83.3% of all

DEE), Epilepsy in Infancy with Migrating Focal Seizures (EIMFS) in 2 (11.1%), Infantile Epileptic Spasm Syndrome (IESS) in 1 (5.6%). In the table below the association of the gene identified and the corresponding epileptic syndrome.

Epilepsy Genes	SeLNE	SeLFNE	SeLNIE	EIDEE	EIMFS	IESS	Total
KCNQ2	2	6		1			9 (23.7)
KCNT1					2		2 (5.3)
SCN2A				3			3 (7.9)
STXBP1				1			1 (2.6)
BRAT				1			1 (2.6)
STAG2				1			1 (2.6)
ALDH7A1				1			1 (2.6)
CACNA1G				1			1 (2.6)
TSC2				1			1 (2.6)
QARS				1			1 (2.6)
GLDC				1			1 (2.6)
Unknown	1		1	3		1	6 (21.4)
Total	3	6	1	15	2	1	28 (100)

Below the table summarizing the genes identified in the 10 patients with genetic disease presenting with acute provoked seizures. In the following page are the tables related to the features of patients with genetic alterations.

Genes in diseases presenting with acute provoked seizures	Count (%)	Intermediate cause	Acute provoked event causing neonatal seizures
22q-deletion	1 (10)	Congenital hypoparathyroidism in DiGeorge Syndrome	Hypocalcemia
CIC	1 (10)	Complex brain malformation, with polimigrogyria as comorbidity	Brain Hemorrhage
ASS1	1 (10)	Citrullinemia	Hyperammoniemia
CPS1	1 (10)	Carbamoyl phosphate synthetase	Hyperammoniemia
Trisomy 21	1 (10)	Cardiac malformation	hemorrhage (in probable ischemia)

Unknown	2 (50.0)	Leucinosis	Acute metabolic derangement
Unknown	1 (10)	Methylmalonic acidemia	Acute metabolic derangement
Unknown	1 (10)	Urea Cycle Defect	Hyperammoniemia
Unknown	1 (10)	Congenital panhypopituitarism in Stalk pituitary syndrome	Hypoglicemia
Total	10 (100)		

In the table below are detailed the features of subjects with genetic neonatal-onset epilepsy.

record_id	Sex	GA	Consanguinity	Familiarity for epile	DOL at seizure onse	Spec. Neo Seiz Clas.	ILAE Epilepsy Syndrome	SeLNE type	DEE type	Provoked Seizures	Acute injury	Inborn Error of Metabolism	Brain malformation	Years from birth at molecular diagnos	GENE	Transcript	Genetic Variant	Inheritance
666-2	1	40,0	0	0	2	4	2		1	0				7,4	*SCN2A*	NM_001040142.2	c.4886G>A (p.Arg1629His)	Heterozigous; de novo
666-6 *	1	41,0	0	1	5	4	1	2		0				0,3	*KCNQ2*	NM_172107.4	c.472_473insG	Heterozigous; inherited, three family members described in the table
666-20 *	0	40,0	0	1	5	4	1	2		0				0,2	*KCNQ2*	NM_172107.4	c.472_473insG	Heterozigous; inherited, three family members described in the table
666-21	1	37,0	0	0	13	0	2		1	0				9,3	*BRAT1*	NM_152743.4	c.638dupA (p.Val214Gly-fsTer189); c. 294dupA (p.Leu99Thr-fsTer92)	Recessive: inherited compoundheterozigous mutations
666-22	1	40,0	0	0	34	2	2		1	0				2,8	*STXBP1*	NM_003165.6	c.922A>T, (p.Lys308(+))	Heterozigous; de novo
666-24	1	38,0	0	1	1	2	2		1	0			frontotemporal pachygiria	10,3	*QARS*	NM_005051.3	c.1841T>C, p(Ile614Thr); c.-15T>Ap.?	Recessive: inherited compound heterozigous mutations
666-27	0	37,1	0	0	0	2	2		1	0			Lissencephaly subcortical band heterotopia		Unknown			
666-34	0	39,0	0	0	21	5	2		3	0								
666-37	1	38,0	0	0	3	4				0				1,3	*KCNQ2*	NM_172107.4	c.1505 C>T, (p.A502V)	Heterozigous; inherited
666-47	1	39,0	0	0	1	4	2		1	0				0,9	*SCN2A*	NM_001040142.2	c.4633A>G, (p.Met1545 Val)	Heterozigous; de novo
666-53 *	1	40,3	0	1	2	4	1	2		0				0,5	*KCNQ2*	NM_172107.4	c.472_473insG	Heterozigous; inherited, three family members described in this table
666-57 §	1	39,0	0	1	2	4	1	2		0				0,6	*KCNQ2*	NM_172107.4	c.1525+5 G>A	Heterozigous; inherited, two family members described in this table
666-61	0	39,0	0	1	3	4	1	3		0					Unknown		Unknown	
666-64 §	0	38,1	0	1	2	4	1	2		0				1,0	*KCNQ2*	NM_172107.4	c.1525+5 G>A	Heterozigous; inherited, two family members described in this table
666-70	0	40,4	0	0	0	4	2		1	0				0,4	*SCN2A*	NM_001040142.2	c.1028A>T	Heterozigous; de novo
666-72	1	39,3	0	0	2	4	2		1	0				1,6	*CACNA1G*	NM_018896.5	c.4591A>G, p.(Met1531Val)	Heterozigous; de novo
666-76	1	38,6	0	0	1	7	2		2	0				0,2	*KCNT1*	NM_020822.3	(c.2849G>A, (p.Arg950Gln)	Heterozigous; de novo
666-85	1	35,7	0	0	6	0	2		1	0				1,5	*TSC2*	NM_000548.5	c.2540T>C, (p.Leu847Pro)	Heterozigous, unknown inheritance as parents not studied
666-87	1	39,0	0	0	1	7	2		1	0					Unknown			
666-88	0	40,0	0	0	3	4	1	1		0					Unknown			
666-89	0	39,0	0	0	2	2	2		1	0					Unknown			
666-98	0	39,7	0	1	4	6	2		1	0				0,7	*KCNQ2*	NM_172107.4	c.817-?_c.927+?del;p.? (deletion of the region including exon 6)	Heterozigous; inherited from an asymptomatic mother; an older brother with the a similar KCNQ2-DEE
666-113	1	37,3	0	1	1	4	1	2		0				0,1	*KCNQ2*	NM_172107.4	c.1217+1G>A	Heterozigous; inherited
666-118	0	37,1	1	0	0	5	2		1	0		Pyridoxine dependent epilepsy		0,1	*ALDH7A1*	NM_001182.5	p.Glu427Gln	Recessive: inherited homozigous mutations
666-123	0	40,4	0	0	2	4	1	1		0				0,1	*KCNQ2*	NM_172107.4	p.Gly271cys	Heterozigous; de novo
666-124	1	41,3	0	0	5	2	2		1	0		Non-ketotic hyperglicinemia		0,1	*GLDC*	NM_000170.3	c.806C>T (p.Thr269Met); deletion of exons 1 and 2 compound in heterozygosity	Recessive: inherited compound heterozigous mutations
666-139	0	38,9	0	0	2	4	2		2	0				0,1	*KCNT1*	NM_020822.3	c.2800G>A (p.Ala934Thr)	Heterozigous; de novo
666-147	0	39,6	1	0	1	2	2		1	0			Holoprosencepha	Prenatal	STAG2	NM_001375375.1	c.3034C>T (p.Arg1012ter)	Heterozigous; de novo

In the table below are indicated the features of the patients that had a genetic disease as primary cause, but that manifested acute provoked seizures mediated by another cause in the neonatal period.

record_id	Sex	GA	Consan-guinity	Familiarity for epilep	DOL at seizure onset	Neonatal seizure Classif	Provoked Seizures	Acute event related to neonatal seizures	Inborn Error of Metabolism	Brain malformation	Years from birth at molecular diagnos	GENE	Transcript	Genetic Variant	Inheritance
666-46	0	32,0	0	0	55	2	1	Hypocalcemia due to DiGeorge		polymicrogyria	0,3	2q11.2 deletion		DiGeorge Syndrome	Heterozigous; de novo
666-55 *	1	36,0	1	0	16	0	1	Acute metabolic derangement	Leucinosis			Unknown		Unknown	Recessive pattern, two family members described in this table
666-56 *	1	36,0	1	0	14	0	1	Acute metabolic derangement	Leucinosis			Unknown		Unknown	Recessive pattern, two family members described in this table
666-60	0	37,4	0	0	4	2	1	Hyperammoniemia	Methylmalonic acidemia			Unknown		Unknown	
666-67	1	32,6	0	0	9	6	1	Hyperammoniemia,	Urea cycle defect			Unknown		Unknown	
666-68	0	38,9	0	0	3	0	1	Hyperammoniemia, hyperpotassemia	Citrullinemia		0,2	*ASS1*	NM_054012.4	c.1168G>A (p.Gly390Arg)	Recessive: inherited homozigous mutations
666-77	0	39,0	0	0	1	2	1	Hypoglicemia due to panhypopituitaris		Pituitary stalk interruption syndrome		Unknown		Unknown	
666-122	1	38,9	0	0	3	2	1	Hyperammoniemia	Carbamoyl phosphate synthetase I deficiency		0,2	*CPS1*	NM_001875.5	Thr481Cysfs*58); p.(Arg1089C	Recessive: inherited compound heterozigous mutations
666-125	1	34,0	0	0	2	6	1	Brain hemorrhage in cardiac malformation, renal dysplasia,			Prenatal	21 Trisomy			
666-142	1	39,9	0	0	2	2	1	Brain hemorrhage in venous thrombosis		Polymalformative syndrome with Polymicrogyria and cerebellar hypoplasia	0,1	CIC	NM_001379482.1	c.6017C>T (p.Ser2006Phe)	Heterozigous; de novo

The overall population with genetic etiology had less electrographic seizures and non-motor seizures and more motor seizures in comparison with all the other etiologies all together; Pearson chi square test = 11.12, p= 0.004.

Among all seizure types, sequential seizures were significantly associated with genetic etiology in comparison with the other etiologies, 15/38 (39.5%) for genetic etiology versus 3/107 (2.8%) for the other etiologies; Pearson chi square = 45.13 p= 0.000. We compare the seizure onset sign (different from the prominent sign, on which the ILAE classification for neonatal seizures is based) and found that was significant different in genetic versus non-genetic cases. In genetic etiology the most frequent onset sign was tonic 16/38 (42.1%) and clonic 8/38 (21%), whereas in non-genetic etiology the most frequent onset sign was electrographic 39/107 (36.4%) and clonic 35/107 (32.7%), Pearson chi square test= 31.8, p = 0.000. The first onset sign could be classified as electrographic only when the seizure type was electrographic without ictal signs, otherwise the first onset sign was the first sign that appeared despite a previous electrographic discharge.

Interestingly, KCNQ2 and SCN2A cases (12 patients), that represented 31,6% of all genetic cases and the 42.8% of all genetic epilepsy patients, had sequential seizures with tonic onset (11 patient) or tonic seizures (1 KCNQ2 DEE).

Brain Malformation

Brain malformations were identified in 7/145 patients (4.8%), although we classified brain malformation as the major cause of epilepsy in 4 of them and as comorbidity in 3. Here are presented their specific features:

- 1 case of alobar holoprosencephaly related to mutation of STAG2 (pathogenetic according to the ACMG guidelines) presenting with severe refractory epilepsy who died within the first month of life (cortical malformation indicated as major cause);
- 1 case of Tuberous sclerosis related to TSC2 mutation presenting with neonatal epilepsy with electrographic seizures at onset and evolving toward IESS at 6 months of age (cortical malformation indicated as major cause).
- 1 case of subcortical band heterotopia in 1 presenting with very severe refractory epilepsy who died within the first month of life (CGH array performed, but unfortunately not a gene panel for neuronal migration disorders or NGS exome sequencing was not performed) (cortical malformation was indicated as the major cause);
- 1 case of brain malformation with frontotemporal pachygyria with refractory epilepsy since the first days of life, progressive microcephaly and severe neurodevelopmental disorder related to compound heterozygous QARS mutations with pathogenic potential (gene already in literature associated with recessive disease with this phenotype); an older sister of the patient has the same phenotype-genotype (brain malformation indicated as major cause);
- 1 case of polymicrogyria in 1 22-q deletion - DiGeorge syndrome presenting, with acute provoked seizures due hypocalcemia (related to the hypoparathyroidism-22q deletion syndrome), no further seizures after the neonatal age except for febrile seizures (cortical malformation as comorbidity).
- 1 case of complex cerebral malformation with polymicrogyria and cerebellar hypoplasia associated with a CIC de novo heterozygous mutation with pathogenetic potential (of

unknown significant at the moment according to the ACMG guidelines), presenting with acute provoked seizures due to brain hemorrhage successfully treated during the neonatal seizure with following development of ISS at 4 months successfully treated with ACTH and vigabatrin than switched to valproic acid (cortical malformation as comorbidity regarding neonatal seizures);

- 1 case of pituitary stalk interruption syndrome presenting with acute provoked seizures due to hypoglycemia (congenital panhypopituitarism is typically associated with the syndrome).

Other etiologies

In the etiology checklist of other etiologies (as principal cause or comorbidity) were recognized in 9/145 (6.2%) and they were further specified in: *post-natal anoxic encephalopathy* (due to cardiac arrest or heart rhythm disorder) in 5/145 (3.4%), *hypoxia due to severe lung disease* in a Congenital Diaphragmatic Hernia (CDH) in 1 (0.7%) and *head trauma* in 3 (2%).

Unknown etiology

In 3 patients (3/145, 2%) no cause could be demonstrated with certainty, but we believe that transient ischemic attack cause could be the most probable diagnosis, because of the perinatal risk factors encountered in them, the clinical and EEG focal unilateral features and the benign course observed at follow up.

Seizure type and Etiology correlation

We first perform a correlation between the major etiology (for definition only one for each patient) and the seizure type. We found the following significant associations: HIE as principal etiology was associated in 52.9% of cases with electrographic seizures ($p < 0.001$) , vascular etiology in 56.8% of cases with clonic seizures ($p < 0.001$), genetic etiology (excluding the brain malformations and the IEM) in 68.2% of cases with sequential seizures ($p < 0.001$). If we aggregate sequential seizures

and tonic seizures, the association with genetic etiology (without brain malformation and IEM) was even stronger (16/22 72.7%) in comparison with all the other etiologies (p <0.001).

Variable	HIE N (%)	Vascular N (%)	Infectious N (%)	Metabolic N (%)	Genetic N (%)	P-value
Electrographic	18 (52.9)	5 (13.5)	8 (33.3)	4 (33.3)	1 (4.5)	<0.001*
Automatisms	3 (8.8)	1 (2.7)	1 (4.2)	0 (0.0)	0 (0.0)	
Clonic	4 (11.8)	21 (56.8)	6 (25.0)	6 (50.0)	2 (9.1)	
Myoclonic	1 (2.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Sequential	3 (8.8)	0 (0.0)	0 (0.0)	0 (0.0)	15 (68.2)	
Spasms	0 (0.0)	0 (0.0)	0 (0.0)	1 (8.3)	1 (4.5)	
Tonic	2 (5.9)	4 (10.8)	6 (25.0)	1 (8.3)	1 (4.5)	
Autonomic	2 (5.9)	6 (16.2)	2 (8.3)	0 (0.0)	2 (9.1)	
Behavioral arrest	1 (2.9)	0 (0.0)	1 (4.2)	0 (0.0)	0 (0.0)	

Variable	Brain malformation N (%)	Head trauma N (%)	Post-natal cerebral anoxia N (%)	Brain hypoxia in C.D.H. N (%)	Unknown N (%)
Electrographic	1 (25.0)	1 (33.3)	5 (100.0)	1 (100.0)	0 (0.0)
Automatisms	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Clonic	3 (75.0)	2 (66.7)	0 (0.0)	0 (0.0)	1 (33.3)
Myoclonic	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Sequential	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Spasms	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Tonic	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Autonomic	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (66.7)
Behavioral arrest	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

We performed similar analysis correlation between seizure type and etiology, using the multiple-choice etiology checklist field (where more than one etiology could be expressed for each patient if it was the case). This analysis confirmed significant association between HIE and electrographic seizures, vascular etiology and clonic seizures and genetic etiology and sequential seizures.

		Electrographic (n = 44)	Automatisms (n = 5)	Clonic (n = 45)	Myoclonic (n = 1)	Sequential (n = 18)	Spasms (n = 2)	Tonic (n = 14)	Autonomic (n = 14)	Behavioral arrest (n = 2)	P-value
HIE	Yes (n = 35)	18 (51.4)	3 (8.6)	5 (14.3)	1 (2.9)	3 (8.6)	0 (0.0)	2 (5.7)	2 (5.7)	1 (2.7)	0.004*
	No (n = 110)	26 (23.6)	2 (1.8)	40 (36.4)	0 (0.0)	15 (13.6)	2 (1.8)	12 (10.9)	12 (10.9)	1 (0.9)	

Vascular	Yes (n = 42)	7 (16.3)	1 (2.4)	23 (54.8)	0 (0.0)	0 (0.0)	0 (0.0)	5 (11.9)	6 (14.3)	0 (0.0)	0.001*
	No (n = 103)	37 (35.9)	4 (3.9)	22 (21.4)	1 (1.0)	18 (17.5)	2 (1.9)	9 (8.7)	8 (7.8)	2 (1.9)	
Infection	Yes (n = 24)	8 (33.3)	1 (4.2)	6 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	6 (25.0)	2 (8.3)	1 (4.2)	0.069
	No (n = 121)	36 (29.7)	4 (3.3)	39 (32.2)	1 (0.8)	18 (14.9)	2 (1.6)	8 (6.6)	12 (9.9)	1 (0.8)	
IEM	Yes (n = 8)	3 (37.5)	0 (0.0)	3 (37.5)	0 (0.0)	0 (0.0)	1 (12.5)	1 (12.5)	0 (0.0)	0 (0.0)	0.391
	No (n = 137)	41 (29.9)	5 (3.6)	42 (30.7)	1 (0.7)	18 (13.1)	1 (0.7)	13 (9.5)	14 (10.2)	2 (1.5)	
Genetic	Yes (n = 38)	5 (13.2)	0 (0.0)	11 (28.9)	0 (0.0)	15 (39.5)	2 (5.3)	3 (7.9)	2 (5.3)	0 (0.0)	<0.001*
	No (n = 107)	39 (36.4)	5 (4.7)	34 (31.8)	1 (0.9)	3 (2.8)	0 (0.0)	11 (10.3)	12 (11.2)	2 (1.9)	
Vitamin-related disorders	Yes (n = 1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.034
	No (n = 144)	44 (30.6)	5 (3.5)	45 (31.2)	1 (0.7)	18 (12.5)	1 (0.7)	14 (9.7)	14 (9.7)	2 (1.4)	
Brain malformation	Yes (n = 8)	1 (12.5)	0 (0.0)	6 (75.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)	0 (0.0)	0.385
	No (n = 137)	43 (31.4)	5 (3.6)	39 (28.5)	1 (0.7)	18 (13.1)	2 (1.5)	14 (10.2)	13 (9.5)	2 (1.5)	
Acute metabolic	Yes (n = 12)	5 (41.7)	0 (0.0)	5 (41.7)	0 (0.0)	0 (0.0)	0 (0.0)	2 (16.7)	0 (0.0)	0 (0.0)	0.651
	No (n = 133)	39 (29.3)	5 (3.8)	40 (30.1)	1 (0.7)	18 (13.5)	2 (1.5)	12 (9.0)	14 (10.5)	2 (1.5)	
Other	Yes (n = 9)	7 (77.8)	0 (0.0)	2 (22.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.334
	No (n = 136)	37 (27.2)	5 (3.7)	43 (31.6)	1 (0.7)	18 (13.2)	2 (1.5)	14 (10.3)	14 (10.3)	2 (1.5)	
Unknown	Yes (n = 3)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (66.7)	0 (0.0)	0.222
	No (n = 142)	44 (31.0)	5 (3.5)	44 (31.0)	1 (0.7)	18 (12.7)	2 (1.4)	14 (9.7)	12 (8.4)	2 (1.4)	

We evaluated seizure type distribution for each etiology subtypes. Regarding HIE, we observed that the moderate HIE (Sarnat 2) in comparison severe HIE (Sarnat 3) had a higher rate of electrographic seizures (Sarnat 3 64.3% versus Sarnat 2 42.9%), but the difference did not reach statistical significance.

Variable options	**Electrographic (n = 18)**	**Automatisms (n = 3)**	**Clonic (n = 5)**	**Myoclonic (n = 1)**	**Sequential (n = 3)**	**Tonic (n = 2)**	**Autonomic (n = 2)**	**Behavioral arrest (n = 1)**	**P-value**
Sarnat 2	9 (42.9)	3 (14.3)	3 (14.3)	0 (0.0)	3 (14.3)	1 (4.8)	1 (4.8)	1 (4.8)	0.482
Sarnat 3	9 (64.3)	0 (0.0)	2 (14.3)	1 (7.1)	0 (0.0)	1 (7.1)	1 (7.1)	0 (0.0)	

Regarding cerebrovascular diseases, we found a strong significant association (p= 0.001) between ischemic stroke and clonic seizures (all ischemic seizures were clonic), whereas hemorrhage had an heterogeneous seizure type presentation, mostly manifesting with clonic, autonomic or electrographic seizures.

Variable	Variable options	Electrographic (n = 7) N (%)	Automatisms (n = 1) N (%)	Clonic (n = 23) N (%)	Tonic (n = 5) N (%)	Autonomic (n = 6) N (%)	P-value
Ischemic stroke	Yes	0 (0.0)	0 (0.0)	13 (100.0)	0 (0.0)	0 (0.0)	0.001 *
(n = 13)	No	7 (24.1)	1 (3.4)	10 (34.5)	5 (17.2)	6 (20.7)	
Venous thrombosis	Yes	1 (20.0)	0 (0.0)	3 (60.0)	1 (20.0)	0 (0.0)	0.902
(n = 5)	No	6 (16.2)	1 (2.7)	20 (54.1)	4 (10.8)	6 (16.2)	
Hemorrhage	Yes	7 (26.9)	1 (3.8)	8 (30.8)	4 (15.4)	6 (23.1)	0.001 *
(n = 26)	No	0 (0.0)	0 (0.0)	15 (93.8)	1 (6.2)	0 (0.0)	
IVH 1° grade	No	7 (26.9)	1 (3.8)	8 (30.8)	4 (15.4)	6 (23.1)	NA
IVH 2° grade	Yes	0 (0.0)	0 (0.0)	1 (33.3)	1 (33.3)	1 (33.3)	0.708
	No	7 (30.4)	1 (4.3)	7 (30.4)	3 (13)	5 (21.7)	
IVH 3° grade (+ IVH 4° grade)	Yes	3 (27.3)	1 (9.1)	3 (27.3)	3 (27.3)	1 (9.1)	
	No	4 (26.7)	0 (0.0)	5 (33.3)	1 (6.7)	5 (33.3)	
Subdural haemorrhage	Yes	2 (40.0)	0 (0.0)	1 (20.0)	0 (0.0)	2 (40.0)	0.709
	No	5 (23.8)	1 (4.8)	7 (33.3)	4 (19)	4 (19)	
Subaracnoideal haemorrhage	Yes	0 (0.0)	1 (25.0)	1 (25.0)	0 (0.0)	2 (50.0)	0.065
	No	7 (31.8)	0 (0.0)	7 (31.8)	4 (18.2)	4 (18.2)	
Epidural haemorrhage	Yes	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	1
	No	7 (28.0)	1 (4.0)	7 (28.0)	4 (16.0)	6 (24.0)	
Parenchimal haemorrhage	Yes	3 (23.1)	0 (0.0)	5 (38.5)	1 (7.7)	4 (30.8)	0.596
	No	4 (30.8)	1 (7.7)	3 (23.1)	3 (23.1)	2 (15.4)	

Regarding infection, we studied the association between the microbic agent and seizures type and among the 6 patients with Escherichia Coli infection 3 patient (50%) presented with tonic seizures.

Variable	Variable options	Electrographic	Automatisms	Clonic	Tonic	Autonomic	Behavioral arrest	P-value
Streptococcus agalactiae	Yes	2 (33.3)	0 (0.0)	1 (16.7)	2 (33.3)	1 (16.7)	0 (0.0)	0.964
	No	6 (33.3)	1 (5.6)	5 (27.8)	4 (22.2)	1 (5.6)	1 (5.6)	
Escherichia coli	Yes	0 (0.0)	0 (0.0)	0 (0.0)	3 (60)	1 (20)	1 (20)	0.014*
	No	8 (42.1)	1 (5.3)	6 (31.6)	3 (15.8)	1 (5.3)	0 (0.0)	
Proteus mirabilis	Yes	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.358
	No	8 (36.4)	1 (4.5)	4 (18.2)	6 (27.3)	2 (9.1)	1 (4.5)	
Streptococcus pneumoniae	Yes	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1
	No	7 (30.4)	1 (4.3)	6 (26.1)	6 (26.1)	2 (8.7)	1 (4.3)	
Klepsiella pneumoniae	Yes	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1
	No	7 (30.4)	1 (4.3)	6 (26.1)	6 (26.1)	2 (8.7)	1 (4.3)	
Seeratia marcescens	Yes	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.071
	No	8 (34.8)	0 (0.0)	6 (26.1)	6 (26.1)	2 (8.7)	1 (4.3)	
Citrobacter koseri	Yes	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1
	No	7 (30.4)	1 (4.3)	6 (26.1)	6 (26.1)	2 (8.7)	1 (4.3)	
HHV6	Yes	1 (50.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	1
	No	7 (31.8)	1 (4.5)	5 (22.7)	6 (27.3)	2 (9.1)	1 (4.5)	
Parechovirus	Yes	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.667
	No	8 (34.8)	1 (4.3)	5 (21.7)	6 (26.1)	2 (8.7)	1 (4.3)	
Other	No	8 (33.3)	1 (4.2)	6 (25.0)	6 (25.0)	2 (8.3)	1 (4.2)	NA
Unidentified	Yes	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1
	No	7 (30.4)	1 (4.3)	6 (26.1)	6 (26.1)	2 (8.7)	1 (4.3)	

The IEM were few cases and divided in many subtypes. The case of Pyridoxine-dependent epilepsy (PDE) presented with spasms (and myoclonic) seizures.

Variable options	Electrographic (n = 3)	Clonic (n = 3)	Spasms (n = 1)	Tonic (n = 1)	P-value
Leucinosis (Maple Syrup Urine Disease)	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.671
Methylmalonic acidemia	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	
Non-ketotic hyperglicinemia	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	
Pyridoxine-dependent epilepsy (PDE)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	
Urea cycle disorder	1 (33.3)	1 (33.3)	0 (0.0)	1 (33.3)	

Regarding the seizure type presentation among the cases with genetic etiology, we compared the genetic cases presenting with epilepsy with those presenting with acute provoked seizures. We observed that sequential seizures were associated with genetic epilepsy (p=0.002).

Variable	Electrographic	Clonic	Sequential	Spasms	Tonic	Autonomic	P-value
Acute symptomatic	3 (30.0)	5 (50.0)	0 (0.0)	0 (0.0)	2 (20.0)	0 (0.0)	0.002*
Epilepsy	2 (7.1)	6 (21.4)	15 (53.6)	2 (7.1)	1 (3.6)	2 (7.1)	

We evaluated seizure type distribution for each specific gene and found a very strong association between the KCNQ2 gene and sequential seizures: 8 KCNQ2 SLNE patients had sequential seizures, 1 KCNQ2 DEE presented with tonic seizures. In the 8 KCNQ2 SLNE we could observe figure-of-4-sign seizure as further descriptor of seizures at their onset in this population. The 3 SCN2A DEE presented with sequential seizures with an alternating side fencer-like asymmetric tonic posture at seizure onset. All the KCNQ2 and SCN2A sequential seizures here reported started with tonic sign as first manifestation, more commonly than sequential seizures due to other etiologies.

Variable	Variable options	Electrographic N (%)	Clonic N (%)	Sequential N (%)	Spasms N (%)	Tonic N (%)	Autonomic N (%)	P-value
KCNQ2	Yes	0 (0.0)	0 (0.0)	8 (88.9)	0 (0.0)	1 (11.1)	0 (0.0)	0.011*
	No	5 (17.2)	11 (37.9)	7 (24.1)	2 (6.9)	2 (6.9)	2 (6.9)	
KCNT1	Yes	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	1 (50.0)	0.242
	No	5 (13.9)	11 (30.6)	14 (38.9)	2 (5.6)	3 (8.3)	1 (2.8)	
SCN2A	Yes	0 (0.0)	0 (0.0)	3 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.557
	No	5 (14.3)	11 (31.4)	12 (34.3)	2 (5.7)	3 (8.6)	2 (5.7)	
STXBP1	Yes	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.586
	No	5 (13.5)	10 (27.0)	15 (40.5)	2 (5.1)	3 (8.1)	2 (5.4)	
BRAT	Yes	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.318
	No	4 (10.8)	11 (29.7)	15 (40.5)	2 (5.4)	3 (8.1)	2 (5.4)	
DiGeorge syndrome	Yes	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.599
	No	5 (13.5)	10 (27.0)	15 (40.5)	2 (5.4)	3 (8.1)	2 (5.4)	
STAG2	Yes	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.585
	No	5 (13.5)	10 (27.0)	15 (40.5)	2 (5.4)	3 (8.1)	2 (5.4)	
ALDH7A1	Yes	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0.116
	No	5 (13.5)	11 (29.7)	15 (40.5)	1 (2.7)	3 (8.1)	2 (5.4)	
CACNA1G	Yes	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	1
	No	5 (13.5)	11 (29.7)	14 (37.4)	2 (5.4)	3 (8.1)	2 (5.4)	
TSC2	Yes	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.3
	No	4 (10.81)	11 (29.7)	15 (40.5)	2 (5.4)	3 (8.11)	2 (5.4)	
QARS	Yes	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.626
	No	5 (13.5)	10 (27.0)	15 (40.5)	2 (5.4)	3 (8.1)	2 (5.4)	
CIC	Yes	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.592
	No	5 (13.5)	10 (27.0)	15 (40.5)	2 (5.4)	3 (8.1)	2 (5.4)	
ASS1	Yes	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.32
	No	4 (10.8)	11 (29.7)	15 (40.5)	2 (5.4)	3 (8.1)	2 (5.4)	
CPS1	Yes	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.603
	No	5 (13.5)	10 (27.0)	15 (40.5)	2 (5.4)	3 (8.1)	2 (5.4)	
GLDC	Yes	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.604

	No	5 (13.5)	10 (27.03)	15 (40.5)	2 (5.4)	3 (8.11)	2 (5.4)	
Trisomy 21	Yes	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0.178
	No	5 (13.5)	11 (29.7)	15 (40.5)	2 (5.4)	2 (5.4)	2 (5.4)	
Unknown	Yes	2 (18.2)	4 (36.4)	2 (18.2)	1 (9.1)	1 (9.1)	1 (9.1)	0.455
	No	3 (11.1)	7 (25.9)	13 (48.1)	1 (3.7)	2 (7.4)	1 (3.7)	

Epileptic syndromes: genotype-phenotype correlation, therapy, and outcome

We had 28 neonatal epilepsies: 10 were self-limited neonatal epilepsy (SeLNE) and 18 were developmental and epileptic encephalopathy.

Epileptic syndrome	**N (%)**
Self-limited epilepsy	10 (35.7)
Developmental and epileptic encephalopathy (DEE)	18 (64.3)

We evaluated the epileptic syndrome distribution according to age and gender. Regarding the gender, no significant difference was found. Regarding the gestational age, only one patient with DEE was preterm, the rest of genetic epilepsy were all term neonates.

Epilepsy	**Female (n = 13)**	**Male (n = 14)**	**P-value**
Self-limited epilepsy	5 (50.0)	5 (50.0)	0.695
Developmental and epileptic encephalopathy (DEE)	8 (44.4)	10 (55.6)	

Epilepsy	**Term (n = 26)**	**Preterm (n = 1)**
Self-limited epilepsy	10 (100.0)	0 (0.0)
Developmental and epileptic encephalopathy (DEE)	17 (94.4)	1 (5.6)

In our population DEE could be classified all as Early infantile DEE, except two patient that had epilepsy of infancy with migrating focal seizures (EIMFS) and one had Infantile Epileptic Spasms Syndrome (IESS).

Variable	N (%)
EIDEE	15 (83.3)
EIMFS	2 (11.1)
IESS	1 (5.6)

We evaluated the correlation between the epileptic syndrome and the seizure type, finding a strong association between SeLNE and sequential seizures, whereas DEE presented with variable seizure type. In DEE electrographic presentation was observed in one case of BRAT1 and in one case of TCS2 (the only preterm DEE with seizures), but both patients in the following days for BRAT1 and in the following weeks for TSC2 developed electroclinical seizures in addition to electrographic seizures, despite first-line anti-seizure medication.

Variable	Electrographic (n = 2)	Clonic (n = 6)	Sequential (n = 14)	Spasms (n = 2)	Tonic (n = 1)	Autonomic (n = 2)	P-value
Self-limited epilepsy	0 (0.0)	0 (0.0)	10 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.011*
Developmental and epileptic encephalopathy (DEE)	2 (11.1)	6 (33.3)	5 (27.8)	2 (11.1)	1 (5.6)	2 (11.1)	

Variable	Electrographic (n = 2)	Clonic (n = 6)	Sequential (n = 5)	Spasms (n = 2)	Tonic (n = 1)	Autonomic (n = 2)	P-value
EIDEE	2 (13.3)	6 (40)	4 (26.7)	1 (6.7)	1 (6.7)	1 (6.7)	0.283
EIMFS	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	1 (50.0)	
IESS	00 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	

The association between the gene and the epileptic syndrome for the 28 epilepsy patients of our cohort are summarized in the table below.

Epilepsy Genes	SeLNE	SeLFNE	SeFNIE	EIDEE	EIMFS	IESS	Total
KCNQ2	2	6		1			9 (23.7)

KCNT1					2		2 (5.3)
SCN2A				3			3 (7.9)
STXBP1				1			1 (2.6)
BRAT				1			1 (2.6)
STAG2				1			1 (2.6)
ALDH7A1				1			1 (2.6)
CACNA1G				1			1 (2.6)
TSC2				1			1 (2.6)
QARS				1			1 (2.6)
GLDC				1			1 (2.6)
Unknown	1		1	3		1	6 (21.4)
Total	3	6	1	15	2	1	28 (100)

KCNQ2 epilepsy

The most common specific neonatal genetic etiology was KCNQ2-epilepsy, diagnosed in 9 of 28 neonatal epilepsy patients (32.1%). This rate may reflect the epidemiology of a tertiary center where an expected relevant bias toward more severe DEE may exist. All 9 KCNQ2 epilepsies presented in term neonates. If we consider only the inborn neonatal population to avoid this bias, as we suppose this part of our population better reflect the general population incidence, we found KCNQ2 epilepsy in 8 of 19 inborn genetic epilepsies (42% of all genetic inborn epilepsies). Only 1 of those 8 KCNQ2 inborn neonatal epilepsy (12.5%) presented with DEE, whereas the rest presented with SeLNE. We calculated the incidence of KCNQ2 in our inborn population: 8 KCNQ2-epilepsy were found among 86255 live births at our center in the same period, corresponding to 9.2 per 10000, similar to the incidence of 5.89/10 000 (with CI 2.24–9.56) reported by Symonds et al. in a recent large population-based study on child genetic epilepsy.

SeLNE

Self-limited Familiar Neonatal Epilepsy history in one parent was found in 5 patients (3 sibling from one family and two siblings from another family), and in one sibling in 1 patient (patient 113, in whom the carrier mother did not have a personal history of epilepsy). All 6 were finally diagnosed with KCNQ2-SeLFNE.

Another Self-Limited Infantile Epilepsy in one parent was reported in one patient (Patient 61), but this case remained genetically undetermined (familiar and electroclinical features of SeLNIE).

As 5 of the 8 inherited KCNQ2 SLNE cases of our population had one of the parents with history of SLNE, penetrance of KCNQ2 in our population was 62.5%.

In one inherited KCNQ2-SLNE (patient 37) the carrier father did not have a personal history of epilepsy, but the proband also inherited two KCNQ2 rare variants/polymorphisms from the mother that could have a possible modulator effect (see patient 37 in the KCNQ2 table). One patient with de novo KCNQ2 mutation had SeLNE phenotype confirmed at 2 year-follow-up time.

All the 10 SLNE cases of our population had a normal developmental outcome (2 of them, patient 57 and 64 of the same family have been previously described (Dilena et al., 2022)) at the minimum follow up of 2 years for all (range 2-13 years). 8 of this 10 SLNE (80%) had KCNQ2 mutation (7 inherited mutation, 1 de novo mutation).

4/10 patients (40% of patients) responded easily to phenobarbital chosen as first option according to our NICU protocol: 3 siblings with SeLFNE (patients 6, 20, 53) and a patient with KCNQ2 sporadic inherited SeLNE (patient 37). The remaining 60% of SLNE patients responded to sodium channel blockers: patient 57 as second choice, as responded only partially to phenobarbital, then responded to phenytoin and was switched successfully to carbamazepine (CBZ). Given this family history her younger sister (patient 64) was directly treated with carbamazepine as first choice. A patient with de novo KCNQ2 SeLNE (patient 123) did not respond to phenobarbital too, but responded rapidly to carbamazepine and had a normal neurodevelopmental outcome. Another patient (patient 113) after a single phenobarbital load was switched to CBZ. One patient with sporadic SeLNE phenotype (patient 88) and another patient with neonatal presentation of a familiar SeLNIE (with negative genetic tests) (patient 61) responded only transiently to phenobarbital but reached a rapid and stable seizure control with CBZ. Carbamazepine for all cases was used at the average dose of 10 mg/kg divided in 2 or 3 doses per day and was withdrawn between 6 months and

2 years. In patient 57 a self-limited focal epilepsy developed at 8 years with spontaneous remission (not pharmacologically treated).

KCNQ2-DEE

The only KCNQ2 DEE patient (patient 98) has an exon 6 deletion inherited from the mother who was apparently asymptomatic. She had a brother 3 years older than her with a cognitive impairment, autism spectrum disorder, who previously had an early infantile epilepsy with onset at the age of two months with spontaneous remission. Our patient (patient 98) had seizure onset with frequent asymmetric tonic seizures with cyanosis at 4 days of life; only few seizures evolved with clonic manifestations after a long tonic phase. EEG showed a discontinuous pattern with abundant multifocal epileptiform abnormalities. Phenobarbital was not effective, but phenytoin and then carbamazepine was fully effective. After carbamazepine withdrawal at 2 years, at the age of three years she was still seizure free. She has a moderate-severe cognitive disability with autism spectrum disorder like the brother. She started to walk at two years of age, but language and socialization was poor. Both the proband and her brother had the KCNQ2 exon 6 deletion.

SCN2A DEE

All the 3 patients with de novo SCN2A presented with sequential seizures in the neonatal age, with a neurodevelopmental disorder spectrum from severe to mild. Background EEG had a burst-suppression-like pattern in patient 47, whereas in the other 2 patients had abundant multifocal epileptiform abnormalities. They all did not respond to phenobarbital, but sodium channel blockers were effective. Patient 2 had a partial response to phenytoin in association to phenobarbital before having a significant spontaneous reduction of seizures around 6 months. This patient has at 11 years has a severe neurodevelopmental; he is still treated with phenobarbital. Patient 47 (who has been previously described (Dilena et al., 2017)) was refractory to phenobarbital, but had a full response to high-dose phenytoin then switched to carbamazepine (35 mg/kg); he presented a moderate-severe neurodevelopmental outcome (no language, good social behavior, ataxic gait). At the current age of

9 years is seizure-free (no drug from the age of 4 years). Patient 70 did not respond to phenobarbital, but she had a full response to phenytoin, that was then switched to carbamazepine. She has a mild neurodevelopmental delay, and seizure relapse occurred when at 5 years antiseizure medication withdrawal was tried, so therapy was restored with seizure control.

KCNT1 DEE

2 patients of our series had KCNT1-related EIMFS phenotype with neonatal onset. Patient 76 presented with autonomic seizures (apnea) and automatisms since the first DOL with initial normal EEG and an apparent initial response to phenobarbital. Since the age of two months, refractory very frequent migrating focal seizures and status epilepticus refractory to all conventional antiseizure therapies developed. A partial and transient efficacy of chinidine for months was documented (as previously reported (Dilena et al., 2018)), but then with the time chinidine lost efficacy and was withdrawn, whereas levetiracetam was continued and increased with some partial efficacy. After the age of three years seizures had a spontaneous significant seizure reduction and although global neurological impairment is severe, some little improvement of interaction has been recently observed. Neurodevelopmental delay is still profound with acquired microcephaly. Patient 139 had refractory multifocal seizures developed since the first days of life, refractory to many conventional therapeutic trials (only levetiracetam seem to have a partial and transient efficacy for one week). Seizures tended to insist in the same focus for days and seem to change area and insist in the new area and so on. Ineffective treatment included ketogenic diet, cannabidiol, chinidine and fluoxetine (this drug as chinidine was tried as in vitro functional studies had shown correction of the pathological eccess of potassium currents due to the mutated channel). At one year of age, she is still in a comatose state with intractable sub continuous multifocal migrating focal seizures and frequent long status epilepticus periods, needing enteral feeding.

CACNA1G DEE

The patient 72 with CACNA1G DEE (de novo heterozygous mutation) had seizure onset at two days of life with sequential neonatal seizures (with focal tonic-automatisms-clonic sequence) and an EEG markedly discontinuous background pattern with multifocal spikes. Phenobarbital was effective on the seizures and the EEG improved after phenobarbital toward almost normal organization. He had mild facial dysmorphisms, plagiocephaly, bilateral I-II hand finger syndactyly. Between one and two months seizures relapse and phenytoin was added. Seizures gradually improved with seizure remission after 6 months. Brain MRI showed mild dysmorphic features of corpus callosum and the tentorium, a left cerebellar hemisphere infero-antero-medial alteration of possible gliotic nature and an aspecific alteration of the posterior white matter density. The patient at 4 years has a profound neurodevelopmental delay and a dystonic movement disorder, but is seizure-free.

STXBP1 DEE

The patient with STXBP1 DEE (patient 22, previously reported (Dilena et al., 2016)) presented at the end of neonatal period with clonic seizures refractory to phenobarbital, pyridoxine and phenytoin, but then had dramatic and rapid response to the levetiracetam intravenous load with full epilepsy control and EEG improvement after an initial EEG disorganization with multifocal epileptiform abnormalities. Despite good epilepsy treatment, he developed a severe intellective impairament (absent language) and a mild-moderate motor delay (at 4-5 years of age he started to walk). After the age of 2 years and half antiseizure medication was stopped without further seizure relapse. Genetic diagnosis was reached as 2 years of age with an epilepsy gene panel.

BRAT1 DEE

Patient 21 (previously described in literature (Carapancea et al., 2023)) was born with a weight small for gestational age (2530g). He had poor suction, very poor general movements, arthrogryposis, and no ocular track. The most relevant neurological finding was a very unusual congenital rigidity with deep tendon reflex hyperreflexia, with negative nose-tapping reaction. For

this reason, the patient underwent EEG that showed continuous background activity and unusual high amplitude frontal sharp waves. He performed EEG follow up and at 13 days of life EEG showed interictal multifocal interictal epileptiform abnormalities and repetitive electrographic-only occipital seizures. Phenobarbital was started. In the following days multifocal seizures with variable semeiology as tonic, clonic and automatism manifest. Phenytoin, pyridoxine, folinic acid, levetiracetam, midazolam, pyridoxal phosphate, clonazepam was tried without effect. At the end of the first month multifocal myoclonic jerks worsened. Brain MRI at 4 days of life showed only small epidural hemotoma (probably birth-related) and a brain CT scan at 50 days of life showed cerebral atrophy. The patient evolved toward a profound encephalopathy, rigidity, no feeding ability respiratory distress. The patient died at around 6 months. The genetic tests performed in 2011 were negative. Diagnosis was performed 10 years after death on a new gene panel including BRAT1 not previously included, identifying two heterozygous mutations (recessive pattern inheritance).

ALDH7A1-Pyridoxine-dependent epilepsy (PDE)

The patient (patient 118), born to a consanguineous couple, manifest since the first day of life with spasms and myoclonic semeiology. Neurological examination showed hypotonia and hyporeactivity. The EEG showed a burst suppression with with burst-related epileptic spasms. Brain MRI showed aspecific parenchymal edema and scattered punctate lesions in the white matter of unknown origin (possible microvascular nature). Seizures were controlled by phenobarbital and phenytoin with EEG normalization within 10 days. At one month of age during drug withdrawal seizures relapsed with a different semeiology characterized by focal motor tonic and clonic seizures, that was treated with phenobarbital and pyridoxine after the WES showed inherited homozygous mutation in ALDH7A1 gene (recessive inheritance). At 20 month a mild developmental delay is present with good seizure control.

QARS DEE

Patient 24 since the first day of life had focal seizures with clonic semeiology, disorganized, slow, paroxysmal EEG background, ictal dyscharges with very high amplitude frontal sharp wave, having a poor response to drugs. Neonatal brain MRI showed bilateral fronto-temporal pachygyria. At 10 years he has very severe neurodevelopmental delay, microcephaly, intractable epilepsy. WES has shown two compound heterozygous variants with pathogenic potential in the in silico analysis in the QARS, a gene that with a recessive inheritance has been previously (Zhang et al., 2014) associated with severe development delay, tetraparesis, microcephaly, epilepsy. The proband and her older sister had the same phenotype, with one variant inherited from the asymptomatic mother and the other variant from asymptomatic father.

TSC2 – Tuberous Sclerosis Complex

The patient (patient 85) was born at 35 weeks. Multiple rhabdomyomas were found at echocardiography, pointing toward a diagnosis of Tuberous Sclerosis Complex. EEG was performed as screening test for an high risk neonate: repeated electrographic seizures were detected in the right central regions. Background EEG was quite organized, but slow and epileptiform multifocal abnormalities were detected. Brain MRI showed multiple cortical tubers. A TSC2 mutation was then identified. Phenobarbital was started, but the patient in the following months developed Infantile spasms syndrome and was treated with vigabatrin and ACTH with good efficacy. Seizure relapse occurred in the following months with focal seizures, that were treated with carbamazepine with partial seizure control. The patient at the age of 18 months had moderate cognitive disability.

GLDC - nonketotic hyperglycinemia

Despite is metabolic disease, we decided to include this patient in the epilepsy section (as we have done with the patient with ALDH7A1-Pyridoxine-dependent epilepsy) since epilepsy in this patient was the main symptom at the neonatal presentation. Patient 124 had onset of clonic focal seizures at 5 days of life. The EEG background activity was slow with central interictal abnormalities.

Neurological examination showed hypotonia and hyporeactivity. The seizures were treated with phenobarbital and levetiracetam with full seizure control. Metabolic tests rapidly showed the diagnosis of nonketotic hyperglycinemia, so dextromethorphan and sodium benzoate were added to levetiracetam. GLDC compound heterozygous mutations have been found. At 2-year follow-up he has a moderate-severe cognitive and motor impairment. EEG showed slow activity with a recognizable awake-sleep differentiation, but no detectable sleep spindles.

STAG2 – Holoprosencephaly

During the pregnancy of patient 147, fetal ultrasound at the 20th-week showed a severe brain malformation. Fetal MRI documented semilobar holoprosencephaly with cerebellar vermis hypoplasia in addition to left renal pyelectasis, mild right heart predominance, diffuse subcutaneous edema, maxillary bone hypoplasia with labiopalatoschisis and bilateral exophthalmos. The fetal genetic investigations performed by WES documented a de novo STAG2 mutation compatible with holoprosencephaly. The patient was born at 39 weejs, had dysmorphic facies and labiopalatoschisis. Seizures were documented since the first day of life mainly with clonic manifestations. EEG documented an abnormal background activity with no physiological figures, unusual sharp theta wave rhythmic discharges. The patient was treated with phenobarbital and midazolam and transition to comfort care was applied with patient death at 10 DOL.

Genetically undefined DEE

We had 4 patients over 18 DEE with neonatal onset without genetic diagnosis (22.2%).

Patient 27. The patient was born at 37 weeks of age by normal vaginal delivery. She born with a low Apgar score, hypotonia, hyporeactivy, metabolic acidosis at birth, a very unusual flat EEG. Brain ultrasound first showed signs of possible cortical malformation. On the presumed hypothesis of HIE as comorbidity, hypothermia treatment was performed within 6 hours from birth. Seizures started in the first day characterized initially by modest bilateral clonic movements, preceded at

EEG by high frequency low-amplitude discharges that were followed by spikes/sharp wave discharges, whereas the rest of background activity was always flat. Cyclic seizures having a variable semeiology with electrographic, tonic, automatisms, sequential features occurred. Brain MRI showed a subcortical band heterotopia. Phenobarbital, phenytoin, levetiracetam, midazolam did not were effective. Status epilepticus was also treated with thiopental. The patient died at 20 DOL. CGH array was negative. Epilepsy and brain malformation gene panel was not performed in this case as at that moment (year 2012) were not routinely performed and the patient died early, so following genetic studies have not been performed.

Patient 34. The patient had onset of spasms at the age of three weeks. Neurological examination showed hypotonia and eye contact deficit. EEG showed burst-suppression pattern with burst-related epileptic spasms. At that time the case was diagnosed as Ohtahara syndrome and now reclassified IESS. Seizures did not respond to vitamins, vigabatrin and valproic acid, but responded partially to steroid treatment. CGH array and gene panel did not identified alterations. The patient developed tetraparesis and very profound cognitive and motor disability, with associated respiratory problems related to the severe neurological condition and died at around 7 years.

Patient 87. After a pregnancy complicated by the ultrasound identification of intrauterine growth retardation with a suspicion of congenital aortic coarctation, the patient born at 39 weeks of gestational age by cesarian section to non-consanguineous parents of Asian ancestry without perinatal complications (normal Apgar). The birth weight was small for gestation age (1875 g). He had mild unspecific dysmorphic facies, central hypotonia, iporeactivity. At one DOL he developed recurrent severe autonomic seizures with apnea and cyanosis. EEG showed initially focal right temporal electroclinical autonomic seizures, initially responsive to phenobarbital. In the following weeks refractoriness developed and multifocal ictal and interictal seizures appeared with poor response to all conventional drugs tried. He died at 6 months of life with recurrent seizures and global neurological deterioration. Brain MRI during neonatal age and at three months showed a

reduction of pons size without a brain MRI identifiable cortical malformation. GCH array, epilepsy gene panel and WES did not identified alterations.

Patient 89. Clonic seizures with bilateral symmetric manifestation and ictal unusually synchronous bilateral EEG spike discharges started at 2 days of life. EEG background was initially quite normal with some unusual fast activities. Seizure responded only partially to the conventional treatment (phenobarbital, carbamazepine, levetiracetam, phenytoin, pyridoxine). The clinical and EEG features slowly changed until the development at the age of 3 months of infantile spasms, that responded to vigabatrin and ACTH, that were then switched to valproic acid. At four years no seizure relapse occurred, but she has a severe global developmental delay: language is absent, but she can interact with non-verbal communication, she is able to sit, but she is not able to walk alone yet. Brain MRI is normal. CGH array, epilepsy gene panel and WES did not identified alterations.

Neurological examination (NE)

Neurological examination (NE) was available in 140/145 (96.6%). NE was normal in 34/140 (24.3%), mildly abnormal in 24/140 (17.1), moderately abnormal in 63/140 (45%), severely abnormal in 19/140 (13.6%). To simply the following analysis, we merge the four category in two categories. In this way we had only two categories of NE: normal (corresponding to a NE from normal to mildly abnormal) and abnormal EEG (corresponding to a NE from moderately abnormal to severe abnormal):

No significant differences in NE between males and females have been found (p=0.624).

A significant association between abnormal EEG and ethnicity was found (p=0.021), being higher in non-Caucasian (28/38, 73.7%) then Caucasian neonates (53/102, 51.9%).

An importance difference in the abnormal NE percentage was found between preterm (30%, 30/99) and term (4/41, 9.7%) was found (p=0.065).

A significant difference in abnormal NE between inborn (45/91, 49.4%) and outborn (73.5%) was found (p= 0.006).

A significant difference in abnormal NE was found between neonates admitted in NICU for seizures (15/45, 33.3%) and neonates admitted for other reason (66/94, 70.2%), $p < 0.001$.

Regarding NE and perinatal risk factors significant difference in abnormal NE was found between:

- neonates born with eutocic delivery (27/58%, 46.5%), dystocic delivery (4/5, 80%) and cesarian section (50/76 65.8%), p= 0.049;

- neonates with weight considered normal at birth for GA (59/111, 53.1%), small for GA (17/24, 70.8%) and large for GA (5/5, 100%), p=0.043;

- neonates who needed resuscitation maneuvers at birth (46/63, 73%) in comparison with the others (35/77, 45.4%), p= 0.001;

- neonates with acidotic (30/40, 75%) versus normal (46/95, 48.4%) cord umbilical blood gas analysis, p=0.004;

NE and seizure types

Electrographic seizures were significantly more associated with NE (32/42, 76.2%) than motor (44/83, 59%) and non-motor seizures (5/15, 33.3%), p = 0.006. Among the motor seizure type clonic seizures were associated with anormal NE in 23/45 (51.1%), whereas sequential seizures in only 5/18 (27.8%).

There was a significant association between neonates with more than one seizure type (48/73, 65,7%) in comparison with neonates with only one seizure type (33/67, 49.2%). p = 0.048

NE and etiology

Abnormal EEG was significantly associated with provoked seizures (70/112, 62.5%) in comparison with epilepsy (11/28, 39.3%), p=0.026.

If we considered the category of the single major etiology, a significant association was found between NE and etiology (p=0.001). Very strong association between abnormal NE and HIE (25/32, 78%) or infection (14/23, 60.9%) or metabolic etiology (11/12, 91.7%) in comparison with genetic 7/22 (31.8%) and vascular etiology (17/36, 47.2%).

Regarding epilepsy a strong association was found between abnormal NE and DEE (11/18, 61.1%), in comparison with SeLNE (0/10, 0%), p = 0.002.

Electroencephalography (EEG)

EEG Background (EB)

In the medical records of the patients the description of EB was available for 137/145 (94.5%).

For our analysis we used the "INNESCO" EB score (Dilena et al., 2021), where 0 is normal/mild alteration, 1 moderate alteration (discontinuous EEG), 2 severe alteration (burst-suppression), 3 very severe alteration (markedly depressed /flat).

We also performed our analysis in a simpler way dividing EB score as normal (0) and abnormal were categories 1-2-3 were unified.

No significant difference in EB were found regarding gender (male-female) or ethnicity (Caucasian /non-Caucasian).

Significant EB difference was found between inborn and outborn neonates, attributable mostly to a higher rate of normal EB in inborn (27/91, 31.9%) than in outborn neonates (10/46, 10%) and inversely to a higher rate of abnormal EB in outborn (14/46, 30.4%) than in inborn (6/91, 6.6%); $p = 0.002$.

Regarding reason of admission in NICU, abnormal EB rate was significantly higher in neonates admitted for other reasons than seizures: 18/45 (40%) neonates admitted for seizures had abnormal EB versus 79/91 (86,8%) of neonates admitted for other reasons, $p < 0.001$.

EEG background (EB) and perinatal risk factors in neonates with seizures

As expected, EB was found more significantly abnormal after pregnancy with any clinical complications then physiological pregnancy: 44/48 (91.7%) versus 54/89 (60.7%).

EB was found significantly more abnormal in neonates born by caesarean section (59/74, 79,7%) or by dystocia with vacuum extractor (4/5, 80%) than born by eutocic delivery (35/57, 59.6%); $p= 0.038$.

EB was found significantly more abnormal in neonates born with normal weight for gestational age (GA) (74/110, 67.3%) than small or large weight for GA (24/27, 88.9%) $p = 0.05$.

Neonates needing resuscitation at birth had a rate of severely altered EB significantly higher than the others: 57/64 (89.1%) versus 41/73 (56.2%).

Neonates with acidotic umbilical-cord blood gas analysis had a rate of abnormal EB significantly higher than neonates with normal umbilical-cord blood gas analysis: 38/40 (95%) versus 54/91 (59.34%), $p < 0.001$.

EEG background (EB) and seizure type

Different neonatal seizure types evaluated at seizure-onset are significantly associated with different EB alteration severity. Whereas most motor and non-motor seizure-type are associated with normal-moderate EB alterations (normal or discontinuous EEG), most electrographic only seizures are associated with severe (Burst-suppression pattern) or very severe (depressed/ flat EEG pattern) EB; $p < 0.001$. In details, one third of electrographic seizures had burst-suppression EB (13/39, 33.3%), another one third had depressed-flat EB, whereas only 2/39 (5.1%) had normal EEG and 11/39 (28.2%) had discontinuous EB (moderate alteration).

Conversely, 32/82 (39%) of all motor seizure types had normal EB, 28/82% (34.15%) had discontinuous EB, whereas only 15/82 (18.3%) had burst-suppression EB and 7/39 (8.5%) had a markedly depressed EEG.

Similarly, 5/16 (31.25%) of non-motor seizures (autonomic or behavior arrest seizures) had normal EEG and 9/16 (56.25%) had discontinuous EB, whereas only 2/16 (12.5%) had burst-suppression EB and none of the neonates with non-motor seizures had markedly depressed / flat EEG.

Significant differences have been found also regarding the EB of the different types of motor seizures and non-motor seizures ($p< 0.001$). In details, 22/44 (50%) of neonates with clonic seizures have normal EB, 14/44 (31.8%) have moderate EB abnormalities, whereas only 6/44 (13.6%) have

burst-suppression pattern and 2/44 (4.55%) have markedly depressed EB. Similarly, 9/18 (50%) of the neonates with sequential seizures had normal EB, 7/18 (38.9%) had moderately abnormal EB, whereas only 2/18 (11.2%) had severely or very severely abnormal EB. Autonomic seizures (mostly apneic seizures) were associated in 5/14 (35.7%) to normal EB, in 8/14 (57.1%) to moderately abnormal EB, whereas only 1/14 (7.14) to severely abnormal EB and none to very severely abnormal. Conversely tonic, spasms and automatisms seizures had much higher rate of severely or severely abnormal EB (burst-suppression or markedly depressed EB), respectively 7/12 (58.3%), 2/2 (100%) and 3/5 (60%).

We evaluated the association with EB and presence of more than one seizure type in addition to the prominent seizure type and found that 39/63 (61.9%) of neonates with only one seizure type had normal EB, whereas 59/74 (79.73%) of neonates with more than one seizure type had normal EB; p= 0.021.

EEG background (EB) and epileptic syndrome

Regarding neonates with epilepsy (28 patients) EB was significantly different between the two ILAE epileptic syndrome categories: SeLNE versus DEE.

EB was normal in 8/10 (80%) of SeLNE, but only in 4/18 (22.22%) of DEE; p= 0.003.

The 2/10 abnormal EB of SeLNE (20%) were classified as moderately abnormal, whereas the abnormal EB of DEE were 14/18 (77.8%), classified as moderately abnormal in 11/18 (68.1%), as severely abnormal (burst-suppression) in 2/18 (11.1%) and markedly depressed EEG in 1/18 (5.6%); p= 0.029.

EEG background (EB) and etiology

A significant difference was found between the EB of neonates with acute provoked seizures and neonates with epilepsy (p=0.016).

Normal EB was found normal in 12/28 (48.9%) of neonatal epilepsy patients and in only 27/109 (24.8%) of patients with acute provoked seizures. Abnormal EB was classified as moderately abnormal in 13/28 (46.4%) of neonatal epilepsy cases and in 35/101 (32.1%) of acute provoked seizure cases, as severely abnormal in 2/28 (7.1%) of neonatal epilepsy cases and 28/101 (25.7%) of acute provoked seizure cases, and as very severely abnormal in 1/28 (3.6%) epilepsy cases and 19/109 (17.4%) of acute provoked seizure cases.

EEG background (EB) and single major etiology category

The major etiology of neonatal seizures was significantly associated with the rate of abnormal EB ($p < 0.001$). All HIE (31/31) and post-natal cerebral anoxia/hypoxia (6/6) had an abnormal EB. Infection (18/21) and metabolic etiology (10/12) had respectively 85.7% and 83.3% of abnormal EB. Brain malformation (3/4) had 75% of abnormal EB, whereas genetic etiology without brain malformation or IEM (11/22) had 50% of abnormal EB. All three unknown etiology cases had normal EB.

EEG background (EB) and specific etiology

In addition to the association between EB and the major etiology category above described based on a field of etiology with only one answer possible, we evaluated the association between EB and the specific etiology as it comes from the etiology checklist multiple answer field where more than one etiology could be indicated.

HIE

Severity of EB abnormality was significantly different between moderate and severe HIE. In moderate HIE (Sarnat 2), moderate EB abnormality was found in the majority 11/18 (61.1%), severely abnormal EB in 6/18 (33.3%) and very severely abnormal EB in only 1/18 (5.6%). Conversely in the severe HIE (Sarnat3) moderate EB abnormality was found in only 1/14 (7.1%), whereas EB was severely abnormal EB in 5/14 (35.7%) and very severely abnormal EB in 8/14 (57.1%).

Cerebrovascular diseases

Normal EB was observed in 12/13 (92.3%) of arterial ischemic strokes, in 5/22 (22.73%) of hemorrhages and 2/4 (50%) of venous thrombosis; $p < 0.001$. The abnormal EB found in the one ischemic stroke (7.7%) was classified as moderately abnormal. In neonates with hemorrhages, EB was moderately abnormal in 6/22 (27.3%), severely abnormal in 9/22 (40.9%) and very severely abnormal in 2/22 (9.9%).

Infection

EB in infectious diseases tend to have a lower rate of normal EB (3/21, 14,3%) in comparison with all the other etiologies (36/116, 31%) and mostly were classified in the moderately abnormal category (12/21, 57.1%; versus all the other etiologies 36/116, 31%), although the difference did not reach statistical significance ($p= 0.125$).

IEM

All 8 patients with IEM presenting with seizures had abnormal EEG. The abnormality was shifted toward very severe EB abnormalities (4/8, 50%) in comparison with all the other etiologies (16/129, 12.4%), $p= 0.019$.

Acute metabolic

7/11 (63.6%) of neonatal seizures acute metabolic injury had severely or very severely EB abnormalities in comparison with 43/126 (34.1%) of all the other etiologies ($p= 0.141$). Half of our neonatal seizures (in 1 patient EB information misses) with acute metabolic disorders occurred in IEM patients who are described above.

Genetic etiology

If all the neonates with genetic conditions (both epilepsies and acute provoked conditions in genetic patients) were first analyzed together against all the other etiologies. No significant difference was

found in EB severity grade distribution, although with a tendency toward a lower grade of severity in genetic etiology (p = 0.189) in comparison with non-genetic conditions. This finding is compatible with the previous analysis showing a major impact on EB by acute provoked type (ischemic stroke very different from HIE) and severity of the acute provoked injury and by epileptic syndrome type (SeLNE different than DEE).

Brain Malformation

Brain malformation were a minority of the population: 4 patients in whom brain malformation was considered the major etiology of epilepsy and 4 patients were brain malformation was a comorbidity. Half of them (4/8) had moderately abnormal EEG, 3 (37.5%) had normal EEG and 1 had very severely abnormal EEG. EB appears very heterogeneous, compatibly with the heterogenous features of this population.

Other etiologies

Among other etiologies there were:

- Post-natal anoxia/hypoxia: 2/6 (33.3%) had moderately abnormal EB, 2/6 (33.3%) severe abnormal EB, 2/6 (33.3%) very severe abnormal EB;

- Head trauma with brain parenchymal contusion /hemorrhage from skull fractures: EEG was available in 2 patients, 1 had normal EB and 1 moderately abnormal EB;

- Unknown etiology: all had normal EB (3/3, 100%), whereas in other known etiologies rate of normal EB was 36/134 (26.8%) (p=0.005).

EEG Seizure pattern (ESP)

The information about EEG seizure lpattern (ESP), as the EB information, was collected by the medical records and was available in 128/145 (88.3%). We classified this information according to

the location pattern of ictal abnormalities at seizure onset as follows: 1, Focal/unilateral; 2, Multifocal; 3, Bilateral symmetric; 4, Bilateral asymmetric.

In all the neonatal seizures with this information available the distribution of ESP was: Focal/unilateral (77, 60.2%), Multifocal (29, 22.7%), Bilateral symmetric (10, 7.8%), Bilateral asymmetric (12, 9.4%). So in the overall neonatal population the onset of seizures was mostly focal or multifocal, more rarely bilateral (symmetric or asymmetric).

We evaluated the association between ESP and other clinically relevant factors as done for EB. A significant difference in the classification of ESP was found between neonates admitted in NICU for seizures in comparison with neonates admitted for other pathological reasons (p=0.044): ESP was frequently focal unilateral in neonates admitted for seizures (32/44, 72.7%) in comparison with neonates admitted for other pathological reasons then seizures (45/81, 55.6%), whereas the latter had more frequently multifocal ESP (23/81 – 28.4% - versus 4/44 – 9%).

We found a significant association between seizure type category (electrographic, motor, non-motor) and ESP: focal unilateral pattern was higher in non-motor seizures (11/14, 78,6%) and motor seizures (51/80, 63.7%) than electrographic seizures (15/32, 46.9%); conversely multifocal seizures were higher in electrographic seizures (14/32, 43.7%) and lower in non-motor seizures (3/14, 21%) and motor seizures (10/80, 12.5%). Motor seizures had 10% of symmetric and 13.7% of asymmetric bilateral ictal abnormalities, higher than non-motor seizures and electrographic seizures (p= 0.006). Among motor seizures the stronger association with focal EPS was found with clonic (31/72, 73.8%) and sequential seizures (11/18, 61.1%).

Regarding etiology a significant difference was found between ESP of the population with acute provoked seizures and the population with epilepsy (p=0.033). Provoked seizures had a higher rate of focal (63/98, 64.3%) and multifocal (23/98, 23.5%) ESP in comparison with epilepsy that had respectively 14/28 (50%) and 4/28 (14.3%). At the contrary epilepsy had a higher rate of symmetric

(4/28, 14.3%) and asymmetric (6/28, 21.4%) bilateral ESP in comparison with provoked seizures (that had respectively 6/98 – 6.1% - and 6/98 – 6.1%).

In contrast to the significant association we found between EB and epileptic syndrome, no significant association was found between ESP and epileptic syndrome (SeLNE or DEE) and ESP (0.867).

The association between ESP and major etiology showed a tendency toward statistical significance, with higher association between focal ESP and vascular etiology (28/33, 84.8%) or multifocal ESP and HIE (9/29, 31%) and post-natal anoxia (80%) ($p = 0.081$).

When we evaluated the association between vascular etiology (both as major etiology or comorbidity, from the etiology checklist field) and ESP against all the other etiologies we found a significant association between focal ESP and vascular etiology (31/37, 83.8%), $p = 0.009$.

When comparing genetic etiology with all other etiologies we found a significant association attributable to a higher frequency of symmetric (13.5%) or asymmetric (18.9%) bilateral ESP in the genetic etiology ($p=0.039$), as bilateral ESP were present in only 5.6% in all other etiologies.

Status epilepticus

We evaluated if EEG had shown status epilepticus (defined as EEG seizure activity of 30 minutes or more in 1 hour time). We than studied the association between status epileptic and other variables of interest. We did not find significant difference in the rate of status epilepticus between neonates with different seizure types ($p=0.277$), although with a trend toward a higher rate of status epilepticus in some seizure types (automatisms, tonic, spasm, autonomic) and lower in others (clonic, electrographic). In the population of epileptic patients (in this population a burst-suppression pattern was considered equivalent to status epilepticus) we found status epilepticus or burst suppression in 0/10 among SeLNE and 9/18 (50%) among DEE patients ($p= 0.009$).

Neonates with more than one seizure type (31/75, 41.33%) in addition to the prominent seizure type tend to have a higher rate of status epilepticus in comparison with the neonates with only one seizure type (14/67, 20.9%), p=0.009.

The association between status epilepticus and major etiology was not significant (p = 0.441), although among the most common etiologies, we found a higher rate of status epilepticus in HIE (12/34; 35.3%), cerebrovascular disease (14/36; 38.9%) and infection (9/23; 39,1%), whereas was intermediate in genetic diseases (6/21; 28.5%) and low in metabolic disease (1/12, 8%).

We examined the association of each specific etiology, as indicated in the multiple answer etiology checklist, against all the other etiology. We found a significant increase of status epilepticus rate in moderate HIE (10/21, 47.6%) in comparison with severe HIE (2/14; 14.3%); p = 0.042.

Among the cerebrovascular diseases we found a markedly higher in the rate of status epilepticus in hemorrhage patients (12/24, 50%) than ischemic stroke patients 2/12 (16.7%), although these differences among subtypes of cerebrovascular disease did not reach significance (p = 0.223).

Discussion

This center-based study evaluated the incidence, semiology, EEG and etiology features of EEG-aEEG-confirmed NS occurring between 2009 and 2022 at the "Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico (Milan, Italy), a tertiary referral center for neonatal care where a standard neurophysiological protocol for evaluation and monitoring of neonatal seizures with a complementary use of EEG and aEEG is applied (Dilena et al., 2021).

EEG and/or aEEG is performed in all newborns with suspected seizures or with a medical condition at high-risk for seizures by neonatologists. We chose to consider only aEEG or EEG-confirmed NS, as suggested by the ILAE (Pressler et al., 2021), because clinical evaluation alone has been largely demonstrated unreliable and could lead to false-positive diagnoses. If we had accepted a clinical diagnosis only, we might have overestimated the incidence of NS and have done wrong correlation between semeiology and etiology. The risk of overestimation of NS is an important limitation of the studies based only on a clinical approach (Pisani et al., 2018a). Moreover, a clinical approach does not allow the identification of electrographic-only seizures that mostly represented in critically ill neonates and preterm infants (Shellhaas, 2015). In our sample, 30.3% of patients presented electrographic only seizures at onset. In addition, in our sample 40% of neonates presenting with electroclinical seizures at onset had also electrographic seizures. The high overall rate of electrographic-only seizures at onset was particularly high in the preterm population (in our sample 52.3% of preterm NS presented with electrographic seizures) and in the HIE etiology (52.9% of HIE related-seizures presented with electrographic seizures) in line with previous studies (Clancy et al., 1988; Nash et al., 2011; Saliba et al., 1999)(Nash, Clancy, Glass 2016, Saliba).

Incidence of neonatal seizures

The incidence of neonatal seizures is variable among studies between 0.95 and 5 per 1000 live births (Vasudevan and Levene, 2013), depending on the country income, type of diagnosis (clinical

or EEG), type of study (population or center-based). Among the neonates admitted to our NICU we found an overall incidence of NS of 1.59 per 1000, but if we considered only the inborn neonates (presuming they much better reflect the general population incidence) we found a lower incidence of 1.11 per 1000 live births. In the term neonatal population incidence was lower (0.83 per 1000 live birth), whereas in the preterm population was higher (3.09 per 1000 live births). Incidence was inversely proportional to gestational age and in the extremely preterm (GA < 28 weeks) incidence rose to 17.66 per 1000 live births. A population-based study conducted in Italy (province of Parma, Pisani 2018) on EEG-confirmed neonatal seizures on neonates with seizures treated between the years 2002-2014, the overall incidence of neonatal seizures was higher than our (2.29/1000). We think this difference could be due to a different setting and population and is in the range of variation that are already known by previous studies on incidence (Vasudevan and Levene, 2013). In addition, i the last decade significant progresses in perinatal and neonatal care have been introduced and this could also have a contribution, as our study referred to the period 2009-2022 in the era of hypothermia treatment and continuous neuromonitoring.

Day of life (DOL) at seizure onset

Regarding the DOL at seizure onset there was a marked significant difference between term and preterm neonates, as found by other studies (Glass et al., 2017; Pisani et al., 2016): median of DOL at seizure onset was for term 2 days and for preterm 9.5 days. The distribution of DOL was not parametric with a variance very different between the two populations, with a much higher dispersion in preterm. In-hospital mortality in our neonatal population with seizures was 18.8% in line with other recent studies (Lemmon et al., 2020), being higher in preterm than in term neonates as previously reported (Glass et al., 2017; Pisani et al., 2018b). Regarding DOL at seizure onset we found a significant association with etiology. Infection had the higher DOL at seizure onset (median: 17 days), whereas HIE had a DOL at seizure onset low as expected (median: 1 day), vascular etiology had a DOL at seizure onset a little superior to HIE (median: 2 days), followed by

genetic etiology (median: 2 days) and metabolic etiology (median: 4.5 days). Unlike previous studies (Cornet et al., 2021), a difference in DOL at seizure onset between acute provoked seizures and epilepsy was not significant in our study (p= 0.5), as it could be expected in a larger population given the high variability in DOL among the provoked seizures according to the specific etiology as above indicated.

Perinatal HIE and vascular etiology were the most frequent principal etiologies which is consistent with previous reports (de Corrêa et al., 2022; Glass et al., 2016; Pisani et al., 2018b; Vasudevan and Levene, 2013). An important difference is that in our population perinatal HIE contribution was inferior and accounted for only 23.4% of all neonatal seizures, whereas the HIE proportion among the total number of neonatal seizures was much higher in previous studies, around 40-50% (Pressler et al., 2021; Vasudevan and Levene, 2013). The overall vascular etiologies (Hemorrhage 16.6%, ischemic stroke 9%) had a frequency a little higher than perinatal HIE (accounting for 25.5% all together), but if we consider separately the two main cerebrovascular diseases (brain hemorrhage and ischemic stroke) has done by other studies (Glass et al., 2016; Vasudevan and Levene, 2013) HIE still remains the first cause of neonatal seizures also in our setting, with brain hemorrhage percentage being 16.6% and ischemic stroke 9% near the average-maximum of the values reported by previous incidence studies (Vasudevan and Levene, 2013). HIE lower incidence in our study is one of the main findings of our study. Although it could be due in part to the specific features of our population coming from a tertiary obstetrician-neonatological referral center from a developed country, it seems to reflect quite well the general trend of the last years decade regarding the general reduction of HIE incidence and severity and also the reduction of electrical seizures incidence among moderate-severe HIE (Glass et al., 2022; Shi et al., 2022; Vega-Del-Val et al., 2021; Wang et al., 2022). To verify this hypothesis, we searched in our medical records the number of moderate-severe HIE and calculated at our center an incidence of moderate-severe HIE (needing hypothermia) of 0.81 for 1000 live births. We also calculated a percentage of seizures among moderate-severe HIE of around 30%. Both data appear in line with very recent studies in the era of

hypothermia treatment and critical care neuromonitoring (Vega-Del-Val et al., 2021). At our center we performed a continuous neurophysiological monitoring with aEEG/EEG for 96 hours, as standard procedure in moderate-severe HIE undergoing, so we consider reliable our estimation of neonatal seizures with moderate-severe HIE undergoing hypothermia that is the majority of our moderate-severe HIE population with seizures. As secondary effect of both lower incidence of HIE and reduction of seizures HIE, the proportion of neonatal seizures due to the other etiologies resulted relatively increased. Vascular etiology in our study accounted for 26.73% of term neonatal seizures and 22.7% of preterm neonatal seizures. Although the overall vascular etiology percentage appears similar between term and preterm neonates, different vascular types are involved in the two groups: intraventricular hemorrhage (IVH) accounted entirely for the preterm vascular etiology, whereas in term neonates the vascular etiology was more heterogenous as arterial ischemic stroke accounted from 12.9% of all term neonatal seizures, intraventricular hemorrhage for 4% and parenchymal, subdural, epidural and subarachnoid hemorrhage for the rest (9.8%).

Meningitis, encephalitis, brain abscesses and septicemia accounted for 16.6% (all intracranial infections 14.5%, septicemia alone 2%). Infection etiology of neonatal seizures is very variable among studies (Vasudevan and Levene, 2013) and this could depend mostly on the type of population studied. In our population we found that preterm neonates had a significant higher risk to develop infection-related neonatal seizures than term neonates (infectious etiology found in 31.82% of preterm NS and 9% of term NS).

If we only consider genetic etiology presenting without brain malformations, IEM or provoked seizures, the so called functional genetic epilepsy, we found that 15.2% (22/145) of our cohort of consecutive neonates with seizures was affected. The high proportion of neonatal seizures of genetic origin is in line with recent studies (Shellhaas et al., 2017). In addition, if we look at the genetic etiology more broadly, considering both direct or indirect mechanisms (the primary cause underlying the cascade of pathogenic mechanism leading to seizures), a genetic etiology could be

detected in 38/145 (26.2%), composed by the sum of 22 (15.2%) with the so called functional epilepsy, 4 (2.8%) with genetic brain malformation, 2 (1.4%) with metabolic (IEM) epilepsy, and 10 (6.9%) with provoked seizures, so a genetic contribution to neonatal seizures surpassed the contribution of perinatal HIE. This knowledge can be attributable to the systematic implementation in the last years in the clinical practice of advanced diagnostic genetic studies as next-generation sequencing techniques that had allowed reach to a molecular diagnosis in most cases and a deep global evaluation of etiology factors in each patient. Regarding epilepsy, if we consider all the population (both inborn and outborn) that reflects the epidemiology of a neonatal tertiary center where a selection bias exists toward a concentration of more severe diseases we found: KCNQ2 9 (32.1%), KCNT1 2 (7.1%), SCN2A 3 (10.7%), STXBP1 1 (3.6%), BRAT 1 (1, 3.6%), STAG2 1 (1, 3.6%), ALDH7A1 1 (3.6%), CACNA1G 1 (3.6%), TSC2 1 (3.6%), QARS 1 (3.6%), GLDC 1 (3.6%), Unknown 6 (21.4%). The genetically resolved cases in this case was increased to almost four-fifths (78.6%) similar to previous recent studies (Shellhaas et al., 2017). If we consider only the inborn neonatal epilepsy population, that we think better reflect the general population incidence we found a proportion of KCNQ2 epilepsy in 42% of neonatal epilepsies, with a calculated incidence of 9.2 per 10000, similar to the incidence reported by Symonds et al. (5.89/10 000; 95% CI 2.24–9.56) (Symonds et al., 2019).

ILAE Seizure type and association with etiology

The frequency of seizure types in all the population of neonates was clonic 31%, electrographic 30.3%, sequential 12.4%, tonic 9.7%, autonomic 9.7%, automatism 3.4%, behavioral arrest 1.4%, spasm 1.4%, myoclonic 0.7%. Frequencies were very different among preterm and terms neonates for the following seizure types: electrographic 52.3% in term neonates and 20.8% in term neonates, clonic 36.6% in term neonates and 18.2% in preterm neonates, sequential seizures 17.8% in term and 0% in preterm neonates, tonic 18.2% in preterm and term neonates, autonomic in 11.9% in term and 4.5% for preterm neonates. This is one of the first studies applying the ILAE seizure type

classification to a population, so few similar data exist for comparison. In the recent monocentric study of Correa de Correa et al. (de Corrêa et al., 2022) that applied the ILAE Seizure type classification for neonates in a center in Brasil, absence of a confirmatory EEG or aEEG was not a criterium of exclusion as in our study, so our two studies are only partially comparable, as electrographic seizures in our sample were the main seizure types in preterm and the second major seizure types in term neonates. Regarding electroclinical seizures the main difference between our results and the study of Correa de Correa et al. are the proportion of sequential seizures that was much higher in the Brasil study (44.6% versus our 18.2%). We think that this great difference between the results of our two studies points to the high inter-operator variability that exists in the application of the new category of sequential seizure, that is one of the most relevant topic of the new ILAE classification of seizure types in neonates (Pressler et al., 2021). In neonatal seizures it is common to observe a variable sequence and evolution of signs. As consequence the operator applying the classification must decide if there is or there is not a predominant semiology that deserves the name (as for instance "clonic seizures" if clonic semiology is predominant) or if the sequence of signs itself should be considered the predominant feature and consequently the "sequential seizure" classification should be assigned. In this choice resides an high risk of inter-operator and inter-center variability, as we have seen in the preparatory phase of our study experimenting its application at our center and comparing our clinical practices in classification during the INNESCO meetings (Dilena et al., 2021), where neonatal clinical cases with videoEEG are presented by different centers and collegially evaluated. The problem is that with an extensive use of the term "sequential seizure", is high the risk to define "sequential" most of neonatal seizures, with the result to lose the specificity of the term and its capacity to point toward etiology. After this considerations and experience, we decided to set "high" our threshold for the definition of "sequential seizure", limiting the assignment of this seizure type to those stereotyped electro-clinical sequence of manifestations of tonic, clonic, automatisms or autonomic features (as apnea) with a correspondent typical EEG pattern (low voltage fast activity during tonic phase and spikes-

spike-waves during clonic seizures), often with varying lateralization during a single seizure. We also find very useful to further increase specificity for etiology of sequential seizures with the addition of the ictal onset sign. Actually, we have seen that among our 18 sequential seizures the 8 KCNQ2 epilepsy, the 3 SCN2A cases, the 2 SLNE with unidentified gene had tonic semiology as ictal onset sign. The remaining 5 sequential seizures were 1 CACNA1G DEE with tonic seizure-onset, 1 KCNT1 EIFMS with automatisms as seizure-onset, 3 HIE (1 with clonic, 1 with tonic, 1 with automatisms as ictal onset sign). As further useful descriptor, in our population KCNQ2-sequential seizures tend to start with asymmetric tonic figure of 4-sign semeiology at seizure onset. 2 of the three SCN2A-DEE patients had a characteristic alternating side fencer's posture seizure semeiology within seizure.

The analysis of correlation between seizure type and etiology showed that acute provoked seizures had a significant higher rate of electrographic seizures (35.9%) in comparison non-acute provoked seizures (only 7.1%), in line with previous studies (Cornet et al., 2021). Perinatal HIE is associated with electrographic seizures (52.9%) in line with previous studies (Wusthoff et al., 2011) and all 6 post-natal cerebral anoxia/hypoxia presented with electrographic seizures too. Vascular etiology is significantly associated with focal clonic seizures (56.8%) and the association is very strong for ischemic stroke (100%). Genetic epilepsy (without inborn error of metabolism or brain malformation, the so called functional genetic epilepsy) are significantly associated with sequential seizures (68,2%) and the association is very strong for KCNQ2 (8/9 – 88.8% - KCNQ2 patients had sequential seizures with tonic onset, 1 patient con KCNQ2 DEE had tonic seizures) and for SCN2A DEE (all 3 presented with sequential seizures with tonic onset), is in line with previous studies (Pressler 2021, Nunes 2019).

The analysis of the correlation between seizure type and epileptic syndrome showed a stronger significant association between sequential seizures and self-limited neonatal epilepsy (SeLNE) in line with previous studies (Sands et al., 2016). In SeLNE all seizures were sequential, whereas in

our population of DEE sequential seizures represent only 27.8%, whereas in 33.3% had clonic seizures, but all DEE due to SCN2A-KCNQ2 mutations (2 patients) and also one CACNA1G DEE and one of the 2 KCNT1 DEE patients had sequential seizures.

EEG findings and association with etiology and seizure type

Regarding EEG background (EB) we found that HIE and post-natal cerebral anoxia/hypoxia had abnormal EB. Infection (18/21) and metabolic etiology (10/12) had respectively 85.7% and 83.3% of abnormal EB. EB was abnormal in 75% (3/4) of brain malformation, whereas half of neonates with genetic etiology (11/22) without brain malformation had abnormal EB. Regarding epilepsy, EB depended on the epileptic syndrome, being significantly different between SeLNE and DEE. EB was normal in 80% of SeLNE (moderately abnormal in the 20% of SeLNE), whereas it was abnormal in 77.7% of DEE (moderately abnormal in 68.1%, severely abnormal /burst suppression in 11.1% and markedly in 5.6%). Interestingly the association found between abnormal EEG and etiologies were similar to the association found between neurological examination (NE) and etiologies.

We found a significant association between seizure type category (electrographic, motor, non-motor) and EEG seizure pattern (ESP), distinguished in focal-unilateral, multifocal, symmetric bilateral and asymmetric bilateral activity. Focal unilateral pattern was higher in non-motor seizures (78,6%) and motor seizures (63.7%) than electrographic seizures (46.9%). Conversely multifocal seizures were higher in electrographic seizures (43.7%) and lower in non-motor seizures (21%) and motor seizures (12.5%). Among motor seizures the stronger association with focal ictal EEG abnormalities were found with clonic (73.8%) and sequential seizures (61.1%). Provoked seizures had a higher rate of focal (64.3%) and multifocal (23.5%) ictal EEG abnormalities, at the contrary epilepsy had a higher rate of symmetric (14.3%) and asymmetric (21.4%) bilateral ictal EEG activities.

Regarding status epilepticus defined on the base of EEG/aEEG (as 30 minute EEG/aEEG activity or more in 1-hour time), we found a significant association with hemorrhage patients (50% of patients). Although not significant, a higher rate of status epilepticus was found also in HIE (35.3% of patients) and infection (39,1%), whereas it was lower in the genetic diseases (28.5%) and metabolic diseases (8%).

Conclusions

We built a comprehensive Redcap database for clinical studies on neonatal seizures very useful not only to reach the results of the present studt, but also as a base for future retrospective or prospective, monocentric or multicentric studies on neonatal seizures and epilepsy diagnosis, treatment and outcome, available for the medical scientific community on request to the authors. In this database all important items for neonatal seizure are included: demographic features, gestational and perinatal risk factors, neonatal comorbidities, seizure type, etiology (differentiated in etiological contributors, principal, and primary etiology), EEG, neuroimaging, therapy, and neurodevelopmental outcome.

In the first application of the database we wanted first of to study seizure types, epileptic syndromes neurological examination, EEG and etiologies, whereas neuroimaging, therapy and neurodevelopmental outcome will be analyzed in the future.

One of the original acquisitions of the present study in comparison with previous studies was that in our tertiary center we found a reduction in HIE-related neonatal seizures and a higher contribution of genetic etiology to neonatal seizures mediated by different mechanisms: functional epilepsy, metabolic epilepsy and brain malformation, but also in the form of genetic conditions causing endocrinological, metabolic or vascular complications leading to acute provoked seizures. Our study also confirms the usefulness of clinical and EEG characterization of neonatal seizure types and epileptic syndromes by the new ILAE classification for neonates to address etiology, confirming the association between certain seizure types and etiology. In order to keep high the specificity and usefulness of the term “sequential” seizure, we concluded that its use should not be extensive, but it should be restricted to those highly distinctive clinical-EEG seizure patterns, characterized asymmetric tonic posturing (often asymmetric and with alternating side onset) followed by clonic manifestations, as usually found in neonatal epileptic channelopathies, remarking the central role of videoEEG recordings for classification.

The main limitation of the present study is the retrospective data collection. A prospective collection of data and the direct videoEEG evaluation is the natural next step of this kind of study, but it required much more time and possibly a multicenter design to reduce the time required to collect a minimum number of patients to perform analysis. The monocentric nature of the present study could be considered a limitation, but it can also be considered a strength as starting point of analysis, as it ensures a uniform approach, as we could verify that some items as seizure type and EEG classification could have a high variability without an effective shared training program.

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