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PHD THESIS

ADVANTAGES AND PITFALLS OF ELECTRODIAGNOSTIC TESTS IN THE ASSESSMENT OF CHILDREN WITH NEUROMUSCULAR DISORDERS AND CORRELATION WITH THE MYOPATHOLOGICAL STUDY

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INTRODUCTION

Neuromuscular disorders (NMD) are a very heterogeneous and complex group of diseases, characterized by the presence of structural and functional alterations of one or more motor system components, which consists of upper and lower motor neurons, neuromuscular junction, and muscles. The distinctive clinical and laboratory features of these diseases depend on which and how many components are involved: we can therefore have motor neuron diseases, peripheral neuropathies, muscular junction diseases and myopathic forms.

Regardless of which component is affected, they may be genetic diseases, increasing thanks to new methods of investigation, and/or acquired. Furthermore, the involvement of the neuromuscular system can be found both as the main manifestation of the pathology, and as a clinical sign of systemic pathologies involving other organs and systems including central nervous system, visual and auditory pathways, locomotor apparatus, immune system, liver, kidneys, cardiovascular system, gastrointestinal tract.

Neuromuscular diseases can have a very variable course and prognosis, and can lead to a high degree of disability. Early diagnosis is crucial for a correct and timely care, and in cases where a genetic cause has been found, also for prenatal diagnosis and genetic counselling.

Diagnosis of neuromuscular disease or neuromuscular comorbidity is a complex algorithm: numerous investigations, performed by different specialists, are necessary and must be coordinated in order to arrive at a suspected diagnosis. An accurate medical history and clinical evaluation are essential. These lead to subsequent investigations, such as electrodiagnostic studies (Nerve conduction studies -NCS- and electromyography - EMG), muscle biopsy, morphostructural investigations (muscle ultrasound and muscle MRI), biochemical and genetic investigations.

For genetic diseases, in patients with recognisable phenotypes, such as Duchenne muscular dystrophy or spinal muscular atrophy, molecular genetic testing without preceding EMG and muscle biopsy have become the clinical standard (Hellman et al, 2005); instead for patients with more heterogeneous phenotypes a better anatomical and functional framework of the disorder that may direct future investigations remains essential, and it becomes necessary to recognize the early and subtle changes (Hafner et al, 2019) (Naddaf et al, 2018) (Hausmanowa-Petrusewicz 1971). Therefore, although imaging and genetic testing are increasingly gaining importance (Arnold et al, 2012) (Kassardjian et al, 2016) (Fischer et al, 2016), (Hafner et al, 2019), needle electromyography and muscle biopsy retain an important role in the evaluation of patients with suspected NMD.

Electrodiagnostic studies are an extension of the physical examination and may help establish the diagnosis of neuromuscular disease (Paganoni et al, 2013). They consist of Nerve conduction studies (NCSs) to study the nerves function (sensory and motor), Electromyography (EMG) to study the muscle function. They can evaluates peripheral motor unit physiology from different areas, help differentiate between neurogenic and myopathic diseases and disorders of neuromuscular transmission, localise lesions in the peripheral nervous system, select the appropriate muscle for biopsy (Russel et al, 1992), recognise a pattern of abnormalities that may indicate a specific picture, classify severity and distribution, monitor disease activity and progress, assess the response or side effects of drug treatment, and rule out conditions without neuromuscular involvement (Hafner et al, 2019).

Therefore, Nerve conduction studies (NCS) and Electromyography (EMG) play a role not only in the initial diagnosis, but also in assessing the clinical course and response to treatment.

Until a few years ago, this was mainly true for acquired forms. For both inflammatory neuropathies and myopathies, as well as for autoimmune or deficiency or iatrogenic diseases, NCS and EMG - due to their recognised advantages (low cost, execution to bedside, no need for sedation, repeatable even at close range) - allow follow up for evaluation of disease activity, response to therapy, and functional recovery (Van den Bergh et al, 2021) (Korinthenberg et al, 2020) (Siao et al, 2019).

In recent years, neurophysiology has also been used in the follow-up of genetic forms, for which new therapies are being developed and new pathogenic mechanisms hypothesised: in recent years, therapies have been proposed for spinal muscular atrophy (Finkel et, 2016) (Finkel et, 2021), (Day et al, 2022), and recently also for metabolic diseases, other genetic disorders (Mendell et al, 2021) (Nitschke et al, 2018) (Sevin C, Deiva K., 2021) and Duchenne muscular dystrophy (Elangkovan et al, 2021).

Neurophysiological evaluation offers recognized advantages: low cost, execution to bedside, possibility of execution from day one of patient's life and monitoring over time; furthermore, they do not require sedation and are the only instrument for functional evaluation.

However, electrodiagnosis of neuromuscular disease is typically difficult in young patients (Chang et al, 2011) due to numerous factors.

First of all in childhood, and especially under the age of eight (Pitt, 2012), is very difficult to obtain a partial/complete collaboration of the patient to electrophysiological investigations.

Moreover, the same diseases are different in different age groups and occur in a different way depending on age, particularly in younger children (under two years of age) even when compared with children generally rather than adults (Pitt, 2012).

Finally, accurate normative data is very difficult to find in the paediatric age group, for some reasons: first of all it is not simple to subject healthy children to an "invasive" method; second, many of the current normative data, obtained mostly from adults, have required standardized conditions for the execution of the exam to try to limit the effect of all the known factors that can influence the results; finally, under the age of two there are very significant changes in normal data range for most of EMG measurements (Pitt, 2012), just like it happens also for nerve conductions - for example, the diameter of the muscle fibre is much smaller in newborns and infants than in older children, obtaining a recruitment pattern that may appear myopathic.

Few studies on the normative data of nerve conduction studies in pediatric age are found in the literature (Shulte et al, 1968; Dunn et al, 1964; Yasumoto et al, 2004); the normative data of paediatric electromyography concerning the duration of the motor unit potentials are available from the results obtained by Buchthal and colleagues in Copenhagen (1960s) (Buchtal et al, 1952, 1954, 1955) (Sacco et al, 1962), however, there are few studies concerning the QEMG normative data, and in these only few parameters are analysed (Chang et al, 2011), (Fuglsang-Frederiksen, 2000).

The lack of normative data makes it difficult to interpret the neurophysiological results obtained in children and allows the diagnostic accuracy of EMG in children varies between 10-98% (Hellmann et al, 2005); with less sensitivity specificity for myopathies (80%) compared to neuropathies (more than 90%) (Chang et al, 2011) (Rabie et al, 2007), also with the combined use of EMG and muscle biopsy (Hafner et al,2019) (Hellmann et al 2005).

Muscle biopsy indeed is a complementary investigation to the neurophysiological study and, including immunohistochemistry, electron microscopy, and biochemical analyses, examines morphology, histopathology, biochemistry of the neuromuscular system (Rabie et al, 2007).

Muscle biopsy is central for the definite diagnosis of both an inherited NMD to target genetic analysis and also for acquired conditions affecting the muscle such as inflammatory myopathy. In other NMD with a neurogenic or NMJ pathogenesis the use of muscle biopsy is limited albeit typical lesions appear on the histopathological studies (such as type grouping or neuromuscular junction alterations). Regardless the NMD condition, muscle biopsy can also

provide information on the severity of the underlying disease and the extent of muscle damage/fibrotic substitution.

Previous studies have investigated the accuracy of neurophysiological tests compared to muscle biopsy in NMD patients (Brusa et al., 1963) (Humphrey and Shy, 1962) (Schwartz et al., 1966) (Hausmanowa-Petrusewicz and Karwanska, 1971) (Black et al, 1974) (Micaglio et al., 1980) (Buchthal and Kamieniecka, 1982) (Packer et al., 1982) (Gibertoni et al, 1987) (Werneck et al, 1988) (David and Jones, 1990, 1994) (Russell et al., 1992) (Hellmann et al, 2005) (Chang et al, 2011) (Rabie et al, 2007) (Ghosh et al, 2014) (Constantinides et al, 2018) (Hafner et al, 2019). However, despite the common use of these diagnostic techniques, few studies have investigated if specific electromyographic (EMG) findings may indicate specific pathological changes on muscle biopsy (Sener 2019) (Naddaf et al, 2018) (Dardiotis et al, 2011), especially in the paediatric population, for which few studies are available.

Nerve conduction studies (NCSs) and paediatric normative values in literature

Nerve conduction studies (NCSs) the evoked responses of muscles and nerves following electrical stimulation of peripheral nerve fibres, allowing exploration of both the distal segments (motor and sensory NCSs) and the proximal ones (reflex H, wave F, blink reflex) of the peripheral nervous system.

There are several types of NCS (motor, sensory, and mixed), which reflect the types of axons that are studied. Although the overall techniques are similar, there are important differences in meaning of the responses recorded.

Motor NCS are performed by stimulating a nerve trunk and recording the response from a muscle supplied by that nerve. Because one motor axon innervates multiple muscle fibres and multiple motor units are present in a muscle, the recorded response is a summated response termed the compound muscle action potential (CMAP) or the M wave. Motor NCS are used to test the integrity of the peripheral motor system, including the anterior horn cells, roots, peripheral nerves, neuromuscular junctions, and muscle. Abnormalities at each of these sites may impact the responses (O'Bryan and Kincaid, 2021).

Sensory NCS assess the integrity of the peripheral sensory axons. The responses recorded, termed the sensory nerve action potential (SNAP), are the summated action potentials of the

large myelinated sensory axons within the nerve. Sensory responses are much lower in amplitude (microvolts) than CMAPs (millivolts). Stimulation methods are similar to motor NCS.

Sensory NCS is reliably performed using an orthodromic or antidromic method. In the orthodromic method, a nerve is stimulated at a distal site (eg, using ring electrodes on a digit) and the action potentials are recorded at a more proximal site along the nerve trunk. In the antidromic method, the nerve trunk is stimulated at a proximal site and the response is recorded at a distal site (eg, from the digit). Antidromic digital studies tend to have higher amplitudes, because the recording electrodes are directly adjacent to the digital nerves. In orthodromic digital studies, the surface recording electrodes are separated from the nerve by subcutaneous tissue resulting in lower amplitudes.

Mixed NCS refer to testing a nerve that contains sensory and motor axons. With this technique, a nerve with motor and sensory fibres is stimulated and the resulting action potentials are recorded from another site along the nerve. Mixed studies are not a substitute for SNAPs when information about sensory function is needed (O'Bryan and Kincaid, 2021).

Nerve conduction studies (NCSs) are a simple and reliable test of peripheral nerve function: the amplitude, distal latency (DL), morphology and conduction velocity (CV) of the compound muscle action potential and sensory nerve action potential are recorded. NCSs help to delineate the extent and distribution of the neural lesion and distinguish two major categories of peripheral nerve disease: demyelination and axonal degeneration. Abnormal values and morphologic features of the recorded waveforms can help to define the underlying pathologic process.

In motor and sensory NCS, the DL is the conduction time between the distal (wrist or ankle) stimulation sites and the recorded response, and is a measure of the integrity of the myelin and axons in a distal segment of the nerve. The CMAP amplitude is a measure of the summation of muscle fiber action potentials. It directly reflects the number of functioning muscle fibres and indirectly reflects the number and function of motor axons and neuromuscular junctions (O'Bryan and Kincaid, 2021).

The conduction velocity (CV) of a nerve may be defined as the speed at which an impulse travels along the nerve trunk under certain standard conditions. It is usually measured in meters per second (m/sec). In order to obtain the conduction velocity along the nerve itself and to eliminate the delay at the neuromuscular junction from the calculation one stimulates the same nerve supramaximally at a distal and more proximal point, for instance, the median nerve at the wrist and elbow. If one then subtracts the latency of the first response (X1) from

that of the second (X2) one obtains the time taken for the impulse to travel from the second to the first point of stimulation, and this can be related to the distance between the points of stimulation, thus yielding the conduction velocity. In this way one may explore the rate of conduction in different parts of a nerve trunk. (Dunn et al, 1964). CV is a primary measure of the integrity of the myelin along a nerve.

Paediatric values in literature

In 1850 Helmholtz first reported that he had measured the rate at which nerve impulses travel; later he also measured the nerve conduction velocity in man.

Subsequently neurophysiologists further showed that conduction velocity bears a linear relationship to the diameter of the nerve fiber in which the impulse travels.

However, analyzing this relationship further, Sanders and Whitteridge investigated the conduction velocity in regenerating peroneal nerves of rabbits and concluded that it depended more upon the thickness of the myelin sheath than on the total diameter of the nerve fiber (Sanders et al, 1946).

In 1952-53, Wagman et al (Wagman et al, 1952) published findings concerning the maximum conduction velocity of motor fibres in the human ulnar nerve and demonstrated that after the age of 40 years there was a gradual decrease in conduction velocity.

Since then several further investigators have established normal values for adults (Carpendale et al, 1956) (Thomas PK et al 1959), (Johnson EW et al, 1960), (Christie BC et al, 1960) (Spiegel MH et al, 1962), (Mayor H et al, 1962), (Thomas PK et al, 1961): it may be said that the normal range of conduction velocity in adults below the age of 60 years is about 50-70 (average 60) m/sec in the ulnar and median nerves and about 40-60 (average 50) m/sec in the lateral popliteal, anterior, and posterior tibial nerves. Values below 40 m/sec may be considered abnormal for the large motor nerves of the arm.

The normal range of conduction velocity in analogous motor nerves of different individuals is fairly wide, even in the same age group (Johnson EW et al, 1960), (Wagman IH et al, 1952).

Neurophysiologists described factors that can modify the results in normal persons, mainly in children and newborn:

- Age: the nerve conduction velocity is slower in newborns and in the first years of life, reaching the adult level at 3-5 years (Thomas JE et al, 1960);

- Temperature: conduction velocity is reduced by about 5% per degree centigrade drop in temperature (Johnson et al, 1960); the room temperature should be kept constant and abnormalities of body or skin temperature should be noted;
- Metabolic Factors: peripheral ischaemia, may increase the conduction time significantly (Magladery et al, 1950),other metabolic factors do not seem to change conduction velocity;
- Tested nerves (Waxmanet al, 1980);
- Technique errors.

Standard values of nerve conduction parameters are essential in evaluating infantile neuromuscular disorders (Garcia et al, 2000).

Existing normal value references or paediatric nerve conduction studies (and paediatric electromyography) are based on limited sample size, with uncertain reliability, suggesting a need for better normative data.

In 1957 Pinelli and Salahl first described that the speed in infants was lower than in older children (Pinelli et al, 1957).

In 1960 Thomas and Lambert published an extensive study of conduction velocity in motor fibres of the ulnar nerve in infants and children (Thomas et al, 1960): they analized motor fibres of the ulnar nerve of 146 children, including 6 premature infants, 42 fullterm newborn infants and 98 children up through the age of 14 years, and records of 188 healthy adults; the authors noted that the velocity in the ulnar nerve of the full-term newborn infant was thus about half the adult value and attained the full value at 3-5 years.

Cerra and Johnson studied 11 preterms, founding a correlation between the infant's weight and nerve conduction velocity (Cerra et al, 1962).

In 1964 Dunn et al published conduction velocity values in median nerve: it was shown that full-term infants usually have a median nerve conduction velocity of at least 20 m/sec in the neonatal period, and that this velocity rises to at least 30 m/sec after the first 6 months and at least 35 m/sec after the first year; subsequently, the rate increases more slowly to reach adult levels of at least 40 m/sec after 3 to 5 years.

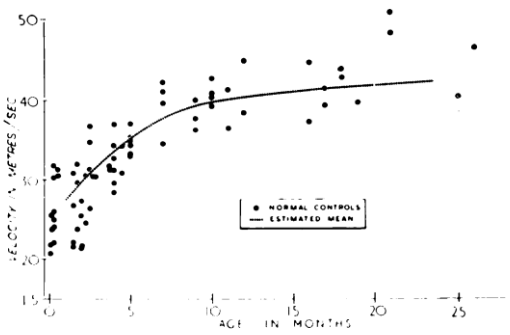

Figure 1 (from Dunn et al): Conduction velocity of median nerve (Normal control).

Later, nerve conduction evolution was investigated in other nerves (Gamstorp 1963), (Gamstorp et al, 1965) (Baer et al, 1965) (Shulte et al, 1968), (Radtke, 1969) (Wagner et al, 1972) (Sachdev et al, 1972) (Cruz Martinez et al., 1977, 1978), (Hakamada et al, 1982), (Vecchierini-Blineau et al, 1984), (Duron et al 1992), (Miller et al, 1986), (Tranier et al, 1989), (Parano et al., 1993), (Kwast, 1995), (Cai et al, 1997), (Smit et al, 1999), (Garcia et al, 2000) (Yasumoto et al, 2004) (Jabre et al, 2020).

In particular Gamstorp studied normal conduction velocity of ulnar, median and peroneal nerves (Gamstorp 1963) and peripheral sensory nerve conduction in the ulnar and median nerves (Gamstorp et al, 1965) through infancy, childhood, and adolescence.

Motor nerve conduction velocities were studied in 174 newborn infants (Blom et al, 1971), 172 ulnar nerves and 99 peroneal nerves. It was found that there was a significant correlation between motor conduction velocity in the neonatal period and gestational age of the infants; the correlation was higher for the peroneal nerve; the range in results at a constant gestational age was rather wide. Motor conduction velocity does not seem to be retarded in small-for-gestational-age infants, in accordance with what has already been described by Shulte et al (Schulte et al, 1968), or in infants who have had signs of perinatal asphyxia, hyperbilirubinemia or minor neurological symptoms in the neonatal period.

It was also described the latency and amplitude of ulnar nerve and NCV (Wagner et al 1972) in preterm and in children: it was demonstrated that maximum motor and sensory conduction along peripheral nerve increased with age, from 20 m/sec at a conceptional age of 33 weeks to 33 m/sec at one month after term; from one month to four years of age the increase was 20 m/sec, the velocity along adult nerve being reached at three to four years of age.

Vecchierini (Vecchierini et al, 1984) studied nerve conduction velocity (NCV) in normal children: motor distal ulnar NCV (in 63 children, from 1 day to 4 years of age), motor distal tibial NCV (in 93 children, from birth to 15 years of age), proprioceptive tibial NCV (in 59 children, from birth to 5 years of age), proximal CV (in 133 children from birth to 15 years of age), as well as VIIth superior branch CV (in 67 children) and XIIth CV (in 56 children) from birth to 3 years of age, were measured. These measurements showed that NCV changes were similar; NCV was 45-50% of the adult value during the first month of life, then NCV increased very quickly, the progression being 90% of the first month's value during the first 18 months of life; the adult value was reached between 2 and 5 years of age.

Duron et al (1992) described the absolute refractory period and the conduction velocities of 99 premature babies and full-term babies (see figure 2 and 3), 43 children from 1 month to 8.5

years, 45 adults, underlining how the marked difference which exists between length development and postnatal increase of the fibre diameter explains the particular evolution of conduction times.

Tableau II. Valeurs moyennes (± SD) des vitesses de conduction, exprimées en m/s, mesurées pour chaque catégorie de fibres et coefficient d'augmentation de vitesse de conduction (m/s/mois) entre 28 semaines d'âge postconceptionnel et le terme.

Nerf	*Âge*					*m/s/mois*
	28 – 31 sem	*31 – 33 sem*	*33 – 35 sem*	*35 – 37 sem*	*Terme*	
Cubital						
Fibres motrices	17,2 ± 1,9	21,5 ± 3,4	23,6 ± 2,0	24,8 ± 3,3	29,2 ± 3,4	4,7
Fibres I_A	18,9 ± 3,3	22,5 ± 5,6	26,7 ± 5,8	28,2 ± 4,8	35,0 ± 4,4	6,4
Fibres cutanées		19,6 ± 3,2	19,4 ± 2,3	23,1 ± 1,7	25,8 ± 4,4	3,9
Médian						
Fibres motrices		18,2 ± 2,9	20,7 ± 2,0	23,2 ± 3,4	26,4 ± 2,8	3,9
Fibres I_A		19,7 ± 2,2	24,6 ± 2,4	27,4 ± 4,6	30,1 ± 3,0	4,7
Fibres cutanées		19,3 ± 3,2	19,9 ± 1,9	23,3 ± 2,2	26,8 ± 4,0	4,3
SPI						
Fibres motrices		16,0 ± 2,7	17,1 ± 2,5	19,1 ± 1,7	21,7 ± 3,5	3,4
Fibres I_A		18,4 ± 3,6	20,0 ± 4,1	22,9 ± 3,0	25,2 ± 4,4	4,7

Fibres I_A: fibres sensitives d'origine musculaire I_A.

Figure 2 (from Duron et al, 1992): normative data in 99 premature babies and full-term babies.

Tableau III. Valeurs moyennes (± SD) de différents temps de conduction, exprimés en ms, mesurés pour les fibres motrices chez les prématurés et les nouveau-nés à terme.

Nerf	*Âge*				
	28 – 31 sem	*31 – 33 sem*	*33 – 35 sem*	*35 – 37 sem*	*Terme*
Cubital	6,3 ± 0,7	5,2 ± 0,7	5,0 ± 0,5	5,0 ± 0,7	4,8 ± 0,5
Médian		5,9 ± 1,1	5,4 ± 0,4	5,2 ± 0,6	5,3 ± 0,6
SPI	10,4 ± 1,4	8,7 ± 1,4	8,1 ± 0,8	7,9 ± 1,2	8,4 ± 1,3

Nerf cubital: temps de conduction coude – muscles de l'éminence hypothénar; nerf médian: temps de conduction coude – muscles de l'éminence thénar; nerf SPI: temps de conduction creux poplité – court fléchisseur du gros orteil.

Figure 3 (from Duron et al, 1992): normative data in 99 premature babies and full-term babies.

Tranier (Trainer et al, 1989) studied 23 preterm, proving that the values for M-NCV in the posterior tibial nerve are comparable with previous values reported in the literature and confirm that maturation in these motor nerve fibres is independent of extra uterine factors;

instead the NCV of the proprioceptive sensory fibres (Ia fibres) of preterm babies (gestational age, < or = 31 weeks) at a post-conceptional age close to term is lower than that in full-term newborns.

Also Smit et al (1999) studied ulnar and posterior tibial MNCVs in preterm born at less than 30 weeks' gestation.

Parano et al (Parano et al, 1993), tested 155 healthy children in seven age groups from 1 week to 14 years (see figure 4) and published normal values of motor and sensory nerve conduction, distal motor latency, F-wave latency, and evoked response amplitude of peripheral nerves commonly tested: it was clearly shown that, in comparison with adult values, the whole group has significantly slower nerve conduction velocities, with reduced muscle and nerve evoked response amplitudes.

TABLE 2
Sensory Conduction Studies in 155 Children

	Median Nerve		Sural Nerve	
Age (N)	CV, m/s	AMP, μV	CV, m/s	AMP, μV
7 d–1 mo (20)	22.31 (2.16)*	6.22 (1.30)	20.26 (1.55)	9.12 (3.02)
1–6 mo (23)	35.52 (6.59)	15.86 (5.18)	34.63 (5.43)	11.66 (3.57)
6–12 mo (25)	40.31 (5.23)	16.00 (5.18)	38.18 (5.00)	15.10 (8.22)
1–2 yr (24)	46.93 (5.03)	24.00 (7.36)	49.73 (5.53)	15.41 (9.98)
2–4 yr (22)	49.51 (3.34)	24.28 (5.49)	52.63 (2.96)	23.27 (6.84)
4–6 yr (20)	51.71 (5.16)	25.12 (5.22)	53.83 (4.34)	22.66 (5.42)
6–14 yr (21)	53.84 (3.26)	26.72 (9.43)	53.85 (4.19)	26.75 (6.59)

*Mean (SD).
CV = conduction velocity; AMP = amplitude.

TABLE 1
Motor Conduction Studies in 155 Children

	Median Nerve				Peroneal Nerve			
Age (N)	DML, m/s	CV, m/s	F, m/s	AMP, mV	DML, m/s	CV, m/s	F, m/s	AMP, mV
7 d–1 mo (20)	2.23 (0.29)*	25.43 (3.84)	16.12 (1.5)	3.00 (0.31)	2.43 (0.48)	22.43 (1.22)	22.07 (1.46)	3.06 (1.26)
1–6 mo (23)	2.21 (0.34)	34.35 (6.61)	16.89 (1.65)	7.37 (3.24)	2.25 (0.48)	35.18 (3.96)	23.11 (1.89)	5.23 (2.37)
6–12 mo (25)	2.13 (0.19)	43.57 (4.78)	17.31 (1.77)	7.67 (4.45)	2.31 (0.62)	43.55 (3.77)	25.86 (1.35)	5.41 (2.01)
1–2 yr (24)	2.04 (0.18)	48.23 (4.58)	17.44 (1.29)	8.90 (3.61)	2.29 (0.43)	51.42 (3.02)	25.98 (1.95)	5.80 (2.48)
2–4 yr (22)	2.18 (0.43)	53.59 (5.29)	17.91 (1.11)	9.55 (4.34)	2.62 (0.75)	55.73 (4.45)	29.52 (2.15)	6.10 (2.99)
4–6 yr (20)	2.27 (0.45)	56.26 (4.61)	19.44 (1.51)	10.37 (3.66)	3.01 (0.43)	56.14 (4.96)	29.98 (2.68)	7.10 (4.76)
6–14 yr (21)	2.73 (0.44)	57.32 (3.35)	23.23 (2.57)	12.37 (4.79)	3.25 (0.51)	57.05 (4.54)	34.27 (4.29)	8.15 (4.19)

*Mean (SD).
DML = distal motor latency; CV = conduction velocity; F = F-latency; AMP = amplitude.

Figure 4 (from Parano et al, 1993): normal values of motor and sensory nerve conduction.

Garcia et al also confirmed that at birth the values are half those of adults (Garcia et al, 2000): the study comprised 92 normal infants and children aged from 1 week to 6 years; using surface electrodes, the investigation included motor conduction velocity (MCV), corrected distal motor latency (DML) to a standard distance, and F-waves of the median, ulnar, peroneal and tibial nerves, sensory conduction velocity (SCV) of the median and tibial nerves; and amplitude and

morphology of the muscle and sensory action potentials. They also found, compared to previous studies (Cai and Zhang 1997) (Parano et al 1993), that F-wave latency decreases in the first year of life, stabilizes and subsequently increases; it could be explained by an increase in MCV, and extremity growth.

Lori (Lori et al, 2018) studied motor/sensory NCV, latencies of cMAPs and SAPs and F waves latencies from plantar medial and median nerves and from tibial and ulnar nerves in 26 neurologically normal preterm infants born at 23–33 weeks of gestational age, repeating the same neurophysiological studies in 19 of the preterm infants every 2 weeks until postnatal term age, and matched these data with a group of ten full-term babies a few days after birth; they demonstrated that sensory-motor conduction parameters were clearly related to gestational age, but extrauterine life did not affect the maturation of the peripheral nervous system in the very preterm babies who were neurologically healthy; in particular the parameters showed a progressive linear maturation that was in agreement with gestational age.

Ryan et al (2019) published the largest paediatric study: 1918 paediatric electrodiagnostic studies, stratified in the following age groups: 0 to <1 month (11 studies), 1 to <6 months (16 studies), 6 to <12 months (39 studies), 12 to<24 months (82 studies), 2 to <3 years (59 studies), 3 to <4 years (53 studies), 4 to <5 years (47 studies), 5 to <10 years (260 studies), 10 to <15 years (509 studies), 15 to <18 years (842 studies); it was a rectrospective study, excluding patients with neuromuscular disorders. Normal reference ranges for amplitude, conduction velocity, and distal latency were established for each age group for 4 motor and 4 sensory nerves (see figure 5 -8).

Table 1. Normal values and cutoffs for lower limb motor nerves.

	Amplitude (mV)			Conduction velocity (m/s)			Distal latency (ms)		
Nerve/age group	Mean (SD), 2 SD below	5th percentile	*N*	Mean (SD), 2 SD below	5th percentile	*N*	Mean (SD), 2 SD above	95th percentile	*N*
Peroneal motor nerve									
0 to <1 mo	2.1 (1.1), 0.0	0.7	7	31 (14), 3	21	7	2.5 (0.5), 3.5	3.3	7
1 to <6 mo	2.8 (1.7), 0.0	1.0	9	41 (4), 33	35	9	2.0 (0.5), 3.0	2.9	9
6 to <12 mo	3.4 (1.2), 1.0	1.4	24	44 (7), 30	32	24	2.0 (0.4), 2.8	2.8	24
12 to <24 mo	3.7 (1.3), 1.1	1.7	65	48 (8), 32	41	65	2.2 (0.4), 3.0	2.9	65
2 to <3 y	3.7 (1.5), 0.7	0.9	51	49 (5), 39	41	49	2.4 (0.4), 3.2	3.3	50
3 to <4 y	4.4 (1.6), 1.2	2.1	46	50 (5), 40	43	46	2.9 (0.7), 4.3	4.7	46
4 to <5 y	4.3 (1.8), 0.7	1.4	38	50 (4), 42	41	38	3.1 (0.4), 3.9	4.2	38
5 to <10 y	4.7 (1.6), 1.5	2.3	204	52 (4), 44	46	202	3.6 (0.6), 4.8	4.7	204
10 to <15 y	5.4 (2.0), 1.4	2.6	410	51 (5), 41	45	408	4.2 (0.7), 5.6	5.6	410
15 to <18 y	6.4 (2.1), 2.2	3.2	559	50 (3), 44	44	558	4.4 (0.7), 5.8	5.8	559
Tibial motor nerve									
0 to <1 mo	5.3 (1.6), 2.1	3.2	4	24 (3), 18	19	4	2.7 (0.2), 3.1	2.9	4
1 to <6 mo	9.5 (0.9), 7.7	8.3	4	40 (5), 30	32	4	2.3 (0.2), 2.7	2.4	4
6 to <12 mo	10.0 (2.8), 4.4	6.2	8	41 (5), 31	32	7	2.7 (0.9), 4.5	4.9	8
12 to <24 mo	11.1 (3.0), 5.0	4.7	22	46 (4), 38	40	22	2.4 (0.4), 3.2	3.3	22
2 to <3 y	11.1 (3.1), 4.8	4.4	20	51 (5), 41	42	20	2.5 (0.4), 3.3	3.4	20
3 to <5 y	13.6 (5.2), 3.2	3.8	36	50 (6), 38	42	35	2.8 (0.4), 3.6	3.7	36
5 to <10 y	12.8 (3.8), 5.2	7.7	119	52 (5), 42	45	118	3.3 (0.6), 4.5	4.5	119
10 to <15 y	11.8 (3.6), 4.6	6.2	274	50 (4), 42	45	273	4.0 (0.7), 5.4	5.6	274
15 to <18 y	13.2 (3.9), 5.4	7.1	414	50 (4), 42	45	413	4.2 (0.6), 5.4	5.4	414

mo, months; y, years.

Figure 5 (from Ryan et al, 2019): amplitude, conduction, distal latency normal values for lower limb motor nerves.

Table 2. Normal values and cutoffs for lower limb sensory nerves.

	Amplitude (μV)			Conduction velocity (m/s)			Distal latency (ms)		
Nerve/age group	Mean (SD), 2 SD below	5th percentile	*N*	Mean (SD), 2 SD below	5th percentile	*N*	Mean (SD), 2 SD above	95th percentile	*N*
Sural sensory nerve									
0 to <6 mo	—	—	—	—	—	—	—	—	—
6 to <12 mo	18 (10), 0	8	4	—	—	—	2.4 (0.8), 4.0	3.8	4
12 to <24 mo	20 (7), 6	11	12	—	—	—	1.9 (0.3), 2.5	2.5	12
2 to <5 y	21 (9), 3	11	24	57 (5), 47	50	8	2.2 (0.3), 2.8	2.8	24
5 to <10 y	21 (10), 1	5	46	56 (7), 42	45	33	2.9 (0.6), 4.1	4.3	46
10 to <15 y	18 (8), 2	9	95	52 (6), 40	43	53	3.6 (0.3), 4.2	4.2	95
15 to <18 y	21 (9), 3	10	165	51 (5), 41	43	49	3.6 (0.3), 4.2	4.2	165
Medial plantar sensory nerve									
0 to <1 mo	10 (8), 0	3	4	—	—	—	3.6 (2.6), 8.8	8.0	4
1 to <6 mo	21 (11), 0	9	12	—	—	—	1.8 (0.6), 3.0	2.9	12
6 to <12 mo	34 (15), 4	12	29	—	—	—	1.7 (0.2), 2.1	2.3	29
12 to <24 mo	32 (16), 0	9	63	—	—	—	1.8 (0.3), 2.4	2.4	63
2 to <3 y	36 (18), 0	15	46	—	—	—	1.9 (0.3), 2.5	2.3	46
3 to <4 y	42 (19), 4	11	43	—	—	—	2.1 (0.3), 2.7	2.5	43
4 to <5 y	38 (17), 4	9	33	—	—	—	2.2 (0.3), 2.8	3.1	33
5 to <10 y	34 (16), 2	12	193	—	—	—	2.5 (0.4), 3.3	3.2	193
10 to <15 y	27 (13), 1	11	345	—	—	—	2.9 (0.4), 3.7	3.7	345
15 to <18 y	28 (13), 2	11	408	—	—	—	3.0 (0.4), 3.8	3.7	408

Dash (—) represents an insufficient sample of data. mo, months; y, years.

Figure 6 (from Ryan et al, 2019): amplitude, conduction, distal latency normal values for lower limb sensory nerves.

Table 3. Normal values and cutoffs for upper limb motor nerves.

	Amplitude (mV)			Conduction velocity (m/s)			Distal latency (ms)		
Nerve/age group	Mean (SD), 2 SD below	5th percentile	*N*	Mean (SD), 2 SD below	5th percentile	*N*	Mean (SD), 2 SD above	95th percentile	*N*
Ulnar motor nerve									
0 to <1 mo	3.8 (1.6), 0.6	0.8	7	35 (7), 21	29	7	2.2 (0.5), 3.2	3.2	7
1 to <6 mo	4.5 (1.9), 0.7	1.7	13	43 (7), 29	29	13	1.8 (0.3), 2.4	2.6	13
6 to <12 mo	5.4 (1.5), 2.4	2.1	29	51 (7), 37	37	29	1.7 (0.2), 2.1	2.4	29
12 to <24 mo	5.8 (1.8), 2.2	3.5	41	53 (7), 39	45	40	1.7 (0.2), 2.1	2.1	41
2 to <3 y	6.2 (1.9), 2.4	3.0	36	56 (6), 44	45	36	1.7 (0.2), 2.1	2.2	36
3 to <4 y	7.8 (1.9), 4.0	3.8	33	58 (6), 46	44	33	1.9 (0.3), 2.5	2.8	33
4 to <5 y	7.2 (1.7), 3.8	2.8	27	60 (6), 48	46	27	1.9 (0.4), 2.7	3.6	27
5 to <10 y	9.2 (2.7), 3.8	5.7	143	61 (6), 49	53	143	2.1 (0.3), 2.7	2.7	143
10 to <15 y	10.7 (2.4), 5.9	7.0	261	62 (5), 52	54	258	2.5 (0.3), 3.1	3.1	261
15 to <18 y	11.9 (2.5), 6.9	8.0	511	63 (5), 53	54	509	2.6 (0.3), 3.2	3.1	510
Median motor nerve									
0 to <1 mo	2.2 (1.6), 0.0	0.2	5	25 (3), 19	22	5	2.2 (0.2), 2.6	2.5	5
1 to <6 mo	3.3 (0.8), 1.7	2.3	4	37 (9), 19	27	4	1.7 (0.1), 1.9	1.8	4
6 to <12 mo	5.9 (2.5), 0.9	3.2	12	45 (13), 19	36	12	2.1 (0.2), 2.5	2.4	12
12 to <24 mo	5.7 (1.9), 1.9	2.4	18	47 (5), 37	38	17	2.2 (0.2), 2.6	2.7	18
2 to <5 y	7.2 (1.7), 3.8	5.2	18	51 (6), 39	40	17	2.3 (0.3), 2.9	3.0	18
5 to <10 y	8.9 (2.8), 3.3	2.1	34	56 (7), 42	48	32	2.9 (0.6), 4.1	5.2	34
10 to <15 y	10.9 (2.7), 5.5	5.7	78	58 (4), 50	51	77	3.3 (0.4), 4.1	4.9	78
15 to <18 y	11.6 (2.9), 5.8	7.3	240	59 (3), 53	54	239	3.3 (0.4), 4.1	4.0	240

mo, months; y, years.

Figure 7 (from Ryan et al, 2019): amplitude, conduction, distal latency normal values for upper limb motor nerves.

Table 4. Normal values and cutoffs for upper limb sensory nerves.

	Amplitude (μV)			Conduction velocity (m/s)			Distal latency (ms)		
Nerve/age group	Mean (SD), 2 SD below	5th percentile	*N*	Mean (SD), 2 SD below	5th percentile	*N*	Mean (SD), 2 SD above	95th percentile	*N*
Ulnar antidromic sensory nerve									
0 to <2 y	—	—	—	—	—	—	—	—	—
2 to <5 y	41 (15), 11	11	11	65 (7), 51	54	9	1.8 (0.2), 2.2	2.3	11
5 to <10 y	41 (15), 11	18	25	65 (5), 55	56	24	2.2 (0.3), 2.8	3.1	25
10 to <15 y	41 (12), 17	24	80	67 (5), 57	57	78	2.6 (0.3), 3.2	3.1	80
15 to <18 y	44 (17), 10	20	214	67 (5), 57	60	209	2.6 (0.2), 3.0	3.0	214
Median antidromic sensory nerve									
0 to <1 mo	24 (7), 10	15	8	26 (4), 18	19	8	2.5 (0.2), 2.9	3.0	8
1 to <6 mo	36 (12), 12	17	14	38 (9), 20	21	14	2.0 (0.3), 2.6	2.8	14
6 to <12 mo	53 (20), 13	30	27	48 (8), 32	33	27	2.1 (0.3), 2.7	2.8	27
12 to <24 mo	54 (23), 8	23	48	55 (6), 43	40	46	2.1 (0.2), 2.5	2.5	48
2 to <3 y	62 (24), 14	17	33	59 (6), 47	46	33	2.0 (0.2), 2.4	2.6	33
3 to <5 y	54 (20), 14	26	60	61 (5), 51	52	60	2.2 (0.3), 2.8	2.8	60
5 to <10 y	55 (19), 17	28	150	64 (5), 54	56	150	2.5 (0.3), 3.1	3.2	150
10 to <15 y	50 (15), 20	28	249	64 (4), 56	58	246	2.9 (0.3), 3.5	3.4	249
15 to <18 y	56 (18), 20	30	454	65 (4), 57	59	453	2.9 (0.3), 3.5	3.3	454

Dash (—) represents an insufficient sample of data. mo, months; y, years.

Figure 8 (from Ryan et al, 2019): amplitude, conduction, distal latency normal values for upper limb sensory nerves.

Finally, Jabre et al (Jabre et al, 2020) performed normal values of nerve conduction studies in a paediatric cohort between birth and 3 years of age using the method of extrapolated norms or e-norms; patients were divided as follows: 0-3 months, 3-6 months, 6-12 months, 12-24 months and 24-36 months. Figures 9 and 10 show the descriptive statistics of nerve conduction e-norms obtained in this age group.

Table 1
E-norms of sensory and motor upper extremity nerve conductions in ≤3 years old infants.

	0–3 months	3–6 months	6–12 months	12–24 months	24–36 months
Median S (P-W) Onset Lat (ms)	0.9 ± 0.08 (74)	0.9 ± 0.09 (64)	0.9 ± 0.08 (89)	0.9 ± 0.08 (93)	0.9 ± 0.09 (106)
Median S (P-W) Amplitude (μV)	19.5 ± 6.5 (79)	23.5 ± 8.1 (72)	28.0 ± 7.8 (85)	28.0 ± 9.1 (90)	28.4 ± 10.0 (63)
5th Percentile Amplitude (μV)	12.6	12.6	14.3	12.7	15.1
Median S (P-W) CV (m/s)	44.9 ± 5.4 (24)	45 ± 5.3 (56)	48.6 ± 5.8 (101)	49.7 ± 6.4 (121)	50.8 ± 6.1 (98)
	0–3 months	3–6 months	6–12 months	12–24 months	24–36 months
Ulnar S (P-W) Onset Lat (ms)	–	0.8 ± 0.1 (8)	0.8 ± 0.2 (5)	0.8 ± 0.2 (19)	0.9 ± 0.1 (48)
Ulnar S (P-W) Amplitude (μV)	–	12.8 ± 3.6 (5)	13.4±2.5 (4)	13.8 ± 4.1 (10)	14.2 ± 3.3 (26)
5th Percentile Amplitude (μV)	–	9.0	10.0	8.5	9.8
Ulnar S (P-W) CV (m/s)	–	49.1 ± 5.1 (9)	51.7 ± 9.7 (5)	56.7 ± 4.8 (20)	57.7 ± 8.0 (48)
	0–3 months	3–6 months	6–12 months	12–24 months	24–36 months
Median M APB Onset Lat (ms)	2.1 ± 0.2 (29)	2.1 ± 0.2 (21)	2.1 ± 0.2 (27)	2.1 ± 0.2 (39)	2.1 ± 0.2 (54)
Median M APB Amplitude (mV)	4.6 ± 1.7 (29)	5.4 ± 1.7 (25)	5.7 ± 1.7 (23)	5.9 ± 1.8 (34)	5.9 ± 1.9 (28)
	0–3 months	3–6 months	6–12 months	12–24 months	24–36 months
Ulnar M ADM Onset Lat (ms)	1.9 ± 0.2 (29)	1.9 ± 0.2 (17)	1.9 ± 0.2 (25)	1.9 ± 0.2 (56)	1.9 ± 0.2 (46)
Ulnar M ADM Amplitude (mV)	3.3 ± 0.9 (56)	3.6 ± 1.1 (40)	3.9 ± 1.2 (68)	4.1 ± 1.1 (82)	4.3 ± 1.1 (50)

Legend: E-norms are listed as mean ± standard deviation (number of studies), and sensory amplitudes 5th Percentile. S: Sensory. P-W: Palm-Wrist. Lat: Latency. CV: Conduction Velocity. M: Motor. APB: Abductor Pollicis Brevis. ADM: Abductor Digiti Minimi. Dash (–): Insufficient Data.

Figure 9: E-norms of sensory and motor upper extremity nerve conductions in _3 years old infants.

Table 2
E-norms of sensory and motor lower extremity nerve conductions in ≤ 3 years old infants.

	0–3 months	3–6 months	6–12 months	12–24 months	24–36 months
Sup Per S Onset Lat (ms)	–	–	1.3 ± 0.5 (18)	1.3 ± 0.3 (80)	1.3 ± 0.3 (116)
Sup Per S Amplitude (μV)	–	8.2 ± 3.8 (8)	13.8 ± 10.5 (18)	15.3 ± 12.4 (81)	17.8 ± 8.4 (115)
5th Percentile Amplitude (μV)	–	3.0	6.2	6.0	6.8
Sup Per S CV (m/s)	–	42.8 ± 8.5 (7)	44.1 ± 7.2 (16)	49.0 ± 10.1 (78)	53.7 ± 9.7 (112)
	0–3 months	3–6 months	6–12 months	12–24 months	24–36 months
Sural S Onset Lat (ms)	–	1.1 ± 0.2 (3)	1.2 ± 0.1 (17)	1.3 ± 0.8 (96)	1.3 ± 0.3 (63)
Sural S Amplitude (μV)	–	8.4 ± 4.4 (3)	12.7 ± 6.4 (18)	15.5 ± 9.1 (97)	18.0 ± 8.4 (132)
5th Percentile Amplitude (μV)	–	6.0	6.0	5.1	6.2
Sural S CV (m/s)	–	43.5 ± 4.1 (2)	44.4 ± 11.3 (17)	48.5 ± 8.7 (92)	50.2 ± 10.1 (130)
	0–3 months	3–6 months	6–12 months	12–24 months	24–36 months
Med Plantar S Onset Lat (ms)	1.3 ± 0.3 (201)	1.3 ± 0.5 (145)	1.3 ± 0.2 (243)	1.3 ± 0.3 (373)	1.3 ± 0.3 (258)
Med Plantar S Amplitude (μV)	8.4 ± 6.3 (213)	12.1 ± 11.2 (145)	13.3 ± 9.4 (244)	15.9 ± 10.0 (374)	16.0 ± 9.5 (258)
5th Percentile Amplitude (μV)	2.2	3.5	4.4	5.8	6.3
Med Plantar S CV (m/s)	32.1 ± 8.9 (208)	40.7 ± 11.2 (143)	45.3 ± 9.3 (236)	50.8 ± 10.8 (369)	53.1 ± 12.6 (254)
	0–3 months	3–6 months	6–12 months	12–24 months	24–36 months
Peroneal M EDB Onset Lat (ms)	2.0 ± 0.2 (9)	2.0 ± 0.1 (11)	2.0 ± 0.1 (29)	2.0 ± 0.1 (55)	2.0 ± 0.2 (40)
Peroneal M EDB Amplitude (mV)	1.4 ± 0.4 (11)	1.4 ± 0.5 (14)	1.5 ± 0.5 (31)	1.5 ± 0.4 (74)	1.6 ± 0.5 (56)
	0–3 months	3–6 months	6–12 months	12–24 months	24–36 months
Tibial M AH Onset Lat (ms)	2.4 ± 0.4 (133)	2.5 ± 0.6 (85)	2.5 ± 0.9 (100)	2.6 ± 0.8 (128)	2.7 ± 0.7 (73)
Tibial M AH Amplitude (mV)	4.6 ± 1.7 (76)	5.3 ± 1.7 (54)	5.7 ± 1.7 (72)	5.9 ± 1.8 (84)	5.9 ± 1.9 (49)

Legend: E-norms are listed as mean ± standard deviation (number of studies), and sensory amplitudes 5th Percentile. Sup Per: Superficial Peroneal. S: Sensory. Lat: Latency. CV: Conduction Velocity. Med Plant: Medial Plantar. M: Motor. EDB: Extensor Digitorum Brevis. AH: Abductor Hallucis. Dash (–): Insufficient Data.

Figure 10: E-norms of sensory and motor lower extremity nerve conductions in _ 3 years old infants.

In conclusion, these studies agrees with the contention that in the neonatal group MCV and SCV mean values are about one-half of those of normal young adults, and that most rapid increase occurs during the first year of life (Thomas and Lambert, 1960; Gamstorp, 1963; Baer and Johnson, 1965; Gamstorp and Shelburne, 1965; Wagner and Buchthal, 1972; Cruz Martinez et al., 1977, 1978; Vecchierini-Blineau and Guiheneuc, 1984; Miller and Kuntz, 1986;

Duron and Khater-Boidin, 1992; Parano et al., 1993; Kwast, 1995; Cai and Zhang, 1997; Garcia et al 2000). These features agree with histological changes determinant of conduction velocity in myelinated fibres (Waxman, 1980); it may be noted that at birth the largest diameter of myelinated fibres of sural nerve is one-half of that of the adult, fibre histogram being therefore unimodal; later, at the end of the first year, 35% of fibres are >8 microm and histogram adopts the characteristic bimodal pattern (Ouvrier et al., 1987).

They demonstrated that NCV are independent of intra/extrauterine factors, but the myelination depends on gestational age (Lori et at, 2018).

These studies demonstrated that adult values for both MCV and SCV are reached during the first 5 years; adult values were reached, however, earlier in the lower limb nerves, this probably being accounted for by the different motor milestones between legs and arms (Gamstorp, 1963; Cruz Martinez et al., 1978a; Cai et al, 1997).

Some studies (Garcia et al, 2000) seem to show that MCV values were in a similar range for peroneal, median and ulnar nerves, and lower for tibial nerve, which has been hypothesized to be related to a less direct anatomic route of the tibial nerve (Baer et al, 1965).

In other studies a similar finding was observed either between tibial and median nerves (Cruz Martinez et al., 1978a), or between tibial and ulnar nerves (Vecchierini-Blineau and Guiheneuc, 1984).

Evolution of F-waves latencies in children has scarcely been studied: in some studies these may remain stable (Cai and Zhang, 1997) or show a linear increase (Parano et al., 1993), in other have a bimodal pattern (Garcia et al, 2000).

The amplitudes of the motor and sensory responses show a less linear pattern and this may be due to higher dispersion for different myelinic fiber maturation at various gestational ages.

However, Smit (Smit et al, 1999) hypothesized that the low amplitude and reproducibility of cMAP in very preterm infants may be due to unstable clinical conditions or local factors.

The amplitude of AH being the first to reach adult values as of age 2 (Cai and Zhang, 1997) (Garcia et al, 2000). In contrast, increase of EBD CMAP amplitude, for unknown reason, occurs throughout the first decade (Thomas and Lambert, 1960) (Wagner and Buchthal,1972) (Cruz Martinez et al., 1978a) (Garcia et al 2000).

SNAP amplitudes reached adult values by age 2 years (Gamstorp and Shelburne, 1965), Cruz Martinez et al., 1978b), (Garcia et al, 2000) earlier than observed for most CMAP; later on, the lesser increase or decrease in SNAP amplitudes can be accounted for by the increase in temporal dispersion of potentials as a result of the longer distances involved in relation to

extremity growth (Cruz Martinez et al., 1978b), and by the differences in the distance from the skin to the nerve (Buchthal and Rosenfalck, 1966; Wilbourn, 1994) (Garcia et al, 2000); for others, the variability in amplitude is because amplitudes depend on not only axonal features, but also the maturity of the neuromuscular junction and the features of the muscle tested (Ryan et al, 2019); confirmation of this hypothesis calls for future studies determining the SNAP areas.

CMAP morphology is biphasic except for ADM where there may be a double negative peak; this should be taken into account to avoid misinterpretation (Garcia et al, 2000).

The major limitations encountered are that is difficult to obtain a healthy study population, for which a retrospective approach was used; not always the number of the sample allowed statistical analysis. However, the sample sizes in these normative studies were relatively small. Furthermore, many laboratories have reported their own pediatric reference values when discussing normal or abnormal results, and only in few cases a clear explanation of the methodology used to obtain these values was reported (Ryan et al, 2019).

Electromyography (EMG) and paediatric normative values in literature

Electromyography is a functional exam that tests the integrity of the entire motor system, which consists of upper and lower motor neurons, neuromuscular junction, and muscle. It consists of recording the electrical activity produced by the muscle fibres.

It allows not only to highlight a pathological condition of the muscle or to exclude conditions without neuromuscular involvement, but plays a pivotal role in differentiating between neurogenic and myopathic diseases and disorders of neuromuscular transmission, in localizing lesions in the peripheral nervous system, in grading severity, in monitoring disease progression.

With conventional EMG we can evaluate: insertional activity caused by movement of needle electrode placed in the muscle, that could be categorize into normal, diminished, enhanced patterns; spontaneous activity recorded at rest, analyzing morphology, stability and firing characteristics (firing rate and firing pattern); isolated voluntary MUPs during mild muscle contraction and of each must be evaluated morphology, amplitude, rise time, duration, area, phases and presence of satellite potential; recruitment of motor units during progressively greater muscle contraction and an interference pattern at the maximum effort.

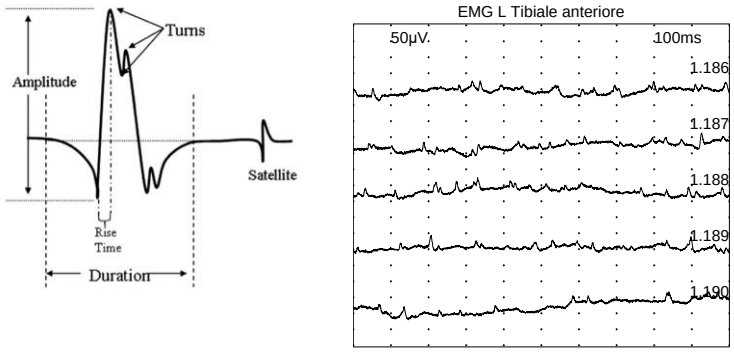

Figure 11: EMG examples.

All these assessments are evaluated by the neurophysiologist and seem to be quite subjective. In the recent years quantitative analyzes of EMG have become more widespread.

Quantitative EMG (QEMG) is defined as ‘‘a systematic method of measuring the recording made by an intramuscular needle electrode. Measurements include motor unit action

potential characteristics such as amplitude, duration, and phases or interference pattern characteristics (Phillips et al, 2001).

Quantitative analysis includes numerous parameters, of which the most used are: quantitative analysis of MUP parameters, multi-MUP analysis (Fuglsang-Frederiksen, 2006), “upper centile amplitude”; quantitative analysis of motor unit firing rate (Fuglsang-Frederiksen, 2006), quantitative analysis of the interference pattern, e.g TAA (Turns/amplitude analysis), clouds, “number of small segments” (NSS), Motor unit number estimation (MUNE).

It appears that QEMG allows more consistency between EMG findings and muscle biopsy when diagnosing myopathy (Jihoon Chang et al, 2011).

Therefore, QEMG this is a study approach that is finding more and more space in the pediatric field (Phongsamart et al, 2003). Therefore, QEMG this is a study approach that is finding more and more space in the pediatric field, because it is potentially useful in the following situations (Phongsamart et al, 2003): evaluation of not cooperative children, because compliance is relatively less important factor for QEMG (and in particular for TAA) than for traditional interference pattern analysis, and this is especially important in patients with impaired cognition or very young, or those who were unable to exhibit gradual muscle contractions(Chang et al, 2011) (Ryu et al, 2007); examination of mild to moderate neuromuscular pathologies in which the electrophysiological abnormalities reveal only slight abnormalities (Barkhaus, 2001); serial re-examination in which the results need to be compared with previous studies to assess the progression of the disease (Stalberg et al, 1995); research applications (Nandedkar et al, 1995) (Stalberg et al, 1995)(Barkhaus, 2001).

We can also register endplates activities, with repetitive nerve stimulation (RNS) and spontaneous or stimulated single fibre EMG (SFEMG) (Chiou-Tan et al, 2015).

Despite the increased availability of more sensitive antibody testing in the diagnosis of Myasthenia Gravis (Rodriguez Cruz et al, 2015), RNS is still considered a rapid non-invasive first-line examination in the diagnostic evaluation of MG and myasthenic syndromes (Medicine AQACAAoE, 2001).

The technique of RNS is similar to that used in conventional neurographic studies, differing only in the application of stimuli trains or paired stimuli, the use of conditioning exercise, and the careful immobilization of the limb to reduce movement artefact.

However, abnormal results from RNS studies are not diagnostic of specific clinical disorders, and abnormalities may be detected in patients with multiple sclerosis, motor neuron disease,

peripheral neuropathy, radiculopathy, or primary muscle membrane disease, in addition to patients with primary disorders of the NMJ such as MG, LES, arthropod envenomation, botulism, congenital myasthenic syndromes, and impaired NMT caused by certain commonly used medications (eg, antibiotics) and toxins (eg, organophosphates) (James F. Howard Jr, 2013).

SFEMG is a highly selective recording technique in which a concentric needle electrode is used to identify and record extracellular APs from individual muscle fibres, to evaluate neuromuscular transmission (NMT). Jitter is the most sensitive clinical electrophysiologic measure of the safety factor of NMT. It is increased whenever the ratio of the AP threshold and the EPP is greater than normal. When NMT is sufficiently impaired, nerve impulses fail to elicit muscle APs and SFEMG demonstrates intermittent impulse blocking. When blocking occurs in many end-plates in a muscle there is clinical weakness. SFEMG can demonstrate abnormal NMT (as increased jitter) in muscles that are clinically normal and have no decrement to RNS.

The selectivity of the technique results from the small recording surface (25 mm in diameter) that is exposed at a port on the side of the recording electrode, 3 mm from the tip (James F. Howard Jr, 2013).

Single fibre EMG reference values for the jitter do not show a very significant change over the age range studied by Stalberg and others which extends down to one year of age (Bromberg et al, 1992).

Paediatric values in literature:

The normative data of paediatric electromyography concerning the duration of the motor unit potentials are available from the results obtained by Buchthal and colleagues in Copenhagen (Buchthal et al, 1941) (Buchthal et al, 1952) (see Figure 12-14): at the beginning he described action potential parameters concerning brachial biceps only , and after that in other human muscles (Buchthal et al, 1955); normal data of the mean duration of motor unit potentials, their tension and shape in a few muscles in infants started to be described (Buchthal et al, 1954), (Hertz et al, 1954), (do Carmo 1960), (Bottone et al, 1960) (Sacco et al, 1962); sometimes that recorded only 1-2 potentials from each muscle (do Carmo, 1960), which is insufficient to characterize action potential parameters in a given muscle.

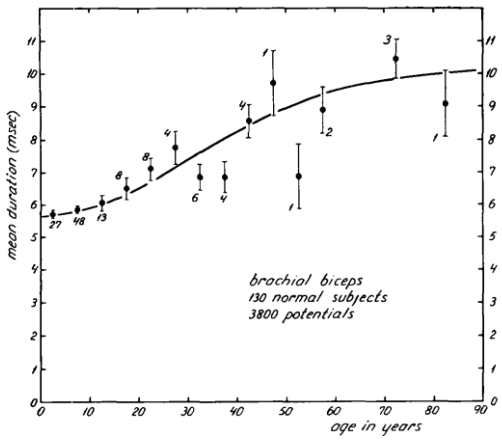

Figure 12 (from Buchthal, 1954): mean action potential duration as a function of age.

Table 1.

Distribution of shape of action potentials for different age groups.

(Brachial biceps; concentric electrodes.)

Age (years)	Total number of potentials	Number of potentials (in per cent)				
		Mono-phasic	Diphasic	Tri-phasic	Tetra-phasic	Poly-phasic
0— 4	170	2.0	31.5	56.5	5.5	4.5
20—22	692	3.2	45.6	41.3	6.5	3.4

Figure 13 (from Buchthal, 1954): distribution of shape of action potentials for different age groups.

LEG AND FOOT MUSCLES

age in years	Glut. max. 22)	Biceps fem. 23)	Rectus fem. 24)	Vastus med. 25)	Vastus int. 26)	Tibial ant. 27)	Peron. long. 28)	Gastro-cnem. 29)	Soleus 32)	Ext.dig. brev. 30)	Abd. hall. 31)	age in years
0	9.2	8.5	8.7	7.9	9.7	9.5	7.6	7.2	7.7	7.2	6.5	0
3	9.8	9.1	9.2	8.4	10.3	10.1	8.1	7.7	8.2	7.7	7.0	3
5	10.2	9.4	9.6	8.7	10.7	10.5	8.4	8.0	8.5	8.0	7.2	5
8	10.7	9.9	10.0	9.1	11.2	11.0	8.7	8.4	8.9	8.4	7.6	8
10	10.9	10.1	10.3	9.3	11.5	11.2	8.9	8.6	9.1	8.6	7.7	10
13	11.3	10.4	10.6	9.6	11.8	11.6	9.2	8.8	9.4	8.8	8.0	13
15	11.5	10.6	10.7	9.8	12.1	11.7	9.4	8.9	9.6	8.9	8.2	15
18	11.8	10.9	11.1	10.0	12.3	12.1	9.6	9.2	9.8	9.2	8.4	18
20	12.0	11.1	11.3	10.2	12.6	12.3	9.8	9.4	10.0	9.4	8.5	20
25	12.4	11.4	11.6	10.5	13.0	12.7	10.1	9.7	10.3	9.7	8.8	25
30	12.7	11.8	12.0	10.8	13.4	13.1	10.4	10.0	10.6	10.0	9.0	30
35	13.1	12.1	12.3	11.1	13.7	13.4	10.7	10.2	10.9	10.2	9.3	35
40	13.3	12.3	12.6	11.3	14.0	13.6	10.9	10.4	11.1	10.4	9.4	40
45	13.4	12.4	12.7	11.4	14.1	13.8	11.0	10.5	11.2	10.5	9.5	45
50	13.7	12.7	12.9	11.6	14.4	14.0	11.2	10.7	11.4	10.7	9.7	50
55	13.9	12.9	13.1	11.8	14.6	14.3	11.4	10.9	11.6	10.9	9.9	55
60	14.3	13.2	13.5	12.1	15.0	14.7	11.7	11.2	11.9	11.2	10.2	60
65	14.6	13.5	13.7	12.4	15.4	15.0	12.0	11.5	12.2	11.5	10.4	65
70	14.9	13.8	14.0	12.6	15.6	15.3	12.2	11.7	12.4	11.7	10.6	70
75	15.1	14.0	14.2	12.8	15.9	15.5	12.4	11.8	12.6	11.8	10.8	75
80	15.4	14.2	14.4	13.0	16.1	15.7	12.6	12.0	12.8	12.0	11.0	80

Figure 14: Motor unit duration tables from laboratory of Clinical Neurophysiology Rigshospital, Copenhagen 1975.

In preliminary investigations (Buchthal and Pinelli, 1951) it was found the mean action potential duration to increase with age over the range two to seventy years.

Buchthal et al (1954) described the influence of some physiological factors, such as age, temperature, degree of contraction and fatigue; in particular the influence of age was studied on the left brachial biceps of 130 subjects between 1 and 85 years of age, they corroborated precedents results, and it was explained in terms of a decrease of propagation velocity of the impulse over the muscle fibre; in the same study (1954) they found that the mean amplitude of the muscle action potentials was independent of the age of the subject; the distribution of the action potentials according to their shape was different in different age groups: in children below 4 years, triphasic potentials were relatively more frequent occurence than diphasic, in adults (20-22 years) triphasic and diphasic were equally represented.

Sacco et al (Sacco et al, 1962) investigated parameters of the motor unit potentials (mean duration, voltage, shapes) in the abductor digiti quinti and the brachial biceps and anterior tibial muscle, in 38 subjects (Four subjects were 3, months, 13 were aged 16-23 years, 5 were 26-40 years, 6 were 47-52 years, and 10 were 61-80 years of age). They stressed the need to use the same method (for example the same type of electrodes), and found that: the mean action potential duration in the anterior tibial muscle is longer than in the first dorsal

interosseus or in the brachial biceps (Buchthal et al, 1955) (Do Carmo, 1960), that can be explained by the difference between bipennate and unipennate muscle; a shorter action potential duration in the abductor digiti quinti than in the brachial biceps (explained in terms of the shorter muscle length and a slightly smaller width of the endplate zone); a significant increase in the duration of motor unit potentials at 20 years of age, as compared with at 3 months of age, that was explained in terms of the increasing width of the endplate zone with growth of the muscle: the average width of the endplate zone of the brachial biceps was 25 mm in adults and 6 mm in stillborn infants (Buchthal et al, 1955); in persons of from 20 to 70 years of age, the mean duration of the action potentials showed a further increase, and it is attributed to an increased fiber density within the motor units caused by a decrease in the volume of the muscle.

One of the major problems regarding EMG concerns the sensitivity and specificity of EMG in the diagnosis of neuromuscular pathologies.

The lack of normative data allows the diagnostic accuracy of EMG in children varies between 10-98% (Hellmann 2005), and it seems that there is less sensitivity and specificity for myopathies (80%) compared to neuropathies (more than 90%) (Chang et al, 2011) (Rabie, 2007), also with the combined use of EMG and muscle biopsy (Hellmann et al, 2005) (Black et al, 1974) (Hafner, 2019).

Previous studies analysing the accuracy of EMG compared to muscle biopsy in patients with neuromuscular diseases are reported in the following section (Brusa et al., 1963) (Humphrey and Shy, 1962) (Schwartz et al., 1966) (Hausmanowa-Petrusewicz and Karwanska, 1971) (Micaglio et al., 1980) (Buchthal and Kamieniecka, 1982) (David and Jones, 1990, 1994) (Packer et al., 1982) (Werneck et al, 1988) (Gibertoni et al, 1987) (Russell et al., 1992) (Hellmann et al, 2005) (Chang et al, 2011) (Hafner et al, 2018) (Rabie et al, 2007) (David et al, 1994) (Ghosh et al, 2014) (Constantinides et al, 2018) (Sener 2019) (Naddaf et al, 2018) (Dardiotis et al, 2011).

Another problem in paediatric age is that very few publications are available on procedural problems of EMG examination in paediatric patients. Hays and colleagues have addressed the problem of pain and stress in young patients and gave suggestions for optimization of the procedures (Hays et al., 1992, 1993).

Hellmann et al (Hellmann et al, 2005) assessed the diagnostic value of EMG and NCS in children, in particular the concordance of the suspected clinical diagnosis, the results of

electrodiagnostic examination, and the final clinical diagnosis, and investigated the incidence of procedural problems: they found, in a large paediatric patient cohort of 498 children, that the diagnostic efficiency and the quality of the results obtained in infants and small children are comparable with those obtained in older and more cooperative children if EMG examination is performed by an experienced investigator, and they therefore concluded that even in very young children procedural problems generally do not impair the diagnostic accuracy of the results obtained.

Therefore, it was demonstrated that QEMG it could be potentially useful in not cooperative children.

Fuglsang-Frederiksen et al (2006) investigated different EMG methods (qualitative and quantitative) in the diagnosis of myopathy: manual analysis of individual motor unit potentials and multimotor unit potential analysis at weak effort; turns–amplitude analyses such as the cloud analysis and the peak ratio analysis at high effort; it was emphasized that EMG is useful to differentiate myopathic to neuropathic forms, but that it can seldom be used to differentiate between different types of myopathy.

It was reported (Chang J. et al, 2011) that the use of the TAA implements the sensitivity of diagnosis in the myopathic forms.

Finally Hafner et al (2018) studies diagnostic value of EMG and muscle biopsy. They confirmed that it is sufficient to examine one muscle provided this is done comprehensively, possible with QEMG, and this is relevant for children of all ages, including the very young under the age of 2 years; infact in this study EMG sensitivity was comparable with others studies that have investigated more muscles (Chang et al, 2011) (Ghosh et al, 2014) (Hellmann et al, 2005), different from Rabie: the fact that the study by Rabie et al. found an EMG sensitivity of 36.4% with qualitative analysis of four muscles strengthens the belief that a thorough examination of one or two muscles is superior to several muscles being examined qualitatively. Furthermore, the subgroup analysis of 22 children under the age of 2 years revealed a EMG sensitivity of 81.8% and a biopsy sensitivity of 86.4% for myopathy (Hafner et al, 2018), so EMG sensitivity in this age group is slightly below the one found in the entire myopathic cohort, but higher than the sensitivity reported by previous studies based on qualitative EMG (David et al, 1994) (Russel et al, 1992) (Kang et a, 2003).

These results emphasise that a concise protocol including nerve conduction studies and EMG of a single muscle in unsedated children offers a diagnostic accuracy for myopathic disorders

that is equivalent to that of a muscle biopsy; nevertheless, whilst EMG can predict myopathic conditions with similar accuracy, muscle biopsy has the advantage of permitting the detection of specific histological findings to further guide focused genetic testing. EMG still remains the more accurate test when determining neurogenic abnormality.

In conclusion, QEMG this is a study approach that is finding more and more space in the pediatric field, because it is potentially useful in the following situations (Phongsamart et al, 2003): evaluation of not cooperative children, because compliance is relatively less important factor for QEMG (and in particular for TAA) than for traditional interference pattern analysis, and this is especially important in patients with impaired cognition or very young, or those who were unable to exhibit gradual muscle contractions (Chang et al, 2011) (Ryu et al, 2007); examination of mild to moderate neuromuscular pathologies in which the electrophysiological abnormalities reveal only slight abnormalities (Barkhaus, 2001); serial re-examination in which the results need to be compared with previous studies to assess the progression of the disease (Stalberg et al, 1995); research applications (Nandedkar et al, 1995) (Stalberg et al, 1995) (Barkhaus et al, 2001).

However, there are few studies concerning the QEMG normative data, and in these only few parameters are analysed (Chang et al, 2011) (Fuglsang-Frederiksen, 2000).

Muscle biopsy

Muscle biopsy plays a critical role in the evaluation and diagnosis of patients suspected of having an underlying neuromuscular disorder.

Muscle biopsy has historically been considered to be the gold standard in the diagnosis of muscle disease (Hafner et al, 2019), allowing for the precise characterisation through histological, biochemical, immunocytochemical and ultrastructural analyses (Joyce et al, 2012) (Thavorntanaburt et al, 2018).

Muscle biopsy is still central to the definitive diagnosis of inherited neuromuscular disorders to guide genetic analysis; moreover, congenital myopathies, caused by genetically determined defects in muscle structural proteins, are classified on the basis of muscle biopsy results (Cassandrini et al, 2017) (North et al, 2014).

Even for acquired conditions that affect the muscle , muscle biopsy plays a critical role, providing diagnostic evidence that either establishes a disease etiology or focuses the

differential diagnosis, allowing the clinician to distinguish between a necrotizing, metabolic or inflammatory myopathy and facilitating rapid, appropriate therapeutic management.
Regardless the NMD condition, muscle biopsy can also provide information on the severity of the underlying disease and the extent of muscle damage/fibrotic substitution.
In NMD with a neurogenic or NMJ pathogenesis the use of muscle biopsy is limited albeit typical lesions appear on the histopathological studies (such as type grouping or neuromuscular junction alterations).
However, muscle biopsy is an expensive, invasive, time-consuming, and resource-dependent procedure, and requires planning. First of all, it needs the involvement of several experts, including the surgeon, the analytical laboratory and the pathologist. Furthermore, muscle biopsy requires a cryo-processing of the fresh specimen to allow for enzymatic histochemistry and metabolic testing (Joyce et al, 2012) (Nix et al, 2020), unlike biopsies of other organs, for which simple preservation in formalin is the routine procedure; therefore, tissue collection, packaging and processing must be organised to ensure that the desired tests are performed and to avoid the need to repeat the procedure due to a limited, inadequate or poor quality sample (Joyce et al, 2012).
Interpretation of the muscle biopsy result is performed as follows.
Routine histochemistry, which is typically performed on frozen tissue, commonly includes the following stains: Hematoxylin and eosin (H&E), Gomori Trichrome and Verhoeff van Gieson (VvG) for the evaluation of morphology; myofibrillar Adenosine triphosphatase (ATPase) (at pH 9.4, 4.6, and 4.3) for fiber Type Enzymes; Oxidative Enzymes: Nicotinamide adenine dehydrogenase (NADH), Succinate dehydrogenase (SDH), Cytochrome oxidase (COX), Mitochondrial pathology; Hydrolytic Enzymes: Esterase, Acid phosphatase, Alkaline phosphatase; Periodic Acid Schiff (PAS) and Oil red O or Sudan Black Lipids for evaluating storage material. Examples of stains are presented in the figure 15.
The numerous techniques make it possible to assess the morphology of muscle fibres and identify many pathological signs, such as those of inflammation, necrosis, obvious mitochondrial abnormalities and abnormalities of glycogen and/or lipid accumulation. Specialised immunohistochemical studies can also be performed, using antibodies against muscle-associated proteins (such as dystrophin, sarcoglycan, major histocompatibility complex 1, etc.) to localise and often quantify these proteins (Joyce et al, 2012) (Meola et al, 2012).

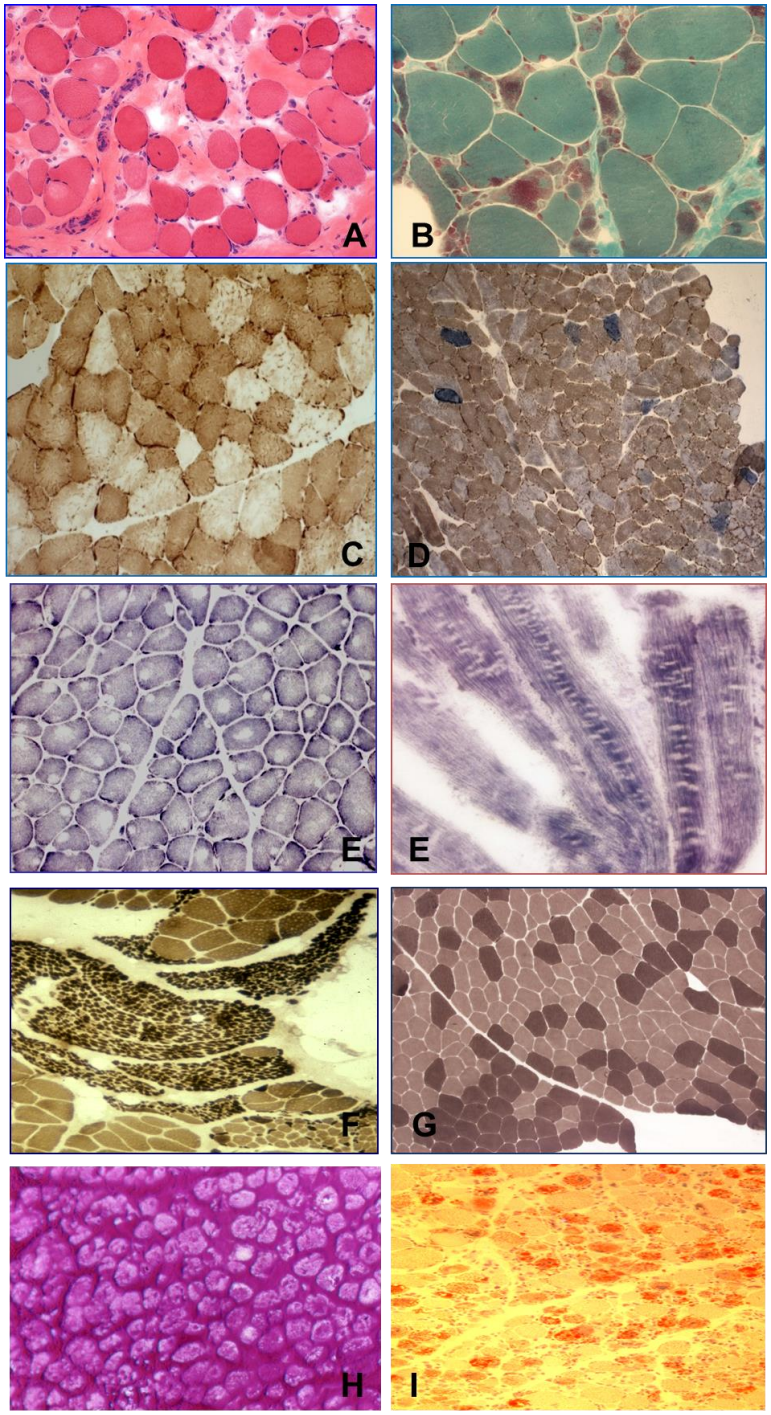

Figure 15: Histology techniques such as Hematoxylin and Eosin, and Gomori Trichrome (A, B), Oxidative enzymes (C, D, E), Muscle fibre types (F, G) PAS and Oil red O (H, I) are shown. A: Muscle biopsy from a patient with Congenital muscular Dystrophy. Section stained with Hematoxylin and eosin (H&E) show Rounded fibres, Internal nuclei Increased connective tissue

(fibrosis) ; B: Gomori Trichrome stain in nemaline myopathy shows Variation in fibrous calibre, Presence of red nemaline bodies or rods. C and D: COX (C) and SDH (D) colourations in Mitochondrial disease with COX deficiency and combined COX/SDH reaction. E: NADH stain of muscle biopsy from a patient with Congenital Myopathy Type central cores multiminicores. F: ATPase stain in Spinal Muscular Atrophy highlights Atrophy of type 2 fibres (fast), Giant type 1 fibres (slow), Type grouping, Indices of motor unit remodelling. G: ATPase stain in congenital myopathy with fibre disproportion demonstrates prevalence of type 1 fibres. H: PAS stain in Pompe Disease highlights glycogen accumulation. I: Oil Red O in patient with Mitochondrial Disease shows deficiency of fatty acid oxidation and lipid accumulation

Normal muscle biopsy

Normal muscle is made up of many muscle fibres grouped together by layers of connective tissue. A muscle fiber, or muscle cell, is a multinucleated, long, tubular structure that varies in diameter from 10–20 mm in the infant to about 50–70 mm in the adult (Swaiman et al, 2012).

The single muscle fibre, is surrounded by the endomysium, a thin layer of mainly reticular fibres, which is hardly visible under the microscope, so the muscle fibres appear to be in direct contact with each other. Groups of muscle fibres are bound together by the perimysium, which is thicker, and form fascicles.

Within the perimysium, which is contiguous with the epimysium, are the nutrient blood vessels, intramuscular nerves, and muscle spindles

The epimysium, a dense connective tissue, encloses the bundles of fascicles. The connective tissue layers offer mechanical protection to the muscle fibres and increase the tensile strength of the muscle (Joyce et al, 2012). The confluence of the perimysial and epimysial connective tissue forms the tendons at either end of the muscle belly (Swaiman et al, 2012).

In cross-section, individual myofibres appear polygonal except in the infant when round fibres are normal (DeGirolani et al, 1997).

The diameter of the cells varies according to age, gender and the specific muscle being assessed (Bossen et al, 2000).

Each muscle fibre contains numerous nuclei and each nucleus provides a segment of the cell with the necessary translated protein products. In normal muscle, these nuclei are located peripherally and immediately adjacent to the sarcolemmal membrane, and only 3-5% of fibres contain nuclei that are located within the cell (Joyce et al, 2012); an increase in the

internalisation of nuclei may be an indicator of a pathological process, and this characteristic is common to many pathological processes.

The sarcoplasm represents approximately 40% of the cell volume and contains myoglobin, mitochondria, lysosomes, lipid vacuoles and glycogen.

To be able to contract a muscle fiber requires the myofibril, its contractile unit; the myofibril is made up of a long chain of sarcomeres that orient parallel to the long axis of the fiber and is built by proteins such as actin, myosin and titin (Joyce et al, 2012).

Under the microscope, muscle fibres create a mosaic of different fibres visible with ATPase staining (Scott et al, 2001). This is because the muscle fibre is an integral and decisive part of the motor unit. The motor unit is by definition made up of a single α-motor neuron and all the corresponding muscle fibres it innervates.

Therefore the neural stimulus determines the metabolic signature of the muscle fibres related to it (Joyce et al, 2012): the muscle fibres from the same single motor unit have the same metabolic type. However, muscle fibres are alternated with fibres from other motor units, creating a mosaic of different fibre types.

There are two main types of muscle fibres and the duration of the muscle fiber contraction varies with the fiber type. Some fibres have have slow contraction times of 50–100 milliseconds and are called “slow-twitch” fibers or type I; these fibres utilise oxidative metabolism and have a greater amount of lipids and mitochondria within the sarcoplasm. Others fibres have have rapid twitch times of 5–10 milliseconds and are called “fast-twitch” fibers or type II; these predominantly utilise glycogen for energy production (Joyce et al, 2012). Finally, there are multiple subtypes of type II fibres, identified by different cellular characteristics, the most common of which is the ability to utilise the oxidative metabolic pathway for ATP production (Scott et al, 2001).

Different ATPase stains are commonly performed at three different pH values, and are used to identify fiber type pH 9.4 ATPase stains type I fibres light and type II fibres dark; pH 4.3 ATPase stains type I fibres dark and type II fibres light; pH 4.6 ATPase stains type I fibres dark, type IIA fibres light and type IIB fibres an intermediate shade(Hilton-Jones et al, 1997).

Myopathologic patterns

There are two major characteristic myopathologic patterns of neuromuscular disease: neurogenic and myopathic.

By neurogenic pattern we mean a series of alterations resulting from diseases of the innervating neuron; with myopathic pattern anomalies due to intrinsic diseases of the muscle fiber, hereditary or acquired, are described.

Sometimes the changes are nonspecific and are consistent with "unspecified myopathy". This finding can occur for several reasons: the disease process is in its early stage and the findings are not yet specific to a single disease process; the tissue may not have been taken from a fulminant region of disease; the disease typically does not cause structural abnormalities (Nix et al, 2020).

Finally, often on muscle biopsy we can find histological results of both neurogenic and myopathic processes; in fact aggressive and chronic myopathies often lead to denervation of the muscle fiber and therefore to neurogenic findings superimposed on a myopathic pattern on biopsy (DeGirolani et al, 1997).

We now report the main histopathological findings.

Neurogenic changes

The first structural change in neurogenic atrophy observed on muscle biopsy is the loss of the polygonal shape of the muscle fiber (DeGirolani et al, 1997). Another early finding is a pattern of atrophic muscle fibers involving both type I and type II fibers; atrophic fibers become small and angular.

After denervation, reinnervation can occur, when a single motor neuron sprouts and reinnervates multiple atrophied muscle fibers; in this way the fiber type is altered (Sorarù et al, 2008) and fiber-type grouping will be marked with ATPase staining, the normal patchwork pattern will be alterated and groups of similar fiber types will be placed adjacent to each other.

As well described by Joyce (Joyce et al, 2012), other structures commonly associated with neurogenic atrophy are: nuclear sacs, which appear as clusters of nuclei surrounded by the remaining sarcolemmal membrane; target fibers or targetoids, which are found in the context of neurogenic atrophy (Nix et al, 2020). The target fibres, which are most commonly type I muscle fibers (Schmitt et al, 1975), are characterized by the presence of three zones, each with variable staining intensity: the central area is pale and is the result of reduced oxidative enzymatic activity, disorganized myofibrils and a paucity of mitochondria; the central area is surrounded by a dark colored area, which is enriched with mitochondria and has increased enzymatic activity; the third zone is located on the periphery and is colored normally.

Targetoid fibres resemble target fibres but have only two discrete regions within the cell (Joyce et al, 2012).

Myopathic Changes

Myopathic changes include alterations that are common to all primary muscle diseases, which may be accompanied by disease-specific structural alterations.

Common myopathic features include: variation in fiber size, with both atrophied and hypertrophic muscle fibers; hypertrophic fibers may eventually split into two fibers and are called "split fibers"; degenerating and regenerating fibers disseminated throughout the muscle. The degeneration usually begins in a segmental manner; regenerating fibers can be easily identified by enlarged nuclei and a bluish stain within the fiber, due to the increased concentration of RNA within the cell (Joyce et al, 2012).

Particularly early in the disease, changes can cause focal damage to myofibers (as in mitochondrial disorders), segmental damage (for example in dystrophies) or multifocal damage (typical of inflammatory myopathies). Old damage can be identified by the increase in internalized nuclei; this characteristic is common to many myopathies (for example in centronuclear myopathy as in myotonic muscular dystrophy). Another change that occurs with the chronic progression of most myopathic diseases is the thickening of the endomysium and perimysium (Joyce et al, 2012).

Some muscle disease will appear normal on muscle biopsy with only minimal hints of disease. This may happen because the disease is segmental and no pathological tissue was obtained during the biopsy, or the disease usually does not cause structural abnormalities (as in some metabolic myopathies). Other myopathies, on the other hand, will present histological anomalies considered highly indicative for a certain disease: ragged red fibres, that are common in mitochondrial disease; rimmed vacuoles, found in inclusion body myositis; central cores, found in central core disease (Joyce et al, 2012). However, each of these histopathological findings can also be observed in other myopathies and this increases the complexity and difficulty of reading a muscle biopsy. In case of diagnostic difficulty, immunohistochemical staining and biochemical tests can provide evidence of a specific disease process (Joyce et al, 2012).

Correlation of neurophysiological studies and muscle biopsy in neuromuscular disorders

Most previous studies have focused on the ability of muscle biopsy and electromyography to distinguish the neuropathic from myopathic nature of the underlying neuromuscular disease.

To our knowledge, there are no systematic studies comparing NCSs and muscle biopsy.

In particular, these studies analysed diagnostic accuracy of EMG versus muscle biopsy in patients with neuromuscular disease (Brusa et al., 1963) (Humphrey and Shy, 1962) (Schwartz et al., 1966) (Hausmanowa-Petrusewicz and Karwanska, 1971) (Micaglio et al., 1980) (Buchthal and Kamieniecka, 1982) (David and Jones, 1990, 1994) (Packer et al., 1982) (Werneck et al, 1988) (Gibertoni et al, 1987) (Russell et al., 1992) (Hellmann et al, 2005) (Chang et al, 2011) (Hafner et al, 2018) (Rabie et al, 2007) (David et al, 1994) (Ghosh et al, 2014) (Constantinides et al, 2018). However, despite the common use of these diagnostic techniques, few studies have investigated the associations specific electromyographic (EMG) findings with histopathologic correlates (Sener 2019) (Naddaf et al, 2018) (Dardiotis et al, 2011). This is particularly true in the paediatric population, for which few studies are available.

The earlier retrospective studies of the diagnostic accuracy of EMG in infants (ranged 0-2 years) with generalized muscle hypotonia ("floppy infants") came to the conclusion that a high rate of concordance exists between the clinical diagnosis and the electrophysiologic examination in peripheral neurogenic disease, whereas in myopathies false-negative results occurred in 50% to 70% (Packer et al, 1982) (Russell et al, 1992) (David and Jones, 1990, 1994).

Packer et al (Packer et al, 1982) studied floppy infants in a retrospective study including 51 patients. The overall compatibility rate between EMG results and clinical diagnosis was 83%, 50% for myogenic diseases and 90% for neurogenic diseases. They also identified congenital myopathies as the most difficult clinical subgroup, and only four out of seven patients with this diagnosis showed a compatible EMG result. The fact that the final diagnosis was determined by the clinical course of the illness, serial examinations, and available laboratory data, including muscle biopsy specimens, should be noted.

Russell et al (1992) published a retrospective study of 79 newborns (<1year) with generalized muscle hypotonia. EMG accurately predicted the final diagnosis in 65% of infants with spinal muscular atrophy (SMA) and was consistent with the diagnosis in another 25%. In contrast, EMG accurately predicted the final diagnosis in only 10% of infants with myopathy and was normal in 88% of infants with central hypotonia. Therefore, muscle biopsy, although it cannot

characterise the exact type of myopathy, it is far more useful than electromyography in establishing the diagnosis of myopathy; electromyography is useful in establishing or excluding a diagnosis of spinal muscular atrophy, and distal motor conduction velocities are of prognostic significance, with decreased velocity associated with a shorter survival time.

David and Jones (1990) in a retrospective study investigated 110 infants with generalized muscle hypotonia. Although the EMG results in their study were compatible in 88% of the neurogenic diseases, they were consistent in only 31% of the myogenic diseases, which, however, included only congenital myopathies.

The same authors reported in a subsequent study (David and Jones, 1994) the diagnostic yield of electrodiagnosis in patients presenting exclusively with "floppy infant syndrome". In a sample of 80 infants investigated for floppy infant syndrome, David et al identified 38 patients investigated by both nerve conduction studies/EMG and muscle biopsy, and overall, a specific diagnosis was obtained by EMG, biopsy or special studies (enzymatic/stool culture) in 30 of the 41 infants. The authors found a very positive correlation rate between nerve conduction studies with electromyography and biopsy results in neurogenic disorders in 93% (14 of 15) in SMA patients and 100% in peripheral neuropathy (3 of 3), respectively; however, only 4 out of 10 infants (40%) with biopsy-proven myopathy had an abnormal EMG. The overall compatibility rate was 76%.

Rabie et al, (Rabie et al, 2007) also described that the EMG sensitivity for detecting myopathic motor unit potentials under 2 years of age was low (1 in 7, 14%).

Cetin et al (Cetin et al, 2009) find a high agreement between final diagnosis and EMG in case of peripheral neurogenic disease, but lower in case of myopathy. In their retrospective study evaluating 37 hypotonic infants (0-24 months), peripheral neurogenic diseases (spinal muscular atrophy or Charcot Marie-Tooth disease) were correctly diagnosed in all cases (13 patients, 100%). Among the 24 children clinically diagnosed with myopathies, five only displayed myogenic alterations (20.8%) when tested before the age of two. Sixteen (66.7%) had normal EMG results and three (12.5%) showed neurogenic alterations.

Finally, a subgroup analysis of 22 children under the age of 2 years diagnosed with a myopathy described by Hafner et al (Hafner et al, 2019) revealed a EMG sensitivity of 81.8% and a biopsy sensitivity of 86.4%. EMG sensitivity in this age group is slightly below the one found in the entire myopathic cohort (see below). This finding is higher than the concordance rates for myopathic disorders in children under the age of 2 years described in the other works.

More recent studies over a wider age range of pediatric patients have revealed a higher rate of correlation in myopathic disorders.

In children and adult patients, numerous authors have found high concordance rates (73–99%) between the EMG findings and final clinical diagnosis (Black et al., 1974; Brusa et al., 1963; Buchthal and Kamieniecka, 1982; Hausmanowa-Petrusewicz and Karwanska, 1971; Humphrey and Shy, 1962; Schwartz et al., 1966). Although peripheral neurogenic diseases could, in general, be more accurately diagnosed by electrophysiological examination than myopathies, ranging from 90% to 100%, even in myopathic diseases the concordance rates ranged from 73% to 98%. As more patients with congenital myopathies were included in the studies, the overall diagnostic yield of the method decreased (Hellmann et al, 2005).

Hausmanowa-Petrusewicz (Hausmanowa-Petrusewicz and Jedrzejowska, 1971), wanted to compare the value of histopathology and EMG in the detection of neuromuscular abnormalities: in their sample, in 331 cases of myopathy EMG provided results supporting the final diagnosis in 324 cases (97.8%) and biopsy indicated myopathy in 299 cases (90%); for neurogenic lesions, EMG gave a correct diagnosis in 98.9% of cases, biopsy in 84.6%. No specific correlations between EMG parameters with histopathological findings were found.

In a sample of 105 patients (children and adults) with muscle weakness examined by Black et al (Black et al, 1974), 101 patients were definitively diagnosed with a neuromuscular disorder; of these, the overall concordance of EMG and histochemistry was greater than 90%, in 5 patients (4.9%) EMG and biopsy were not concordant (notably all 4 cases of Kugelberg-Welander syndrome with neuropathic EMG and myopathic biopsy); the 4 patients without a definitive diagnosis had a myopathic EMG and a neuropathic biopsy.

Previous retrospective studies on the diagnostic value of EMG and muscle biopsy in children of all age are summarized in Table 29.

In the study of Buchthal and Kamieniecka (Buchtal et al, 1982), in 77% of myogenic and in 91% of neurogenic disorders, both EMG and biopsy were concordant with the clinical findings. The electromyogram agreed with the clinical classification in 87% of patients with myopathy and 91% of patients with neurogenic impairment. The agreement of the biopsy with the clinical diagnosis was 79% in patients with myopathy and 92% in patients with neuropathy.

Gibertoni's results (Gibertoni et al, 1987) agree with those reported by other laboratories (Buchthal et al, 1982) (Haumanova-Petrusewicz et al, 1971) (Humphrey et al, 1962). The concordance of the two techniques reaches 94%; in particular, in the neuropathy group, complete concordance between ENG/EMG, muscle biopsy and clinical diagnosis was obtained

in 100% of patients; in patients with primary myopathies, neurophysiological examinations agreed with the clinical diagnosis in 80% and muscle biopsy in 91%, so that neurophysiological techniques and muscle biopsy are complementary to each other, since sometimes one technique is diagnostic when the other gives negative results (e.g. in the 2 myotonic patients, only EMG was diagnostic).

To study the concordance between EMG and muscle biopsy, Werneck and Lima (Werneck et al, 1988) evaluated data from 100 patients, children and adults, with neuromuscular disorders, 58 primary myopathies, 10 myotonic dystrophies and 32 neurogenic disorders. There was an agreement of 80% between the MB and EMG. The agreement between the two methods was 82.75% in the primary myopathies (100% in Duchenne Muscular Dystrophy, 92.85% in Limb-girdle muscular dystrophy, 55.55% in Facio-scapulo-humeral dystrophy, and 50% in Dermato and polymyositis) and 84.37% in the denervation disorders (100% in Amyotrophic lateral sclerosis, 88.88% in SMA and 69.28% in Peripheral neuropathies). The myotonic dystrophy presented only 50% of accordance between the two methods. The chi-square test gave a value of 96.41 ($p<0.01$).

Hellmann et al, (Hellmann et al, 2005) evaluated the diagnostic value of EMG and nerve conduction studies (NCSs) in a retrospective study of 498 pediatric patients. Peripheral neurogenic diseases were correctly diagnosed in all but one case (99.5%). In myogenic diseases, the concordance between EMG and clinical results was lower (80%), because some patients with congenital myopathies showed normal EMG findings in this group. The overall consistency between EMG findings and the final clinical diagnosis in all children examined was 98%. No decrease in diagnostic reliability was found in the younger age group. No evaluation of muscle biopsy sensibility or specificity is described.

Rabie et al (Rabie et al, 2007) described 27 children in which EMG and biopsy was performed;

EMG accurately concurred with final diagnoses in 20 of 27 (74%) and was discordant in 7 children (26%). All patients with discordant EMGs (false-negatives) had diagnoses of myopathy. In these children, the EMGs findings were normal in 3 (27%; 2 congenital myopathies and 1 myopathy of unknown cause) and neurogenic in 4 (36%; 1 with congenital myotubular myopathy and 3 with myopathy of uncertain etiology).

EMG sensitivity for detecting myopathic motor unit potentials was 4 of 11 (36.4%), with the qualitative analysis of four muscles; below 2 years of age was low (1 of 7, 14%), increasing to 3 of 4 (75%) in children over 2 years of age. Mild-to-moderate neurogenic EMG findings occurred frequently in the myopathy category (8 of 11, 73%). In young children < 2 years of age with

myopathy, 3 of 6 (50%) of the false-negative EMGs were neurogenic. In congenital myopathies (5 patients) EMG detected myopathic motor unit potentials in 40%, with false-negative results neurogenic (20%) or normal (40%). EMG sensitivity in detecting neurogenic disease was 100%. Biopsies agreed with final diagnoses in 20 of 23 (87%), 100% in myopathic and normal categories, 25% (1 of 4) for neurogenic diseases; for neuropathic patients (N=4), muscle biopsy was neurogenic in 1 of 4 patients and normal in the others.

Chang et al (Chang et al, 2011) analysed and evaluated the concordance of EMG with muscle biopsy results in 62 young patients (≤18y old), and compared conventional and quantitative EMG, to assess the accuracy of TAA and determine whether or not it was useful for making a diagnosis. They detected 51 myopathic patients, identified by conventional EMG in 96% (50/51), and 3 patients with neurogenic disease, with EMG sensitivity of 66% (2 of 3); with the use of additional TAA (one of the quantitative interference pattern analysis) the sensitivity was 100% for both myopathic (26) and neuropathic (1) patients. There were no significant differences in the sensitivity (P 0.49) between the conventional EMG and TAA groups. TAA may be helpful in cases of no definite conventional EMG findings and less cooperative patients, demonstrating more consistency between EMG findings and muscle tissue biopsy when diagnosing myopathy when compared to previous reports (Rabie et al, 2007), (Ryu et al, 2007), (Fuglsang-Frederiksen, 2000), (Fuglsang-Frederiksen, 2006), (Stalberg et al, 1983), (Garcia et al, 1980).

In their sample of 72 myopathic children (aged between 6 months and 18 years), Ghosh et al (2014) determined the sensitivity and specificity of EMG results with respect to the presence or absence of a clearly defined muscle disease, with the diagnostic gold standard represented by muscle biopsy or pathognomonic genetic testing: paediatric electromyography was 91% sensitive and 67% specific in myopathic disorders; if the EMG was myopathic, the likelihood of the assessment leading to a definitive diagnosis was 2.8 times higher than for a normal EMG. Metabolic myopathies were commonly missed by EMG.

It has also been shown (Pugdahl et al, 2017) that adding VCS and EMG information increased the diagnostic probability of myopathy compared to clinical diagnosis alone in 67 patients (34.4%) and that the greatest increase was seen for myopathies of unknown aetiology.

The study of Constantinides et al (2018), in which 123 patients were included, 89 with a final diagnosis of myopathy and 10 of neurogenic disorder, demonstrated that muscle biopsy had a very high diagnostic yield in neuromuscular disorder compared to electrophysiological

evaluation, with a concordance between biopsy and EMG of 70.7%. Diagnostic accuracy of muscle biopsy was 88.6% (109 of 123) compared to 70.7% (87 of 123) for EMG. Muscle biopsy was highly specific (97.1%) and sensitive (86.5%) for the detection of myopathy, was highly specific for a neurogenic disorder (94.7%) or in patients without neuromuscular disorders (94.1%) with a sensitivity in both cases of 100%. In contrast, EMG had a moderate sensitivity (76.4%) and poor specificity (58.8%) for a myopathy diagnosis. Likewise, it had poor sensitivity (57.1%) with high specificity (92.1%) for patients with no neuromuscular disorder. EMG, however, was highly indicative in cases of neurogenic disorder (sensitivity 100%, specificity 92.9%).

Hafner et al (Hafner et al, 2019) restrospectively assessed the sensitivity, specificity and positive and negative likehood ratio of muscle biopsy and electrodiagnostic tests in a sample of 171 children; with regard to electrodiagnostic tests, these included as a minimum the evaluation of a sensory nerve and a motor nerve in the leg and EMG of the tibialis anterior; for EMG analysis, at least 20 MUPs were quantitatively analysed, but fewer MUPs were evaluated in the presence of very marked abnormalities or where the recording was too short.

In the 98 patients with myopathic disease, EMG had a sensitivity of 87.8% and a specificity of 67.1%, while biopsy showed a sensitivity of 84.5% and a specificity of 75.7%; In the 18 children with neurogenic disease, the sensitivity and specificity were 94.4% and 96.1% for EMG and 33.3% and 99.3% for muscle biopsy, respectively. Indeed, the accuracy of our EMG results in detecting myopathies was comparable to that of EMG protocols examining at least two muscles (Chang et al, 2011) (Ghosh et al, 2014) (Hellmann et al, 2005) (Rabie et al, 2007).

Subgroup analysis of 22 children under the age of 2 years diagnosed with a myopathy revealed a EMG sensitivity of 81.8% and a biopsy sensitivity of 86.4%. EMG sensitivity in this age group is slightly below the one found in the entire myopathic cohort. This finding is higher than the concordance rates for myopathic disorders in children under the age of 2 years described in other works (Cetin et al, 2009) (David et al, 1994) (Russel et al, 1992), where they were often low, between 10% and 56%, even if more muscles were examined; this further supports that the use of quantitative analysis on a single muscle may be superior.

As previously anticipated, the correlation of individual electromyographic and histopathological findings remains poorly explored.

Dardiotis et al (Dardiotis et al, 2011) correlated QEMG with biopsy results in a group of 31 patients in whom a final clinical diagnosis of neuromuscular disorder was reached. The

sensitivity of QEMG was between 24 to 69% depending of the specific method of signal analysis. The highest sensitivity (68,9%) in detecting a myopathic biopsy was obtained using the amplitude outlier method (MUP amplitude of < 300μv). The sensitivity of the amplitude outlier method was superior to the duration outlier (p = 0,000) and mean duration methods (p = 0.007). The positive predictive value of abnormal QEMG was more than 90% while its negative predictive value was only about 20%. Amplitude outlier analysis was particularly sensitive at detecting increased variability in fiber size and more subtle myopathic changes.

Naddaf et al, (Naddaf et al, 2018) focused on the correlation of individual electromyographic and histopathologic findings, which have so far remained poorly explored.

There was a correlation between the presence of fibrillation potentials on EMG and a wide spectrum of histopathologic findings including: atrophic fibres, inflammation, active myopathy (necrotic and regenerating fibres), fiber disruption (fiber splitting, vacuoles and congophilic inclusions), chronicity (increased endomysial connective tissue) and denervation (fibres reacting for NSE).

Short duration motor unit potentials correlated with the presence of atrophic fibres, necrotic and regenerating fibres, increased endomysial connective tissue, and perimysial inflammation.

With regard to long duration motor unit potentials, there was a significant correlation with fiber type grouping but not with fiber type predominance.

They also described a correlation between increased phases and the presence of atrophic fibres, fibres reacting for NSE and increased endomysial connective tissue. Regarding increased turns, there was a correlation with the presence of atrophic fibres, regenerating fibres, target formations, and increased endomysial connective tissue Regarding rapid recruitment, there was a correlation with the presence of regenerating fibres, perimysial inflammation, and increased endomysial connective tissue.

Sener et al (Sener et al, 2018) analysed 218 patients to assess the sensitivity and specificity of EMG in confirming myopathy and the sensitivity, specificity, and positive and negative predictive values of specific EMG findings for pathologic changes on muscle biopsy (inflammation, necrosis, splitting, and vacuolar changes).

The sensitivity of EMG interpretation to confirm the diagnosis of myopathy was 95.3% and the specificity 48.5%.

Short-duration motor unit potentials (MUP) were sensitive (83%–93.5%) but not specific (34%–49%) for pathologic changes. The absence of fibrillation potentials had high negative predictive

value (82%–93%) for inflammation, splitting, or vacuolar changes, so the absence of fibrillation potentials suggests other myopathologic changes (e.g., congenital myopathy).

AIM

This project sets out two global aims.

The first aim is:

- to collect and define the normative data for sensory-motor conduction study during the maturation phases from newborn to three years of age and to collect and define the normative data for the electromyographic study during the maturation phases from newborn to six years of age, also by applying quantitative EMG in the pediatric field to obtain the normative values of the main parameters

The second aim is:

- to assess the diagnostic accuracy of the concise paediatric NCS and EMG protocol used over recent years in our institution, identifying the ability of electrodiagnostic studies to detect the pathological processes in neuromuscular disorders, and compared it to muscle biopsy results and the final clinical diagnosis, to assess their concordance and increase their sensitivity and specificity in neuromuscular disorders; in addiction, to evaluate whether specific neurophysiological findings correlate with pathological findings on muscle biopsy and whether there are EMG and NCS parameters that can predict specific forms of neuromuscular disorders

MATERIAL AND METHODS

Study outline and data collection for first aim

The study from which this thesis was developed is a longitudinal, retrospective and prospective study.

For electromyography, we enrolled patients who had access and are followed from 1st January 2016 to 31st October 2019 by Neurophysiology Unit of the "Giannina Gaslini Institute" Genoa Italy.

For Nerve conduction studies, we enrolled patients from a multicentre study from 1st January 2016 to 31st October 2019, that involved Neurophysiology Unit of the "Giannina Gaslini Institute" Genoa Italy, of Carlo Besta Institute (Milan), Policlinico (Milan), Bambino Gesù Hospital (Rome), Careggi Hospital (Florence), University of Padua.

Only the investigations necessary from the clinical point of view will be performed.

Individual chart reviews were performed.

Demographic data included age at time of study, use of sedation, gender, and height and weight (available in most cases).

Inclusion criteria:

- suspected neuromuscular disease or requirement to exclude neuromuscular involvement in syndromic patients, aided by clinical history and expert team evaluation.
- for electroneurographic evaluation, age between 0 and 3 years, trying to get a enough homogeneous sample for ages.
- for electromyographic evaluation, age between 0 and 6 years, trying to get a enough homogeneous sample for ages.

Exclusion criteria:

- non-cooperative patient but not sufficient clinical motivation for sedation

Study outline and data collection for second aim

This longitudinal, retrospective study was conducted in children aged ≤ 18 who had who had access and are followed from 1st January 2011 to 31st December 2021 at "Giannina Gaslini Institute", Genoa Italy.

Of the pediatric muscle biopsy patients identified, only those patients with an electrodiagnostic test performed were included in the analysis.

Of these, we reviewed clinical, neurophysiological and histopathological findings.

Only the investigations necessary from the clinical point of view will be performed.

Tissue and blood samples were obtained for diagnostic and research purposes after ethical approval from the Ethic Committee of our Institution and written informed consent from the patient parents.

Individual chart reviews were performed.

Demographic data included age at time of study, sedation use, gender, height and weight (available in most cases), biochemical test results, muscle biopsy reports, NCSs results and EMG results, genetic test results, and final diagnoses.

Determination of each patient's final diagnosis was based on review of the medical record notes.

Inclusion criteria:

- suspected neuromuscular disease or requirement to exclude neuromuscular involvement in syndromic patients, aided by clinical history and expert team evaluation
- age between 0 and 18 years
- muscle biopsy and electrodiagnostic test performed in the patient

Exclusion criteria:

- non-cooperative patient but not sufficient clinical motivation for sedation for electrodiagnostic test

For both of these projects, informed consent was obtained from the parents of all patients and

Each patient was identified with a progressive number and the demographic data was stored in a protected personal computer.

NCSs and EMG protocol

NCSs and EMG data will be recorded by trained staff.
NCSs and EMG will acquire from all participants using Synergy EDX/ Viking EDX NicoletTM.

NCS recordings:
-use of adhesive surface electrodes or ring electrodes.
- separation of the recording electrodes: use of inter- electrode distances of 10 mm (under 2 years) or 26 mm (over 2 years).
- The frequency filters for motor nerve conduction studies are set to 3 HZ for low pass and 10 kHZ for high pass. The frequency filters for sensory nerve conduction studies are set to 20 HZ for low pass and 3 kHZ for high pass.
- at least one sensory nerve (antidromic): sural nerve or superficial peroneal but if not possible try the medial plantar; for upper limb median nerve is studied;
- at least one motor nerve: Peroneal nerve and/or tibial nerve; for upper limb median nerve is studied , if there is any abnormality ulnar nerve is tested;
- F weaves of at least one nerve.
- nerve conduction values for motor and sensory nerves included amplitude, conduction velocity, distal latency and for motor nerves also duration.
- For all motor nerves, the amplitude recorded was the amplitude for the response from the distal stimulation site, expressed in millivolts (mV). Distal latency was measured from the onset (or rise of the negative deflection) of the compound muscle action potential (CMAP), expressed in milliseconds (ms). Motor conduction velocity (CMV) was determined by using the distance between the distal and proximal stimulation sites, divided by difference in latency, expressed in meters per second (m/s).
- For all sensory nerves, the amplitude recorded was the peak-to-peak amplitude at the distal stimulation site, expressed in microvolts (μV). All sensory distal latencies were recorded as the peak latency of the distal site, expressed in milliseconds. Conduction velocity was determined in the same manner as described for motor NCS, using the onset latencies when calculating the sensory conduction velocity (SCV).

- due to the age of the sample, the limbs have not grown completely, so our laboratory practice is to use anatomical landmarks to determine the proper site of nerve stimulation.
- in the upper limbs, the sites of stimulation were the elbow and wrist, and for lower limbs it was the knee and ankle.
- The minimal acceptable temperatures are 35°C in the upper limb and lower limb.

EMG recordings:

-needle electrodes (25 mm x 26G), which allow the extracellular recording of motor unit potentials.
- recordings were made using standard concentric needle electrodes (CNEs) inserted into the midportion of the muscle;
- EMG signals were band-pass filtered from 2 Hz to 10 kHz and stored for offline analysis;
- screening muscle: anterior tibial muscles; if there are specific clinical symptoms and signs, they guide the optimal selection of specific muscle groups; if pathological, we will study a proximal and a bulbar muscle.
- For EMG analysis at least 20 MUPs were analysed quantitatively, but fewer MUPs were evaluated in the presence of very pronounced abnormalities or where the recording was too short.
- The minimal acceptable temperatures are 35°C in the upper and lower limb.
- we analized: insertional activity; spontaneous activity recorded at rest, analyzing morphology, stability and firing characteristics (firing rate and firing pattern); voluntary MUPs during the reflected contraction and of each must be evaluated morphology, amplitude, rise time, duration, area, phases, polyphasicity, number of turns, discharge frequency and presence of satellite potential; recruitment of motor units during progressively greater muscle contraction and an interference pattern at the maximum effort (if was possible); QEMG of which we have evaluated Turns/sec, Amp/Turn, NSS, NSS/Att.

NCSs were classified as: axonal, demyelinating or mixed motor polyneuropathy; axonal, demyelinating or mixed sensory polyneuropathy, or normal. All values of the nerve conduction studies of the patients were adjusted to the normative values of the reference age. Furthermore, EMGs were classified as Myopathic, Neurogenic, Non-specific, Normal.

Myopathic: there are several needle EMG criteria for myopathy (Buchthal et al, 1985) (Rabie et al, 2007) (Chang et al, 2011) (Dumitru et al, 2002). In this study, a myopathic disorder was defined as a needle study showing: short duration, polyphasic, low-amplitude motor unit potentials, usually early appearance of maximum interference pattern in weak muscles, and normal or abnormal spontaneous activity (Rabie et al, 2007) (Chang et al, 2011). A disorder of myopathic origin on TAA was defined as more than 10% of the plots of turns per second versus the mean amplitude being located downward from the reference cloud after more than 20 plots (Stalberg et al, 1983) (Chang et al, 2011).

Neurogenic: A neurogenic disorder at needle EMG was defined as the presence of normal or abnormal spontaneous potential during the resting phase, high amplitude polyphasic motor unit potential with a long duration, and reduced recruitment (Rabie et al, 2007). A disorder of neuropathic origin on TAA was defined as more than 10% of the plots of the turns per second versus the mean amplitude being located upward from the reference cloud after more than 20 plots.

Non-specific: This category includes all nonspecific cases for either neurogenic or myopathic disease; EMG may present isolated scattered abnormal spontaneous activity, or isolated amplitude/duration/phase changes of the motor unit potential.

Myopathological study

Open muscle biopsies performed by quadriceps muscle, in the majority of cases.
All histopathological analyses were performed in the Giannina Gaslini Istitute Muscle Laboratory by muscle pathologists using standard preparation, staining, and interpretation.
Routine morphology, histochemical stains and spectrophotometric determination of RC complexes in skeletal muscle biopsy were performed according to standard protocols.
Muscle biopsy pathology reports were reviewed for each patient and the presence of
fibral hypotrophy, polydimensionality of fibres, fibrosis, grouping, predominance, necrosis,
and reduction of cytochrome c oxidase as well as the final pathologic diagnosis were recorded.
In our study, all patients underwent open air surgical sampling of the quadriceps muscle. Histological and histochemical methods were performed according to standard procedure in all cases but one in which no muscle tissue was available due to severe fatty replacement.

These included hematoxylin and eosin, Gomori's trichrome, myofibrillar ATPase (at pH 9.4, 4.6, and 4.3), cytochrome c oxidase (COX), succinate dehydrogenase (SDH), NADH-TR, periodic acid–Schiff (PAS), Oil Red O, acid phosphatase, esterase, and phosphorylase.

Immunohistochemical study was performed by indirect immunofluorescence. The following antibodies were tested: dystrophin COOH, dystrophin Mid Rod, dystrophin NH, alpha-sarcoglycan, beta-sarcoglycan, gamma-sarcoglycan, deltasarcoglycan, alpha-dystroglycan, merosin, dysferlin, caveolin, and collagen 6 (Veneruso et al, 2021).

A biochemical assay of the respiratory chain enzymes activity was performed according to Spinazzi et al. (Spinazzi et al, 2012). Ultrastructural investigation was performed on biopsy samples indicating a congenital myopathy.

Four categories were established: normal, myopathic, neurogenic, mixed/unspecific.

Normal or nearly normal biopsy with minimal changes were classified if the amount of connective tissue was little, the morphology of fibres was polygonal with similar diameter and in normal value. Nuclei must be peripherally located, and all the histochemical reactions gave normal results.

Myopathic alteration included round and small fibres, centrally located nuclei and increased fibrosis or necrosis

Neurogenic alterations included groupe atrophy, angulated fibres and presence of type grouping with ATPase staining.

Unspecific or mixed biopsy defined a condition with alteration of both origin including few neurogenic changes and myopathic features, alteration of the myofibrillar network or the oxidative staining.

Statistical analysis for first aim

Normal ranges of many biomedical markers are unavailable for some subpopulations such as infants and children due to the extra burden of a measurement that may inflict on this cohort.

For this reason, we will use a technique developed by Jabre and referred to as extrapolated norms or e-norms (Jabre et al, 2015) (Jabre 2019), which has already been used to find jitter reference values (Pitt et al, 2017) (Jabre et al, 2020).

The e-norms method (Jabre et al., 2015) has to date been validated by several workers in the field (Nandedkar et al., 2015), (Zaccarini et al., 2016), (Pitt and Jabre, 2017), (Jabre et al., 2015), (Jabre et al., 2018), (Punga et al., 2019) (Jabre et al, 2020), and cited in the recently

published ‘‘Standards for quantification of EMG and neurography” by Stålberg et al as one of the ‘‘Novel methods that do not require the tedious collection of reference values from healthy individuals..”(Stålberg et al., 2019).

In fact, against this background, the discovery of the extrapolated normal methodology which allows the use of results collected from attendees to the electromyographic lab without the necessity of screening for the likelihood of pathology to produce the likely normative values for any particular parameter, is particularly timely (Pitt et al, 2018), (Jabre et al., 2015, 2016); (Zaccarini et al., 2016) (Pitt and Jabre, 2017) (Shammas et al, 2020).

Pitt and Jabre (2018) described the application of this method to any laboratory’s database.

The E-norms technique exploits another property of variables derived from normal individuals that distinguishes them from variables derived from patients with pathology, a property called ‘‘normal clustering” (Jabre, 2018), that is a range with low first-order difference.

To date, numerous studies conducted in different labs from around the world have shown that data extracted using the e-norms technique reveals normal values that show little difference between them and normal values collected in the traditional way (Jabre, 2018).

Data will also be described as absolute and relative frequencies for categorical variables, while means, standard deviation (SD), medians and range will used for continuous variables.

Statistical analysis for second aim

Descriptive statistics were generated for the whole cohort and data were expressed as mean and standard deviation for continuous variables. Median value and range were calculated and reported, as were absolute or relative frequencies for categorical variables.

Sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV) were calculated.

To performed the correlation between EMG and muscle biopsy variables we performed Fisher’s exact test. Significance level was set at $p<0.05$.

Statistical analysis was performed using SPSS for Windows (SPSS Inc., Chicago, Illinois USA).

RESULTS

NERVE CONDUCTION STUDIES - NORMATIVE VALUES

Sample Description:

From 1st January 2016 to 31st October 2019 717 tests were evaluated in our Multicentric study, performed in children between 0 days and 3 years old.

Premature infants with gestational age at the time of the exam less than 40 weeks of GA were excluded from our study. Two patients were excluded for erroneous patient record number. No patients needed sedation.

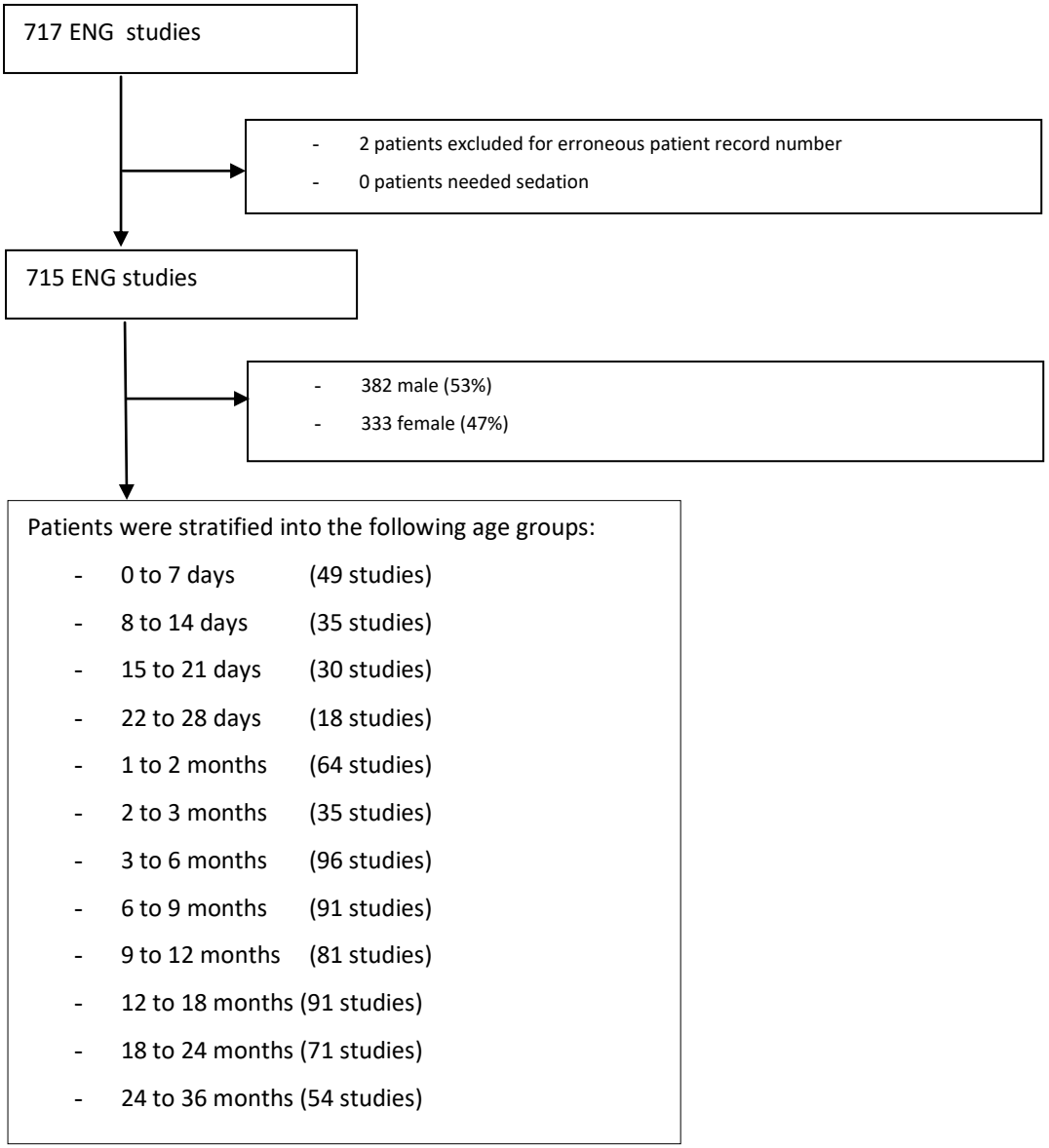

Figure 16: Flowchart of case selection, demographics, and age stratification.

The final sample consists of 715 studies; 382 performed on males (53%), 333 on females (47%).

Figure 16 shows the selection of cases, demographics and stratification by age.

Only the nerves that had a sufficient sample were analyzed; typically at least 20 values, although for younger age groups we included sample sizes with fewer than 20 values.

Motor Nerves:

Below are reported for each motor nerve with an adequate sample size the data regarding conduction velocity and amplitude (in particular mean, standard deviation-SD, 2 SD below or above the mean, variance, minimum and maximum values found).

PERONEAL NERVE

Motor Conduction Velocity:

For the speed of conduction, it must be emphasized that: we observe a mean of 23.16 m/sec in the first week of life, it gradually increasing up to a mean of 43.98 m/sec at 12 months, still increasing up to 3 years, more gradually, with an average of 47.8 m/sec at 3 years of age.

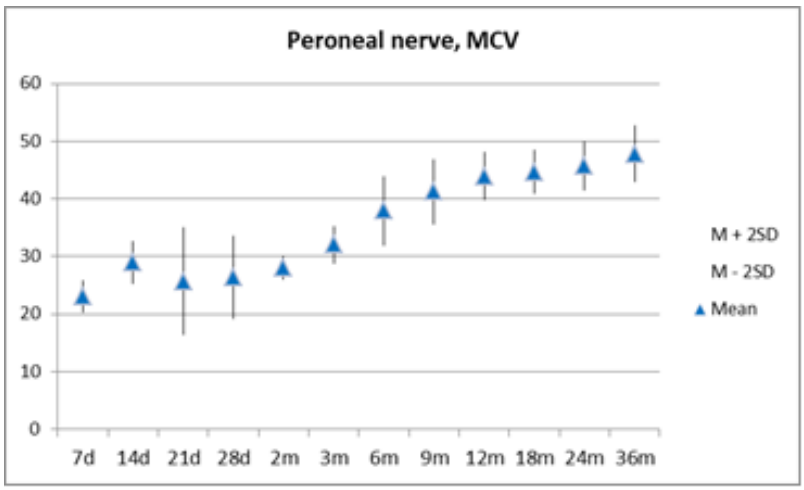

Figure 17: MCV of Peroneal Nerve in relation to age; mean and SD.

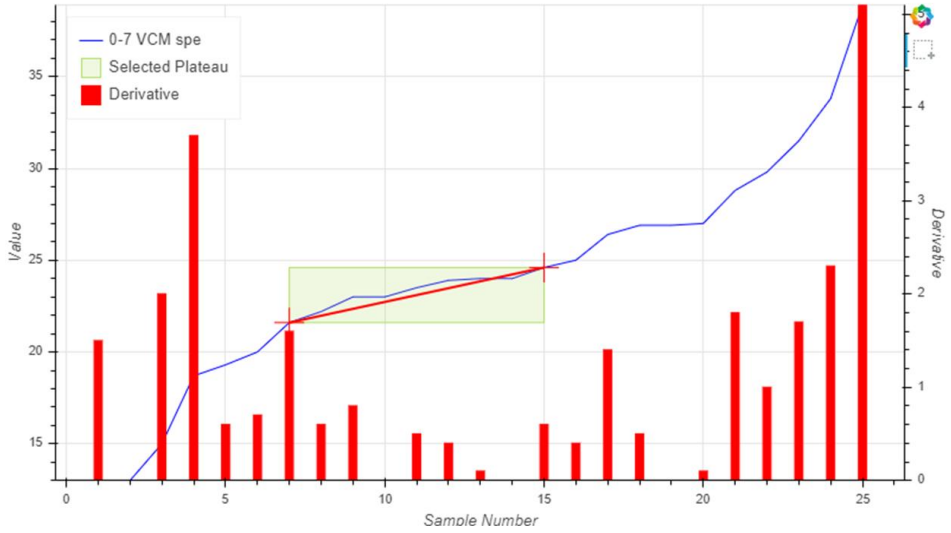

Figure 18: example of E-norms values: MCV of peroneal nerve (0-7 days).

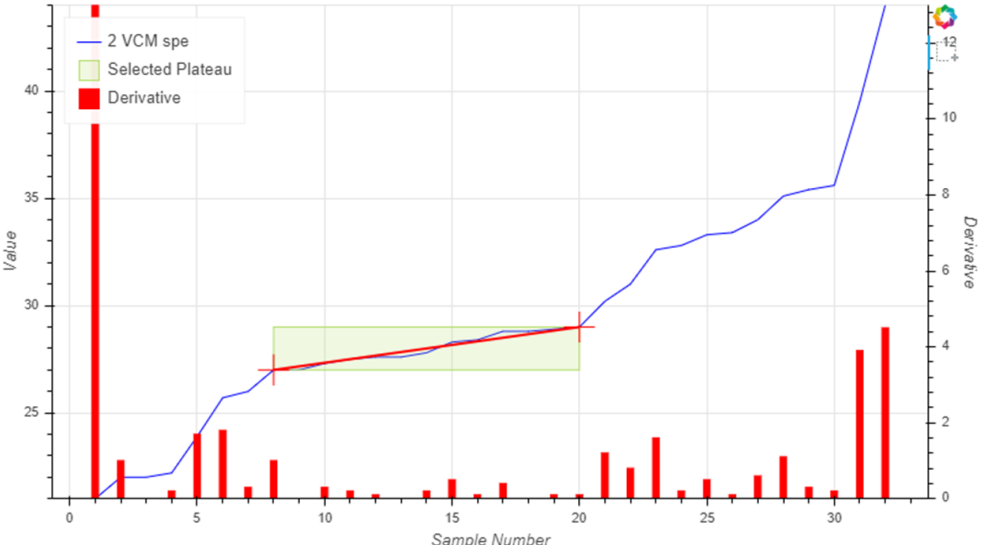

Figure 19: example of E-norms values: MCV of peroneal nerve (12-24 months).

Table 1: Motor Nerve conduction velocity of Peroneal Nerve from birth to 3 years of age

MCV	7d	14d	21d	28d	2m	3m	6m	9m	12m	18m	24m	36m
Total N	26	20	9	10	33	20	45	61	50	42	42	48
Mean	23,16	29	25,72	26,36	28,01	32,04	37,99	41,24	43,98	44,74	45,76	47,8
StDev	1,38	1,82	4,63	3,61	1,01	1,63	2,99	2,84	2,05	1,9	2,13	2,44
RMSE	0,851	1,073	3,851	1,913	0,427	1,296	0,894	1,231	0,386	0,373	0,519	1,46
Variance	6.0%	6.0%	18.0%	14.0%	4.0%	5.0%	8.0%	7.0%	5.0%	4.0%	5.0%	5.0%
M - 2SD	20,4	25,36	16,46	19,14	25,99	28,78	32,01	35,56	39,88	40,94	41,5	42,92
M + 2SD	25,92	32,64	34,98	33,58	30,03	35,3	43,97	46,92	48,08	48,54	50,02	52,68
Min	20	25	21,7	21,3	26	28,3	33,3	36,2	40,4	41,5	42	42
Max	25	31,3	34,8	32,6	30,2	34,6	44	48	47,9	48	50	51
Plateau N	11	9	5	5	15	8	22	41	24	22	22	20
Normal Slope	0,5	0,79	3,27	2,83	0,3	0,9	0,51	0,29	0,33	0,31	0,38	0,47
Min Row	6	8	4	2	7	5	17	9	12	5	3	12
Max Row	16	16	8	6	21	12	38	49	35	26	24	31

Amplitude:

Regarding the amplitude, it should be noted that the mean on the seventh day of life is 1.57 microV, seems to remain stable until the month of life, then there is a gradual increase, reaching a mean value at 6 months of 2.56 microV , at 12 months of 3.09 microV, which subsequently remains.

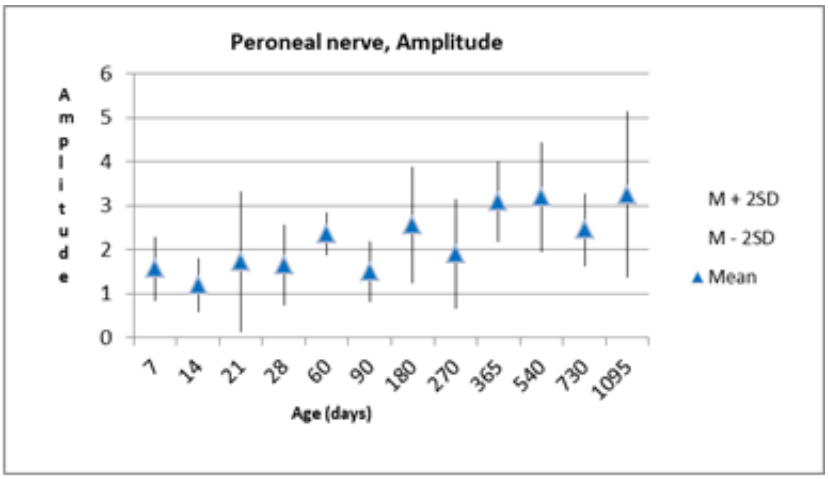

Figure 20: Amplitude of Peroneal Nerve in relation to age, mean and SD.

Table 2: Amplitude (µV) of motor Peroneal Nerve from birth to 3 years of age.

Ampl (µV)	7d	14d	21d	28d	2m	3m	6m	9m	12m	18m	24m	36m
Total N	26	20	9	11	33	20	46	62	50	43	43	49
Mean	1,57	1,19	1,72	1,65	2,36	1,49	2,56	1,9	3,09	3,19	2,45	3,25
StDev	0,36	0,3	0,8	0,46	0,24	0,34	0,66	0,62	0,46	0,62	0,41	0,94
RMSE	0,13	0,112	0,482	0,191	0,118	0,294	0,222	0,459	0,11	0,113	0,235	0,406
Variance	23%	25.0%	47.0%	28.0%	10.0%	23.0%	26.0%	33.0%	15.0%	19.0%	17.0%	29.0%
M - 2SD	0,85	0,59	0,12	0,73	1,88	0,81	1,24	0,66	2,17	1,95	1,63	1,37
M + 2SD	2,29	1,79	3,32	2,57	2,84	2,17	3,88	3,14	4,01	4,43	3,27	5,13
Min	0,9	0,7	0,8	1	1,9	1,1	1,3	1,1	2,2	2,1	1,9	1,1
Max	2,2	1,8	3,2	2,5	2,9	2,3	4	3,5	3,8	4,3	3,4	4,7
Plateau N	9	11	5	6	14	8	24	35	29	21	15	30
Normal Slope	0,16	0,11	0,6	0,3	0,08	0,17	0,12	0,07	0,06	0,11	0,11	0,12
Min Row	11	2	2	2	14	3	11	11	17	9	10	13
Max Row	19	12	6	7	27	10	34	45	45	29	24	42

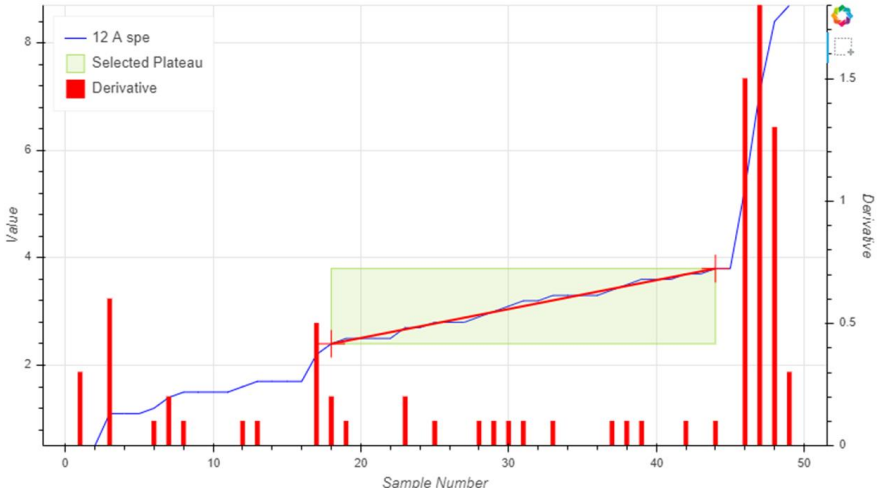

Figure 21: example of E-norms values: Amplitude of peroneal nerve (6-12 months).

POSTERIOR TIBIAL NERVE:

Motor Conduction Velocity:

Regarding the motor conduction velocity of the tibial nerve, we can see that at 7 days of life the mean of the MCV is 18.45 m/s, at 14 days we obtain a mean of 25.5 m/s, exceeding 30 m/s at 3 months (in particular at 3 months there is a mean of 31.04 m/s), at six months 34.01m/s, at 12 months 44.05 m/ s, increasing again up to 3 years, where an MCV mean of 47.59 m/ s is obtained.

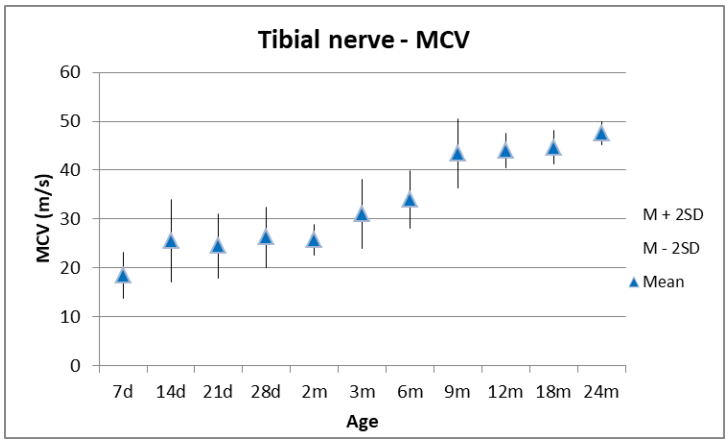

Figure 22: MCV (m/s) of Tibial Nerve in relation to age; mean and SD.

Table 3: Motor Nerve conduction velocity (m/s) of Tibial Nerve from birth to 3 years of age.

VCM	7d	14d	21d	28d	2m	3m	6m	9m	12m	18m	24m
Total N	8	8	11	6	15	12	13	19	18	55	37
Mean	18,45	25,5	24,49	26,28	25,7	31,04	34,01	43,36	44,05	44,67	47,59
StDev	2,37	4,24	3,32	3,11	1,55	3,49	2,94	3,53	1,76	1,75	1,2
RMSE	1,146	2,197	2,265	1,067	0,469	1,481	3,025	2,197	0,558	0,616	0,647
Variance	13%	17.0%	14.0%	12.0%	6.0%	11.0%	9.0%	8.0%	4.0%	4.0%	3.0%
M - 2SD	13,71	17,02	17,85	20,06	22,6	24,06	28,13	36,3	40,53	41,17	45,19
M + 2SD	23,19	33,98	31,13	32,5	28,8	38,02	39,89	50,42	47,57	48,17	49,99
Min	14,3	21,1	20	21,3	23,3	24,3	31	38,9	40,9	42,1	44,8
Max	22,4	32,5	32	29,7	28,6	36,2	41,4	51,3	46,7	48,3	50
Plateau N	6	4	8	5	8	7	8	10	8	30	15
Normal Slope	1,62	3,8	1,71	2,1	0,76	1,98	1,49	1,38	0,83	0,21	0,37
Min Row	1	3	3	1	3	4	2	6	7	8	16
Max Row	6	6	10	5	10	10	9	15	14	37	30

Amplitude:

Regarding the amplitude, we obtained in our sample at 7 days of life a mean of 5.22 µV (SD 0.69), 5.47 µV (SD 1.32) at 28 days, 7.64 µV at two months, 9.09 µV (SD 1, 65) at six months, 10.06 µV at 12 months (SD 0.62), which remains stable thereafter.

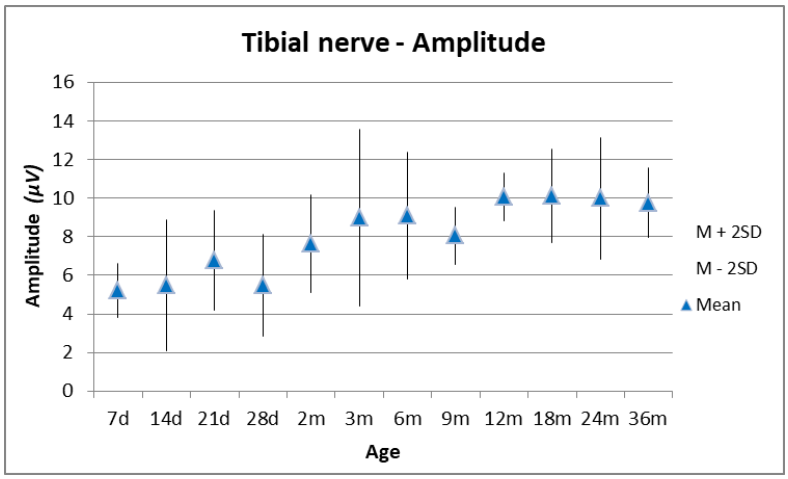

Figure 23: amplitude (µV) of Tibial Nerve in relation to age; mean and SD.

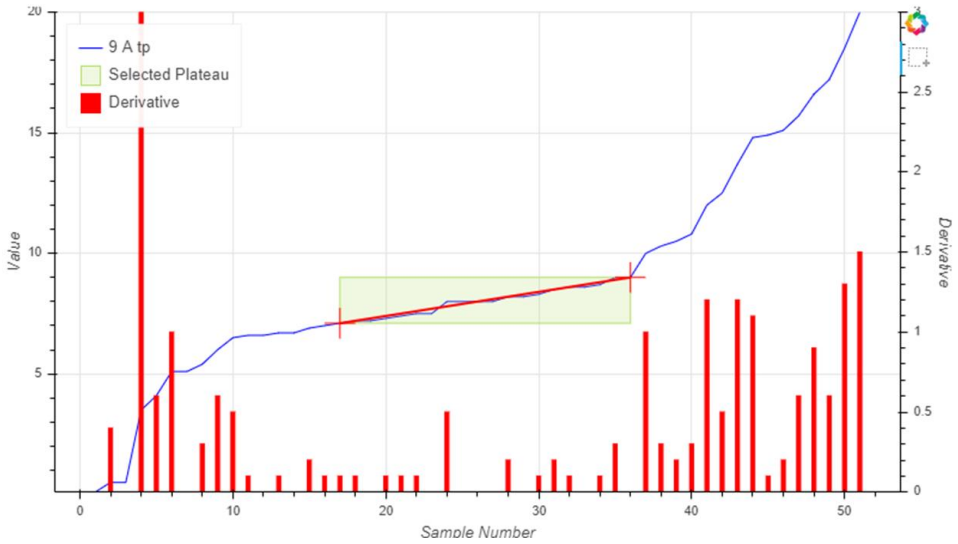

Figure 24: example of E-norms values: Amplitude of tibial nerve (6-9 months).

Table 4: Amplitude (µV) of motor Tibial Nerve from birth to 3 years of age.

Ampl (*µV*)	7d	14d	21d	28d	2m	3m	6m	9m	12m	18m	24m	36m
Total N	19	15	14	12	26	23	43	52	41	48	48	36
Mean	5,22	5,5	6,78	5,47	7,64	9,01	9,09	8,06	10,06	10,11	10	9,76
StDev	0,69	1,7	1,29	1,32	1,27	2,29	1,65	0,74	0,62	1,22	1,58	0,91
RMSE	0,576	0,332	0,424	0,522	0,971	0,542	0,259	0,522	0,512	0,392	0,536	0,225
Variance	13.0 %	31.0%	19.0%	24.0%	17.0%	25.0%	18.0%	9.0%	6.0%	12.0%	16.0%	9.0%
M - 2SD	3,84	2,1	4,2	2,83	5,1	4,43	5,79	6,58	8,82	7,67	6,84	7,94
M + 2SD	6,6	8,9	9,36	8,11	10,18	13,59	12,39	9,54	11,3	12,55	13,16	11,58
Min	3,6	2,9	4,7	4	5,7	6,1	6,6	7	8,6	8,4	7,4	8,6
Max	6,1	7,9	8,7	7,7	11,1	12	11,9	10	11,4	12,5	13,4	11,1
Plateau N	8	11	5	6	18	8	22	22	11	21	27	12
Normal Slope	0,36	0,5	1	0,74	0,32	0,84	0,25	0,14	0,28	0,2	0,23	0,23
Min Row	6	3	5	4	7	14	19	16	19	15	11	15
Max Row	13	13	9	9	24	21	40	37	29	35	37	26

<u>Comparison between tibial and peroneal nerve amplitude in relation to the age:</u>

The amplitude of the tibial nerve is observed greater than that of the peroneal nerve: between 0-7 days of life tibial nerve amplitude is 5.22 µV, peroneal nerve amplitude is 1.57 µV; this difference is maintained during maturation: we can see tibial nerve amplitude 5.47 µV at one month, peroneal nerve amplitude 1.65 µV; at 6 months 9.09 µV for tibial nerve, 2.56 µV for peroneal nerve; at 12 months 10.06 µV for tibial nerve , 3.09 tibial nerve amplitude for peroneal nerve; then both remain stable.

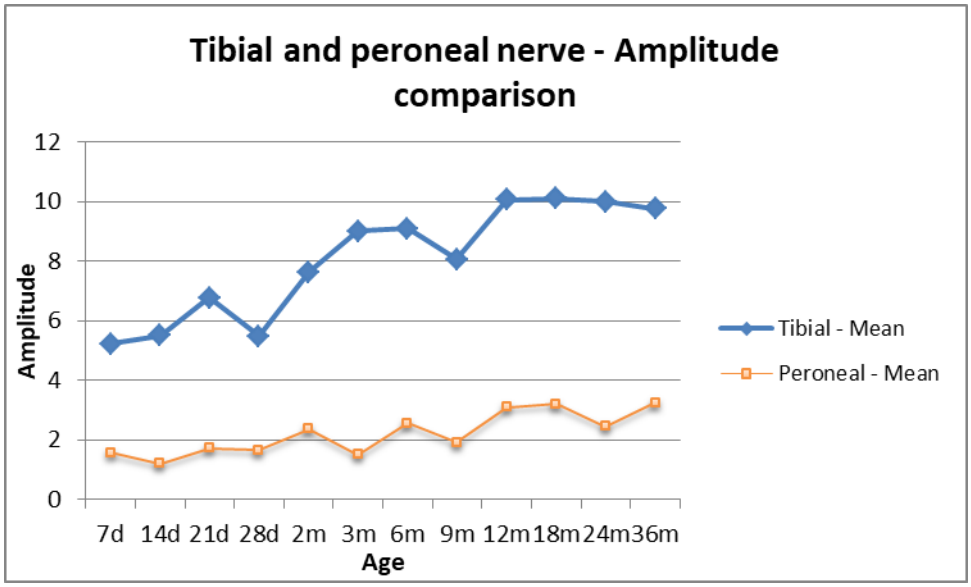

Figure 25: comparison between Tibial and Peroneal nerve amplitude.

Table 5: amplitude of Tibial and Peroneal Nerve from birth to 3 years of age.

	7d	14d	21d	28d	2m	3m	6m	9m	12m	18m	24m	36m
Tibial - Mean	5,22	5,5	6,78	5,47	7,64	9,01	9,09	8,06	10,06	10,11	10	9,76
Peroneal - Mean	1,57	1,19	1,72	1,65	2,36	1,49	2,56	1,9	3,09	3,19	2,45	3,25

MEDIAN NERVE

Motor Conduction Velocity:

Regarding the motor conduction velocity of median nerve, we can see that at 7 days of life the mean of the MCV is 22.33 m/s, at 14 days we obtain a mean of 28,26 m/s, that increases progressively: 35.67 mm/s at 6 months, 44,63 m/s at 12 months, until a mean of 51,53 m/s at 24 months, then it remains stable.

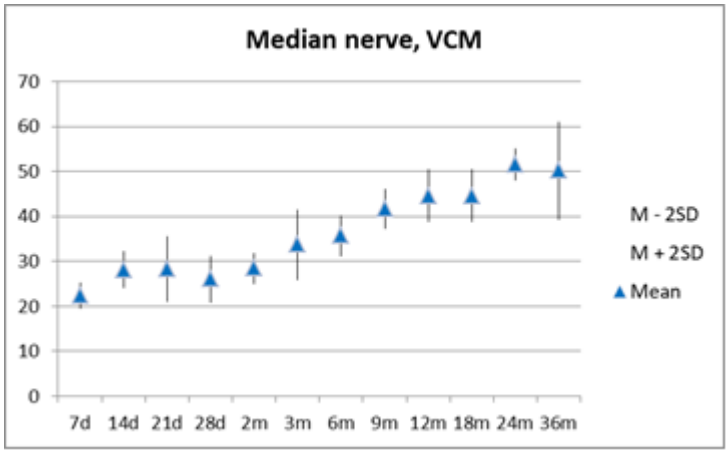

Figure 26: MCV (m/s) of Median Nerve in relation to age; mean and SD.

Table 6: Motor Nerve conduction velocity (m/s) of Median Nerve from birth to 3 years of age.

MCV	7d	14d	21d	28d	2m	3m	6m	9m	12m	18m	24m	36m
Total N	29	16	14	11	35	15	54	47	33	33	18	18
Mean	22,33	28,14	28,26	26	28,39	33,67	35,67	41,73	44,63	44,63	51,53	50,18
StDev	1,42	2,07	3,62	2,56	1,68	3,88	2,22	2,19	2,92	2,92	1,75	5,42
RMSE	0,46	1,086	1,919	1,869	0,418	1,338	0,464	0,907	0,554	0,554	0,355	6,26
Variance	6%	7.0%	13%	10%	6%	12%	6%	5.0%	7.0%	7.0%	3.0%	11%
M - 2SD	19,49	24	21,02	20,88	25,03	25,91	31,23	37,35	38,79	38,79	48,03	39,34
M + 2SD	25,17	32,28	35,5	31,12	31,75	41,43	40,11	46,11	50,47	50,47	55,03	61,02
Min	20	25	22,4	23,4	25	28	31,8	36,6	40	40	48,6	35
Max	25,2	32,6	36	31,3	31,4	38,1	40	45,8	50	50	54,5	55
Plateau N	15	9	9	6	21	7	35	30	21	21	9	11
Normal Slope	0,37	0,95	1,7	1,58	0,32	1,68	0,24	0,32	0,5	0,5	0,74	2
Min Row	5	4	4	2	6	5	6	9	6	6	5	6
Max Row	19	12	12	7	26	11	40	38	26	26	13	16

<u>Amplitude:</u>

Regarding the amplitude, we obtained in our sample at 7 days of life a mean of 2,94 µV (SD 0.55), 2,1 µV (SD 0,33) at 28 days, 2,66 µV at two months (0,48 SD), 3,47 µV (SD 0,63) at six months, 5,84 µV at 12 months (SD 1,21), which remains stable thereafter, 6,6 µV at 24 months and increases again until 36 months, when we obtained a mean of 7,58 µV (1,36 SD).

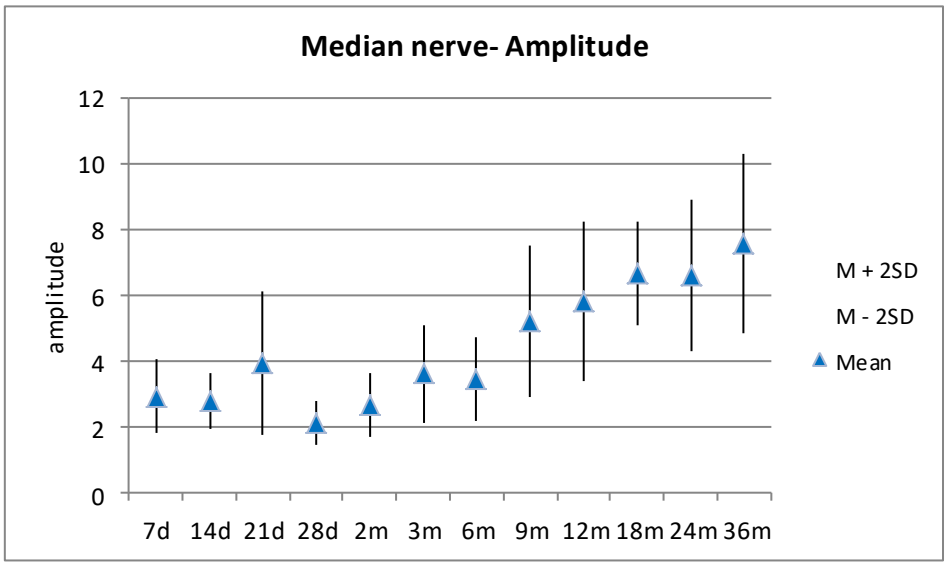

Figure 27: amplitude (µV) of Motor Median Nerve in relation to age; mean and SD.

Table 7: Amplitude (µV) of motor Median Nerve from birth to 3 years of age.

Ampl	7d	14d	21d	28d	2m	3m	6m	9m	12m	18m	24m	36m
Total N	29	17	15	12	39	16	60	50	32	36	19	19
Mean	2,94	2,81	3,93	2,1	2,66	3,61	3,47	5,2	5,84	6,66	6,6	7,58
StDev	0,55	0,42	1,09	0,33	0,48	0,75	0,63	1,15	1,21	0,79	1,16	1,36
RMSE	0,267	0,145	1,042	0,182	0,317	0,678	0,204	0,507	0,365	0,151	0,217	0,968
Variance	19%	15.0%	28.0%	16.0%	18.0%	21.0%	18.0%	22.0%	21.0%	12.0%	18%	18.0%
M - 2SD	1,84	1,97	1,75	1,44	1,7	2,11	2,21	2,9	3,42	5,08	4,28	4,86
M + 2SD	4,04	3,65	6,11	2,76	3,62	5,11	4,73	7,5	8,26	8,24	8,92	10,3
Min	1,7	2,3	2,3	1,7	1,9	1,8	2,2	2,5	3,4	5,2	4,8	4,2
Max	4,1	3,5	6,7	2,7	3,9	4,5	4,4	7,2	7,8	7,9	8,5	9,6
Plateau N	16	9	9	5	16	8	23	25	19	19	12	12
Normal Slope	0,16	0,15	0,55	0,25	0,13	0,39	0,1	0,2	0,24	0,15	0,34	0,49
Min Row	9	4	3	4	6	3	10	10	6	8	4	4
Max Row	24	12	11	8	21	10	32	34	24	26	15	15

Comparison between median and peroneal nerve conduction velocity in relation to the age:

NCV in peroneal and median nerve seems to have an almost overlapping maturity. At 28 days the mean MCV for both nerves is of 26 m/s, at 6 months is about 36 m/s, at 12 months of 44 m/s. The only different value is at 24 months, where the MCV for the median nerve is of 50,18 m/s, for the peroneal nerve is of 47,8 m/s.

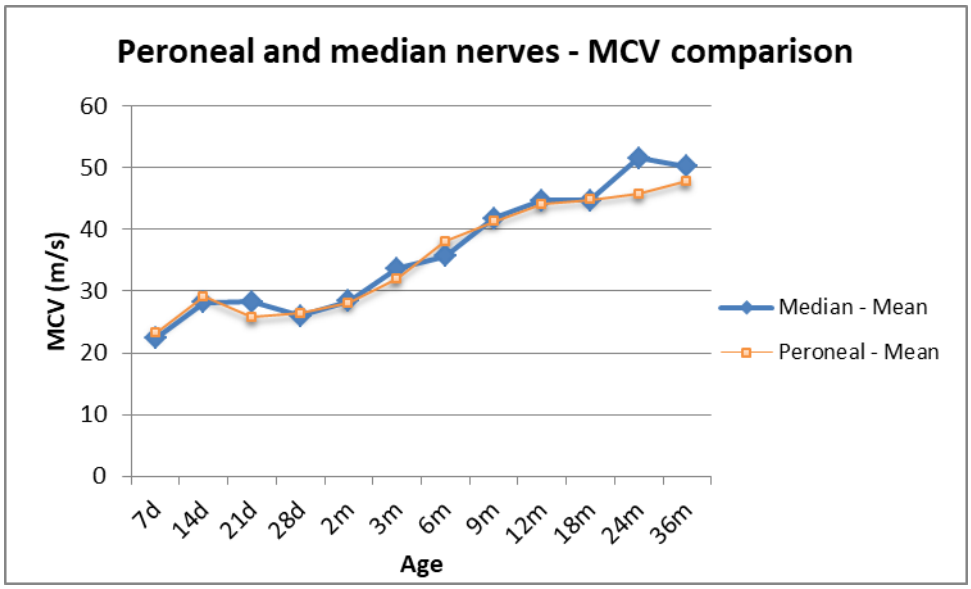

Figure 28: comparison between tibial and peroneal NCV in relation to age.

Table 8: MCV (m/s) of Tibial and Peroneal Nerve from birth to 3 years of age.

VCM	7d	14d	21d	28d	2m	3m	6m	9m	12m	18m	24m	36m
Median - Mean	22,33	28,14	28,26	26	28,39	33,67	35,67	41,73	44,63	44,63	51,53	50,18
Median total N	29	16	14	11	35	15	54	47	33	33	18	18
Peron-Mean	23,16	29	25,72	26,36	28,01	32,04	37,99	41,24	43,98	44,74	45,76	47,8
Peron total N	26	20	9	10	33	20	45	61	50	42	42	48

Sensory Nerves:

Below for each sensory nerve with an adequate sample size data regarding conduction velocity and amplitude are reported (in particular mean, standard deviation-SD, 2 SD below or above the mean, variance, minimum and maximum values found).

SURAL NERVE:

Sensory conduction velocity:

Regarding sensory conduction velocity of sural nerve, we obtained data from an adequate sample size from the age of 9 months.

At 9 months we measured a mean SVC of 33.72 m/s, at 12 months 46,58 m/s (SD 3,63), at 24 months a mean of 44,62 m/s (SD 3,13), at the age of 36 months a mean of 50,77 m/s (SD 2,81).

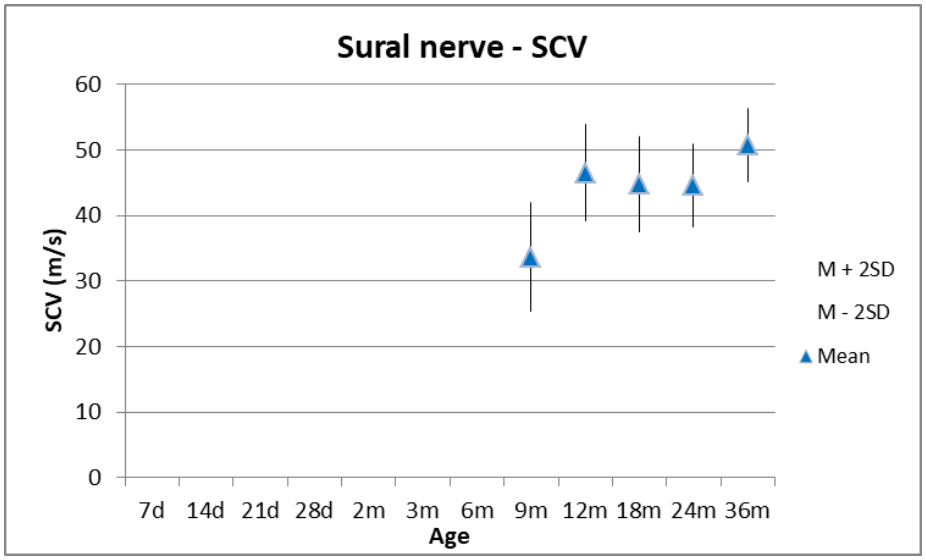

Figure 29: Sensory Conduction Velocity (m/s) of Sural Nerve in relation to age; mean and SD.

Table 9: Sensory Nerve conduction velocity of Sural Nerve from birth to 3 years of age.

VCS sur	7d	14d	21d	28d	2m	3m	6m	9m	12m	18m	24m	36m
Total N	-	-	-	-	-	-	-	26	21	47	43	34
Mean	-	-	-	-	-	-	-	33,72	46,58	44,81	44,62	50,77
StDev	-	-	-	-	-	-	-	4,17	3,67	3,6	3,13	2,81
RMSE	-	-	-	-	-	-	-	1,15	1,394	3,395	0,677	1,11
Variance	-	-	-	-	-	-	-	12.0%	8.0%	8.0%	7.0%	6.0%
M - 2SD	-	-	-	-	-	-	-	25,38	39,24	37,61	38,36	45,15
M + 2SD	-	-	-	-	-	-	-	42,06	53,92	52,01	50,88	56,39
Min	-	-	-	-	-	-	-	26	41	34	40	45
Max	-	-	-	-	-	-	-	41,4	54	50	50	56
Plateau N	-	-	-	-	-	-	-	12	10	29	15	13
Normal Slope	-	-	-	-	-	-	-	1,4	1,44	0,57	0,71	0,92
Min Row	-	-	-	-	-	-	-	5	7	6	4	13
Max Row	-	-	-	-	-	-	-	16	16	34	18	25

Amplitude:

Regarding sural nerve amplitude, we obtained data from an adequate sample size from the age of 9 months. We can see at 9 months a mean amplitude of 9,94 µV (2,05 SD), at the age of 12 months a mean of 12,24 µV (SD 2,39), that arrives at the age of 24 months to 15,02 µV (SD 3,58).

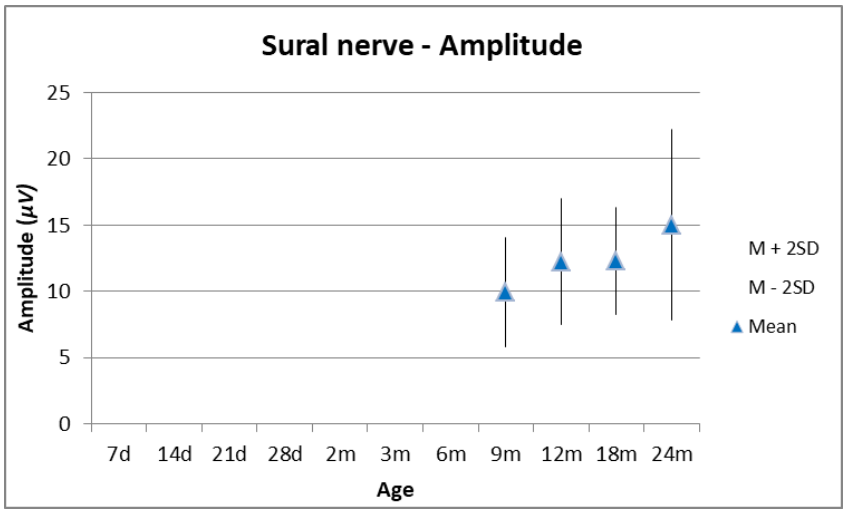

Figure 30: amplitude (µV) of Sural Nerve in relation to age; mean and SD.

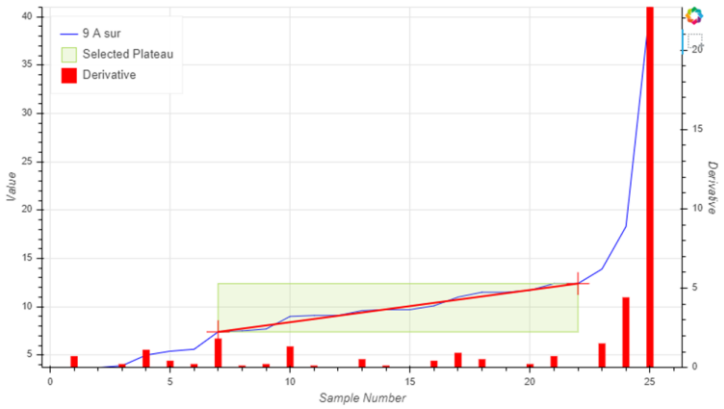

Figure 31: example of E-norms values: Amplitude of sural nerve (6-9 months).

Table 10: Amplitude of Sural Nerve from birth to 3 years of age.

Ampl	7d	14d	21d	28d	2m	3m	6m	9m	12m	18m	24m	36m
Total N	-	-	-	-	-	-	-	26	21	47	34	-
Mean	-	-	-	-	-	-	-	9,94	12,24	12,3	15,02	-
StDev	-	-	-	-	-	-	-	2,05	2,39	2,03	3,58	-
RMSE	-	-	-	-	-	-	-	0,714	1,275	0,383	1,186	-
Variance	-	-	-	-	-	-	-	21.0%	20.0%	17.0%	24.0%	-
M - 2SD	-	-	-	-	-	-	-	5,84	7,46	8,24	7,86	-
M + 2SD	-	-	-	-	-	-	-	14,04	17,02	16,36	22,18	-
Min	-	-	-	-	-	-	-	5,6	6,8	8,6	9,6	-
Max	-	-	-	-	-	-	-	13,9	16	16,2	22,4	-
Plateau N	-	-	-	-	-	-	-	18	12	23	20	-
Normal Slope	-	-	-	-	-	-	-	0,49	0,84	0,35	0,67	-
Min Row	-	-	-	-	-	-	-	6	6	13	8	-
Max Row	-	-	-	-	-	-	-	23	17	35	27	-

MEDIAL PLANTAR NERVE:

Sensory conduction velocity:

Regarding sensory conduction velocity of sural nerve, we obtained data from samples consisting of less than 20 patients; after 24 months the sample was not assessable.

It starts at 0-7 days with a mean SVC of 19,67 m/s (SD 4,49), at 1 months a mean of 3,57 (SD 10,49), at 12 months a mean of 37,4 m/s (SD 6,09) at 24 months a mean of 46,3 m/s (SD 3,95).

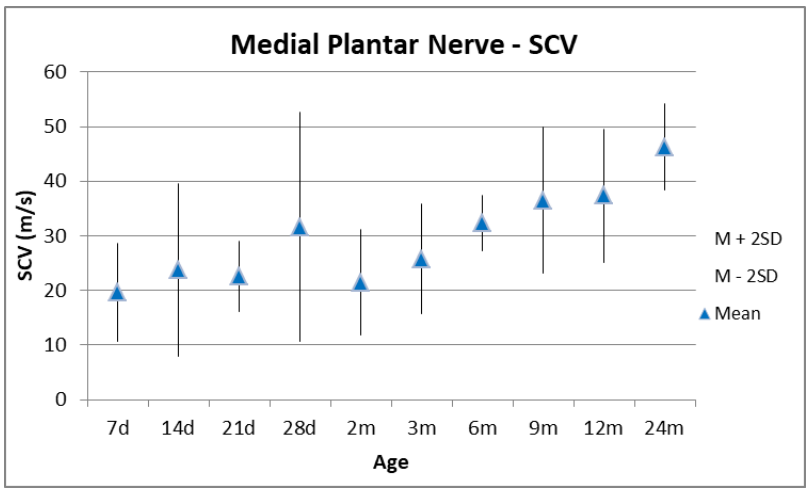

Figure 32: Sensory Conduction Velocity (m/s) of Sural Nerve in relation to age; mean and SD.

Table 11: Sensory Nerve conduction velocity of Medial Plantar Nerve from birth to 3 years of age.

SCV	7d	14d	21d	28d	2m	3m	6m	9m	12m	24m	36m
Total N	13	5	8	4	16	9	17	12	17	5	-
Mean	19,67	23,75	22,6	31,57	21,43	25,74	32,34	36,5	37,4	46,3	-
StDev	4,49	7,9	3,26	10,49	4,83	5,04	2,51	6,68	6,09	3,95	-
RMSE	3,148	2,186	0,659	6,261	0,94	2,995	1,507	4,374	1,005	2,467	-
Variance	23.0%	33.0%	14.0%	33.0%	23.0%	20.0%	8.0%	18.0%	16.0%	9.0%	-
M - 2SD	10,69	7,95	16,08	10,59	11,77	15,66	27,32	23,14	25,22	38,4	-
M + 2SD	28,65	39,55	29,12	52,55	31,09	35,82	37,36	49,86	49,58	54,2	-
Min	14,1	12,9	17,5	14,3	13	21	29	26	29	42,9	-
Max	30	32,3	27	40	28,4	35	38	49	46,7	53	-
Plateau N	9	4	5	4	9	5	10	6	8	4	-
Normal Slope	1,99	6,47	2,38	8,57	1,92	3,5	1	4,6	2,53	3,37	-
Min Row	2	1	2	0	2	2	2	1	3	1	-
Max Row	10	4	6	3	10	6	11	6	10	4	-

Amplitude:

Even for sural nerve amplitude, we obtained data from samples consisting of less than 20 patients; after 24 months the sample was not assessable.

We can see an average of 1.91 µV at 0-7 days of life, which subsequently increases gradually: we got a mean of 3,35 µV (SD 0,98) at 1 month, 3,82 µV (SD 0,57) at 6 months, a mean of 7,06 µV (SD 4,06) at 12 months.

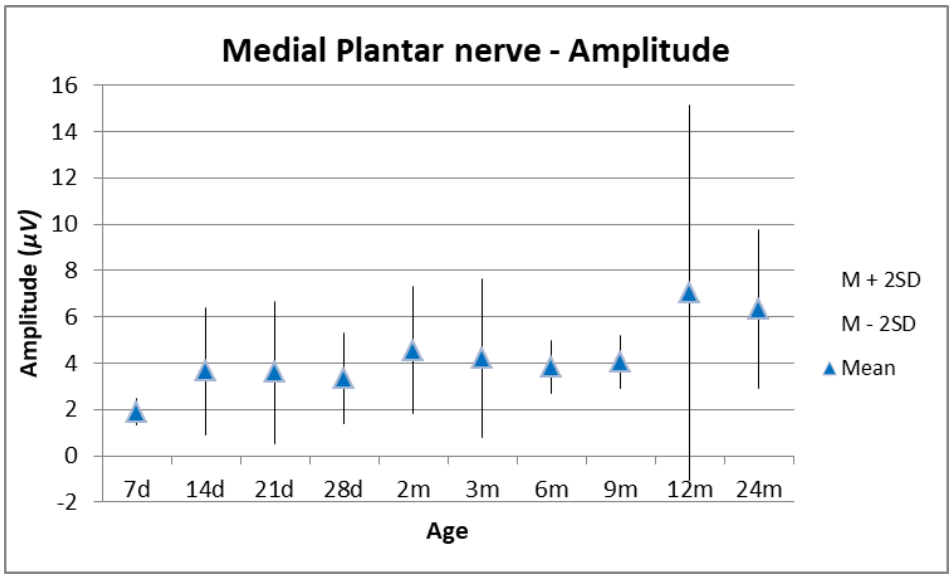

Figure 33: amplitude (µV) of Medial Plantar Nerve in relation to age; mean and SD.

Table 12: Amplitude (µV) of Medial Plantar Nerve from birth to 3 years of age.

Ampl	7d	14d	21d	28d	2m	3m	6m	9m	12m	24m
Total N	13	5	8	4	16	9	17	12	17	5
Mean	1,91	3,67	3,6	3,35	4,57	4,22	3,82	4,04	7,06	6,33
StDev	0,28	1,37	1,54	0,98	1,37	1,7	0,57	0,57	4,05	1,72
RMSE	0,108	0,467	0,963	0,5	0,334	1,07	0,213	0,281	0,937	0,815
Variance	15.0%	37.0%	43.0%	29.0%	30.0%	40.0%	15.0%	14.0%	57.0%	27.0%
M - 2SD	1,35	0,93	0,52	1,39	1,83	0,82	2,68	2,9	-1,04	2,89
M + 2SD	2,47	6,41	6,68	5,31	7,31	7,62	4,96	5,18	15,16	9,77
Min	1,4	2	1,9	1,8	2,6	2,7	2,7	3,1	1	4,7
Max	2,4	5,8	6,5	4,2	6,3	7,1	4,7	5,2	12,3	9,1
Plateau N	7	4	5	4	7	4	9	8	8	4
Normal Slope	0,17	1,27	1,15	0,8	0,62	1,47	0,25	0,3	1,61	1,47
Min Row	2	1	1	0	4	2	4	3	4	1
Max Row	8	4	5	3	10	5	12	10	11	4

MEDIAN NERVE:

Sensory Conduction Velocity:

Regarding sensory conduction velocity of median nerve, we can see that at 7 days of life the SCV mean is 20,9 m/s (SD 1,11), at 28 days we obtain a mean of 25,63 m/s (SD 1,98), that increases progressively: 33,87 mm/s at 6 months (SD 2,13), 42,13 m/s (SD 2,57) at 12 months, 48,99 m/s at 24 months (SD 2,97), 50,38 m/s (SD 4,83) at 36 months.

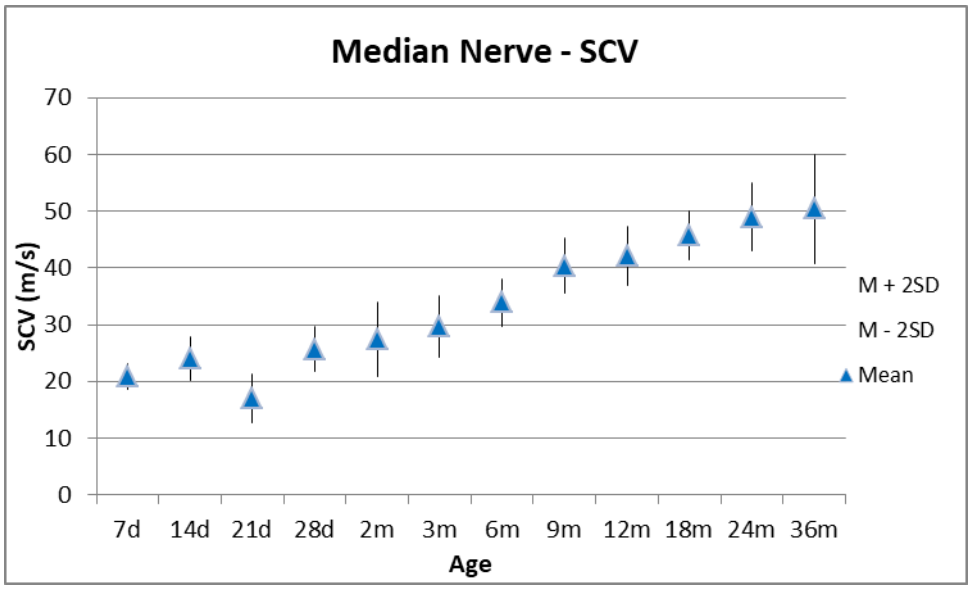

Figure 34: Sensory Conduction Velocity (m/s) of Median Nerve in relation to age; mean and SD.

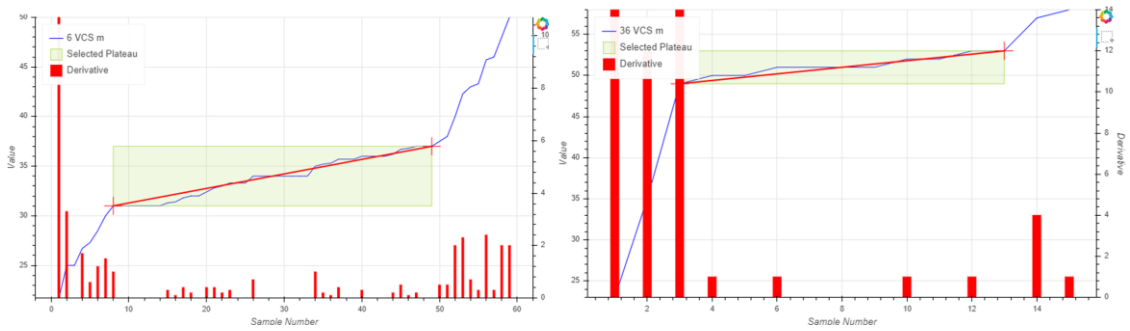

Figure 35: examples of E-norms values: SCV of medial nerve (3-6 months and 24-36 months).

Table 13: Sensory Nerve conduction velocity of Median Nerve from birth to 3 years of age.

VCS	7d	14d	21d	28d	2m	3m	6m	9m	12m	18m	24m	36m
Total N	29	18	13	7	38	17	60	41	31	33	21	16
Mean	20,9	24,03	16,95	25,63	27,46	29,65	33,87	40,39	42,13	45,74	48,99	50,38
StDev	1,11	1,91	2,14	1,98	3,28	2,69	2,13	2,41	2,57	2,11	2,97	4,83
RMSE	0,784	1,041	0,393	0,344	1,948	0,898	0,319	1,133	0,693	0,967	0,764	6,409
Variance	5.0%	8.0%	13.0%	8.0%	12.0%	9.0%	6.0%	6.0%	6.0%	5.0%	6.0%	10.0%
M - 2SD	18,68	20,21	12,67	21,67	20,9	24,27	29,61	35,57	36,99	41,52	43,05	40,72
M + 2SD	23,12	27,85	21,23	29,59	34,02	35,03	38,13	45,21	47,27	49,96	54,93	60,04
Min	19	20	13,5	23	21	25	30	35	38	42,7	44	35
Max	23,3	27,5	20	28,5	36,1	34	37,5	44,1	46,2	50	54	57
Plateau N	8	9	8	4	27	8	44	21	12	15	10	13
Normal Slope	0,61	0,94	0,93	1,83	0,58	1,29	0,17	0,46	0,75	0,52	1,11	1,83
Min Row	10	6	2	2	6	5	7	7	7	6	6	2
Max Row	17	14	9	5	32	12	50	27	18	20	15	14

Amplitude:

Regarding the amplitude, we obtained in our sample at 7 days of life a mean of 9,23 µV (SD 2,46), 14,28 µV at two months (4,54 SD), 19,18 µV (SD 4,84) at six months, 29 µV at 12 months (SD 9,83), 42,47 µV (SD 4,77) at 18 months, then it remains stable.

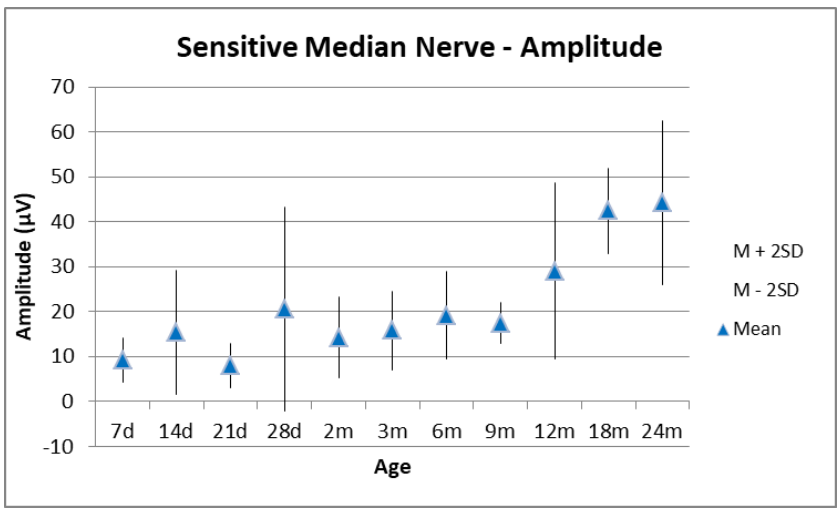

Figure 36: Amplitude (µV) of Sensory Medial Nerve in relation to age; mean and SD.

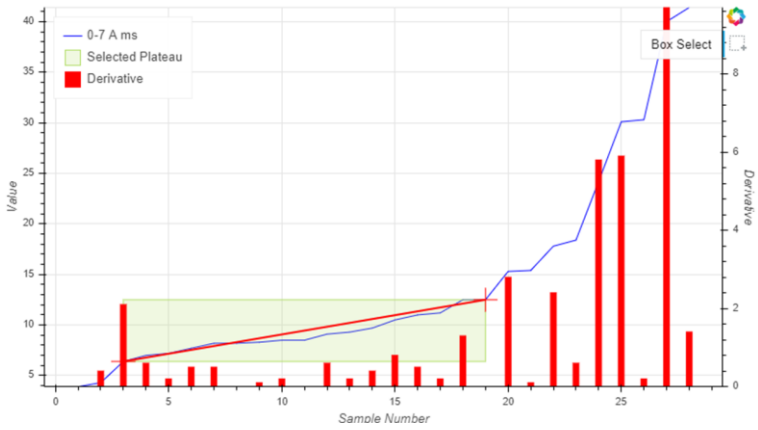

Figure 37: example of E-norms values: amplitude of sensory medial nerve (0-7 days).

Table 14: Amplitude of Sensory Medial Nerve from birth to 3 years of age.

Ampl	7d	14d	21d	28d	2m	3m	6m	9m	12m	18m	24m	36m
Total N	29	18	13	7	38	17	60	41	31	33	21	16
Mean	9,23	15,4	7,97	20,54	14,28	15,78	19,18	17,48	29,01	42,47	44,25	29,68
StDev	2,46	6,93	2,5	11,38	4,54	4,42	4,84	2,25	9,83	4,77	9,08	8,4
RMSE	1,391	5,921	1,211	7,521	0,781	1,83	2,299	0,95	1,526	2,273	2,484	7,794
Variance	27.0%	45.0 %	31.0%	55.0%	32.0%	28.0%	25.0%	13.0%	34.0%	11.0%	21.0%	28.0%
M - 2SD	4,31	1,54	2,97	-2,22	5,2	6,94	9,5	12,98	9,35	32,93	26,09	12,88
M + 2SD	14,15	29,26	12,97	43,3	23,36	24,62	28,86	21,98	48,67	52,01	62,41	46,48
Min	4,3	5,5	4,8	10,2	7,5	7	11,4	13,7	13,4	32,4	31,5	22
Max	15,3	33,9	13	42	21,6	22,6	30,8	22,4	47	52,5	60,9	50,3
Plateau N	19	12	9	5	22	8	35	18	18	13	13	9
Normal Slope	0,61	2,58	1,02	7,95	0,67	2,23	0,57	0,51	1,98	1,68	2,45	3,54
Min Row	2	4	3	2	10	3	9	7	6	14	5	3
Max Row	20	15	11	6	31	10	43	24	23	26	17	11

ELECTROMYOGRAPHY – NORMATIVE VALUES

Sample Description:

From 1st January 2016 to 31st October 2019 3848 MUPs registration in the tibial anterior muscle were recorded by Neurophysiology Unit of the "Giannina Gaslini Institute" Genoa Italy, performed in children between 0 days and 6 years old (78 months).

Premature infants with gestational age at the time of the exam less than 40 weeks of GA were excluded from our study. No patients needed sedation.

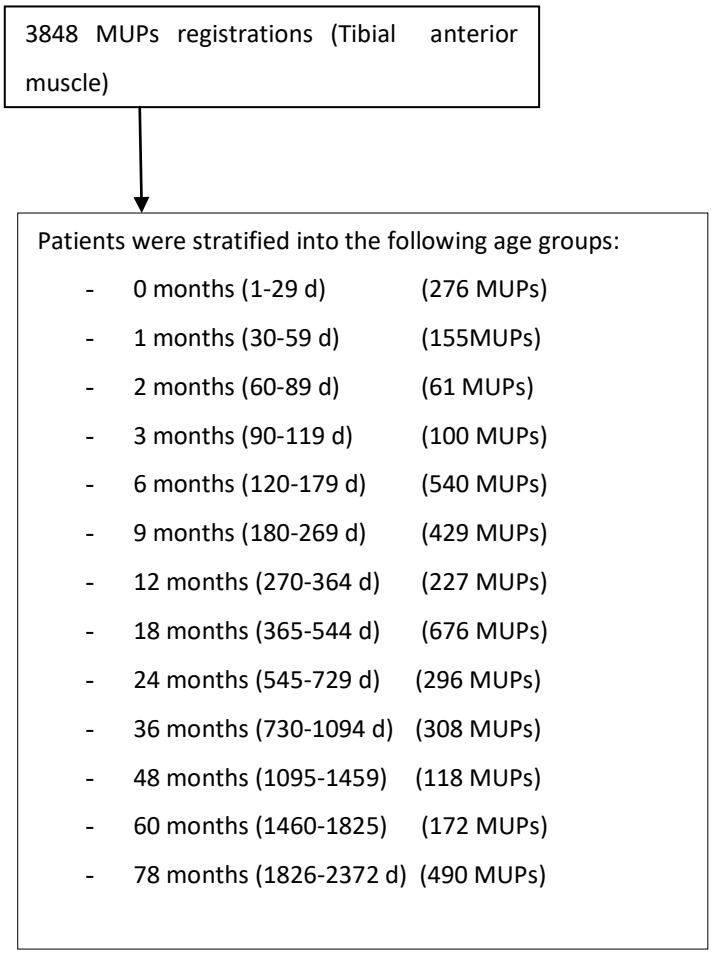

Figure 38: Flowchart of case selection and age stratification.

Semiquantitative EMG:

Amplitude:

Regarding MUPs amplitude, we obtained a mean of 484,34 µV at 1 months, a mean of 516, 99 µV at 6 months (SD 152), a mean of 674 µV at 12 months, that increases at 24 months to 522, 33 µV (SD 174,39)and to 840,54 µV (SD 92) at 48 months.

We can see in Table 15 the values reported in literature regarding adults (young, middle age, elderly, from Howard JE 1988).

Table 15: MUPs Amplitude from birth to 6 years of age.

Ampl	0m	1m	2m	3m	6m	9m	12m	18m	24m	36m	48m	60m	72m	Y	MA	E
Mean	201,59	484,34	163,99	319,82	516,99	667,1	674	614,28	522,33	423,42	840,54	819,43	762,97	633	753	849
StDev	43,19	148,04	25,48	60,51	152	233,7	159,9	194,9	174,39	96,02	92	160,58	195,66	-174	-401	-269
RMSE	26,7	55,8	17,8	20,092	47,975	32,089	16,722	36,48	105,8	35,811	19,721	24,687	44,166	174	401	269
Variance	21%	31%	16%	19%	29%	35%	24%	32%	33%	23%	11%	20%	26%	174	401	269
M - 2SD	115,21	188,26	113,03	198,8	212,99	199,7	354,08	224,32	173,55	231,38	656,54	498,27	371,65			
M + 2SD	287,97	780,42	214,95	440,84	820,99	1134,5	993,92	1004,24	871,11	615,46	1024,54	1140,59	1154,29			
Min	145	253,4	118	213	284,7	271,8	400,8	293,1	278	266,4	700	545	458,8			
Max	305,3	805,3	229,8	456	833	1102,3	964,1	995,4	959,5	642,7	1013	1100	1148,1			
Plateau N	120	26	14	47	181	142	67	204	144	59	19	29	102			
Total N	234	62	30	86	278	198	94	307	204	83	45	61	169			
Normal Slope	1,35	22,08	8,6	5,28	3,05	5,89	8,53	3,46	4,77	6,49	17,39	19,82	6,82			
Min Row	27	8	4	7	32	28	7	48	16	9	6	16	14			
Max Row	146	33	17	53	212	169	73	251	159	67	24	44	115			

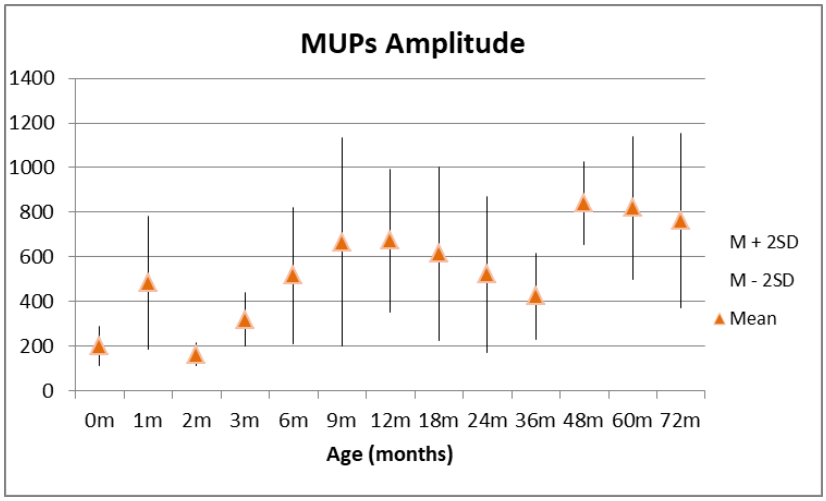

Figure 39: MUPs Amplitude in relation to age; mean and SD.

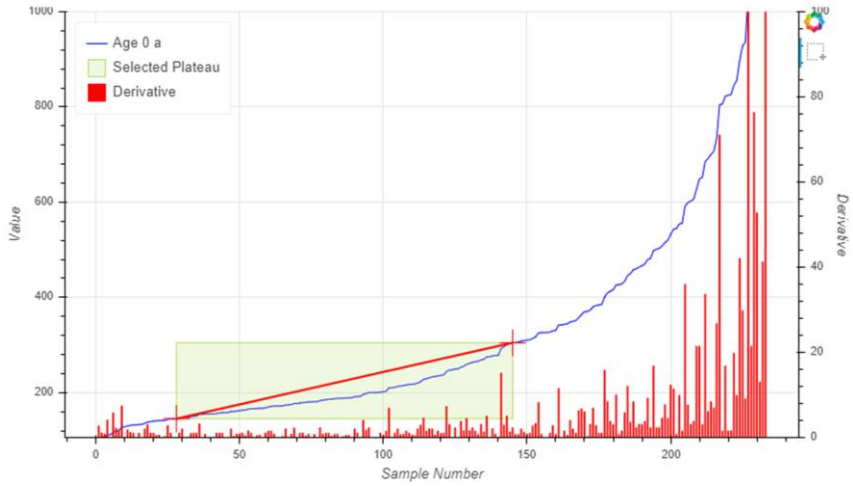

Figure 40: example of E-norms values: MUPs amplitude (0-1 months).

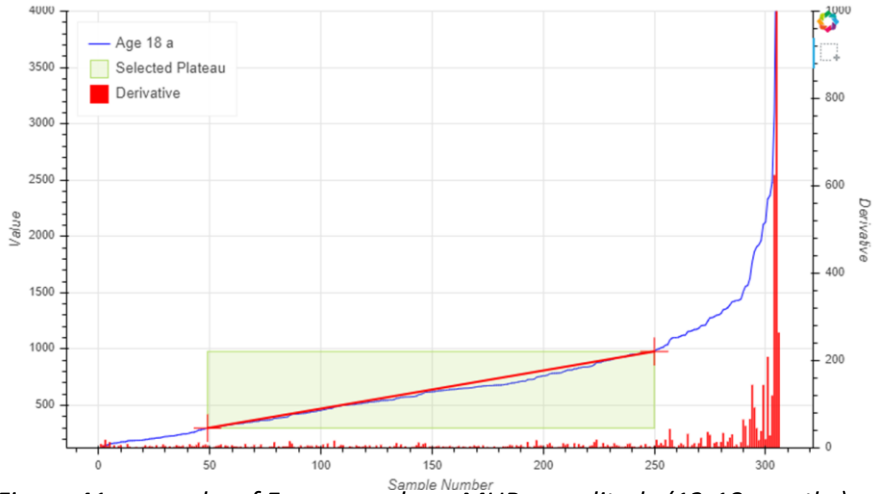

Figure 41: example of E-norms values: MUPs amplitude (12-18 months).

Duration:

In table 16 we can see MUPs Duration from birth to 6 years of life. We obtain a mean of MUPs Duration of 8,1 ms at birth (SD 1,12), 10 ms (SD 1,05) at 6 months, 11,48 ms (SD1,15) at 12 months, 12,28 ms (SD 1,21) at 18 months, after that in our sample it remains stable.

Table 16: MUPs Duration from birth to 6 years of age.

Dur	0m	1m	2m	3m	6m	9m	12m	18m	24m	36m	48m	60m	78m	Y	MA	E
Mean	8,17	9,95	7,38	10,05	10	8,03	11,48	12,28	10,38	11,99	10,04	13	12,05	13,1	12,7	13,9
StDev	1,12	0,34	0,98	1,01	1,05	1,08	1,14	1,21	1,15	0,72	0,57	1,64	1,1	1,2	1,2	1
RMSE	0,151	0,1 83	0,7 33	0,13	0,1 98	0,1 48	0,3 09	0,1 37	0,2 35	0,4 69	0,2 47	0,1 8	0,1 75	1,2	1,2	1
Variance	14%	3%	13%	10%	11%	13%	10%	10%	11%	6%	6%	13%	9%			
M - 2SD	5,93	9,27	5,42	8,03	7,9	5,87	9,2	9,86	8,08	10,55	8,9	9,72	9,85			
M + 2SD	10,41	10,63	9,34	12,07	12,1	10,19	13,76	14,7	12,68	13,43	11,18	16,28	14,25			
Min	6,46	9,17	6,25	8,33	8,23	6,25	9,17	10	8,75	11,04	9,27	10,21	9,9			
Max	10	10,42	9,79	11,67	12,08	10	13,33	14,48	12,4	13,75	11,25	15,73	14,06			
Plateau N	62	24	15	28	114	104	42	219	64	43	17	55	120			
Total N	129	62	30	55	249	198	94	445	111	105	45	111	219			
Normal Slope	0,06	0,05	0,25	0,12	0,03	0,04	0,1	0,02	0,06	0,06	0,12	0,1	0,03			
Min Row	20	18	5	10	41	47	35	81	20	21	11	23	40			
Max Row	81	41	19	37	154	150	76	299	83	63	27	77	159			

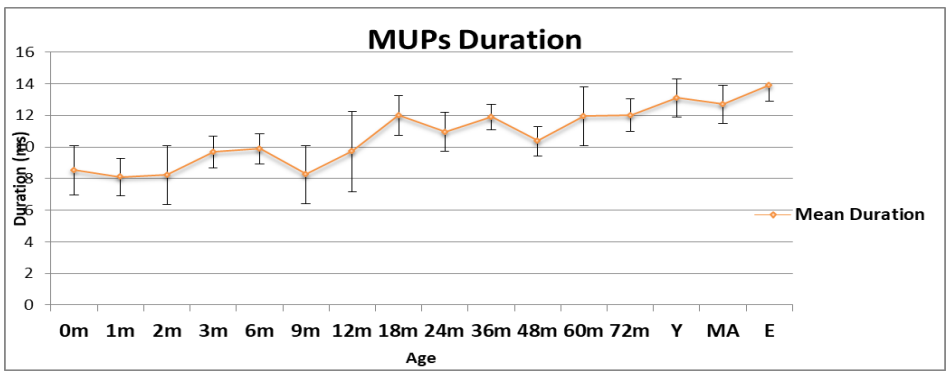

Figure 42: MUPs Duration in relation to age; mean and SD.

Figure 43: example of E-norms values: MUPs duration (12-18 months).

Quantitative EMG

Turns/Second:

Regarding Turns/second, we can see a mean of 104,74 at birth, a mean of 294 at 6 months, a mean of 241,71 at 12 months, a mean of 315,01 (SD 63,55) at 24 months, a mean of 242,62 at 36 months, that remains stable thereafter.

Table 17: T/S from birth to 6 years of age

T/S	0m	1m	2m	3m	6m	9m	12m	18m	24m	36m	48m	60m	72m
Mean	104,74	339	177,06	338,18	294,04	282,16	241,71	252,42	315,01	242,5	262,62	204,41	226,15
StDev	35,95	54,87	17,37	80,96	71,36	71,24	40,88	77,21	99,33	63,55	67,41	26,39	64,11
RMSE	2,392	6,086	4,69	25,377	12,328	6,244	11,54	6,842	10,498	6,828	18,178	5,359	9,005
Variance	34%	16%	10%	24%	24%	25%	17%	31%	32%	26%	26%	13%	28%
M - 2SD	32,84	229,26	142,32	176,26	151,32	139,68	159,95	98	116,35	115,4	127,8	151,63	97,93
M + 2SD	176,64	448,74	211,8	500,1	436,76	424,64	323,47	406,84	513,67	369,6	397,44	257,19	354,37
Min	44	240	144	200	156	162	158	126	134	134	162	154	124
Max	166	436	202	516	412	412	310	388	496	362	396	248	344
Plateau N	123	38	17	45	168	161	42	213	192	138	55	64	152
Total N	255	93	47	74	367	312	100	389	260	277	118	171	364
Normal Slope	1	5,3	3,63	7,18	1,53	1,56	3,71	1,24	1,9	1,66	4,33	1,49	1,46
Min Row	59	18	9	20	124	70	34	105	25	74	26	36	90
Max Row	181	55	25	64	291	230	75	317	216	211	80	99	241

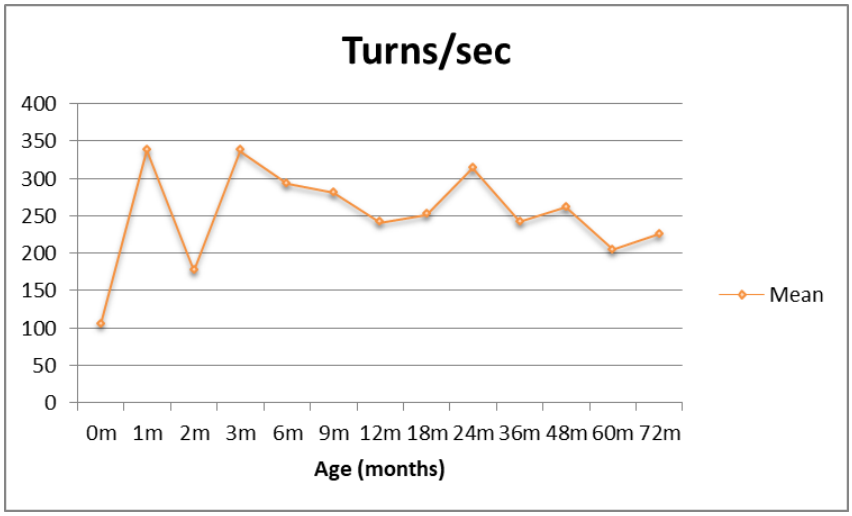

Figure 44: Turns/sec in relation to age; mean.

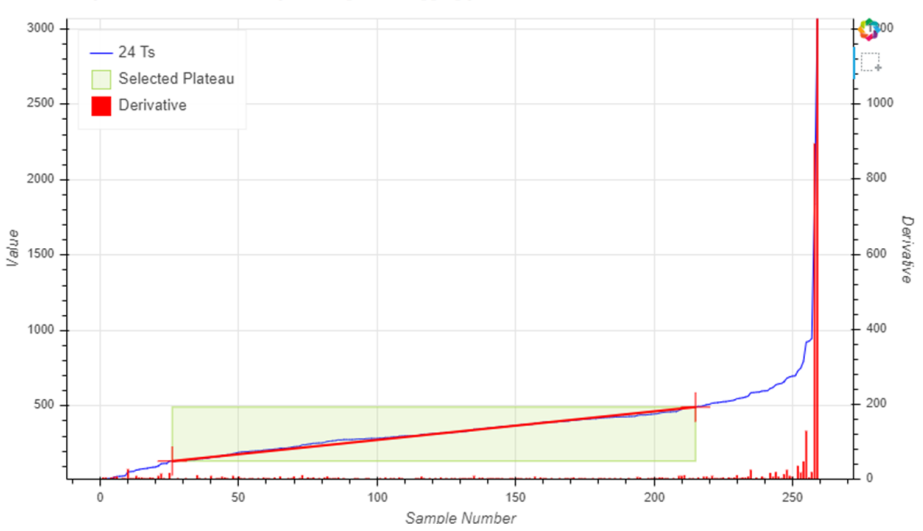

Figure 45: example of E-norms values: Turns/sec (18-24 months).

Turn Amplitude:

The technique was first described by R.G. Willison in 1964 and Rose and Willison in 1967 where the number of turns and the mean amplitude (total amplitude divided by the number of turns) were used.

We obtain a mean of 196 a birth, that increases at 278,7 at 1 months; after that we can see a mean of 355,5 at 6 months, of 501,9 at 12 months, a mean of 408,8 at 24 months, without any others variations thereafter. We can see A/T values in Table 18.

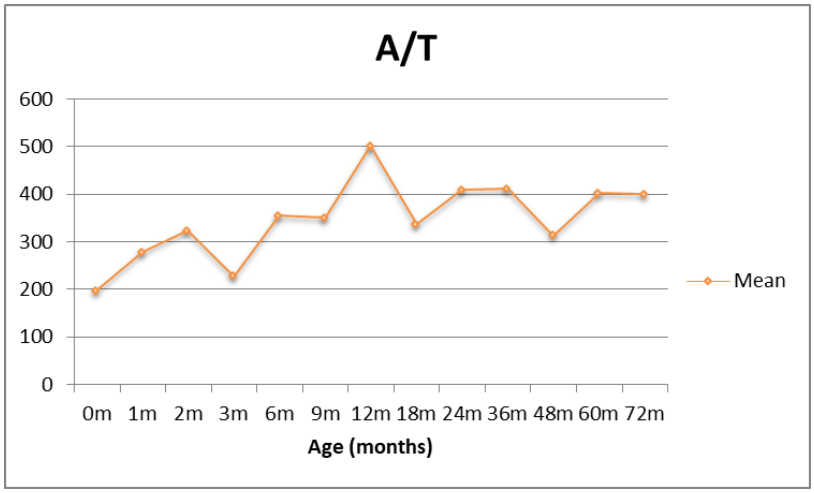

Figure 46: Turns/sec in relation to age; mean.

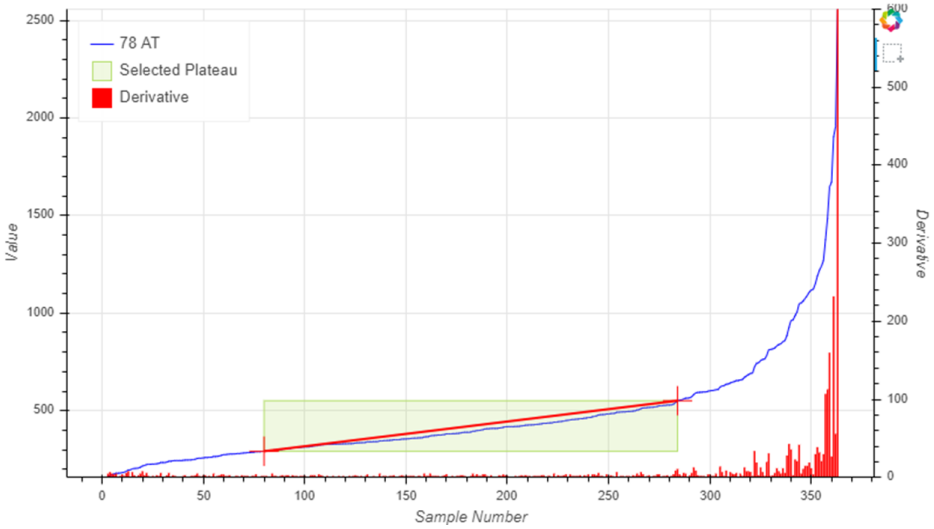

Figure 47: example of E-norms values: Amplitude/Turns (60-78 months).

Table 18: Amplitude/Turns from birth to 6 years of age

A/T	0m	1m	2m	3m	6m	9m	12m	18m	24m	36m	48m	60m	78m
Mean	196,8	278,7	323,9	227,4	355,5	350,56	501,9	336,6	408,8	411,8	313,3	402,	400,6
StDev	28,98	33,21	18,52	18,37	61,82	65,72	69,44	75,91	72,6	73,31	52,67	53,53	70,58
RMSE	6,973	11,598	4,113	7,775	10,672	11,309	6,732	32,601	23,725	7,044	20,554	6,66	24,319
Variance	15%	12%	6%	8%	17%	19%	14%	23%	18%	18%	17%	13%	18%
M - 2SD	138,89	212,37	286,91	190,62	231,88	219,12	363,05	184,78	263,58	265,17	207,93	294,96	259,48
M + 2SD	254,81	345,21	360,99	264,1	479,16	482	640,81	488,42	553,98	558,41	418,61	509,08	541,8
Min	148	223	296	202	249	243	385	223	299	285	243	310	291
Max	255	346	359	266	477	478	617	510	561	545	416	503	554
Plateau N	82	19	21	14	185	183	45	237	65	162	44	102	207
Total N	144	93	47	43	338	312	99	389	167	276	118	171	364
Normal Slope	1,32	6,83	3,15	4,92	1,24	1,29	5,27	1,22	4,09	1,61	4,02	1,91	1,28
Min Row	13	11	3	3	50	51	22	87	32	64	31	23	79
Max Row	94	29	23	16	234	233	66	323	96	225	74	124	285

NEUROPHYSIOLOGICAL AND HYSTOPATHOLOGICAL CORRELATION

Sample description

From January 2011 to December 2021, 537 children underwent muscle biopsy at the Giannina Gaslini Institute, Genoa, Italy. Eighteen patients were excluded from this study because they underwent muscle biopsy as part of clinical trials.

We identified 82 patients within the study period, 50 (61%) boys and 32 (39%) girls, that fulfilled the inclusion criteria. The mean age is 6.6 years (SD +/- 5.8), median 4.7 years.

Electromyography (EMG) was available in 63 patients, Nerve conduction studies (NCSs) in 79 patients. In the majority of cases (63 patients, 76,8%) neurophysiological studies preceded the muscle biopsy.

The mean distance between neurophysiological study and muscle biopsy was 1.05 years, (standard deviation of 2 years, median of 0.4 years).

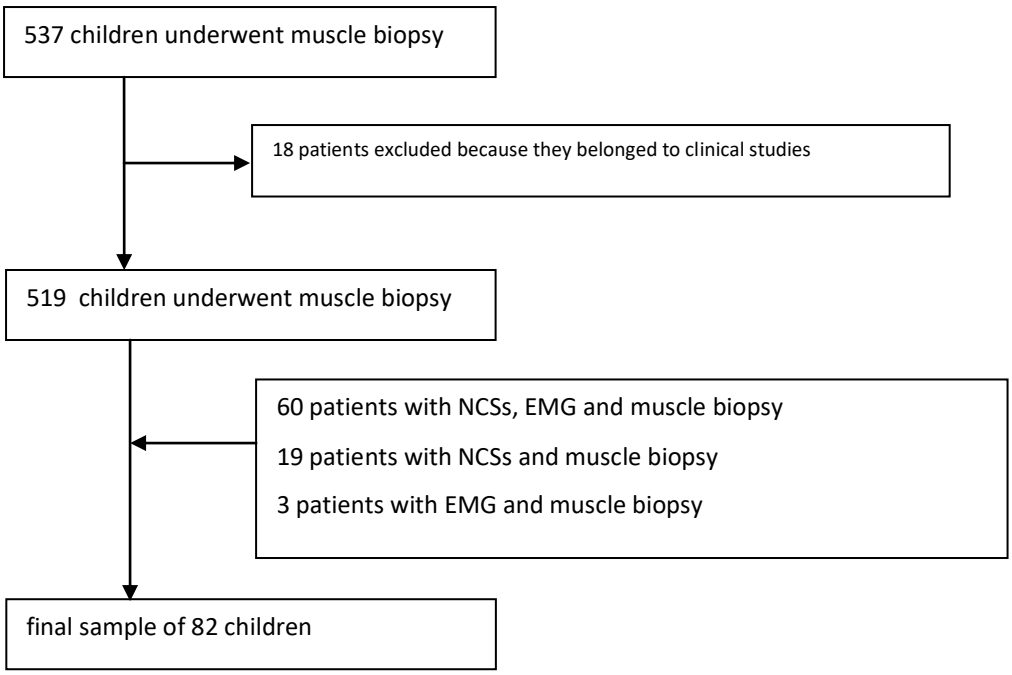

Figure 48: Flowchart of case selection.

AETIOLOGICAL DIAGNOSIS

Forty-six (56.1%) children had a final diagnosis of myopathic disorder; eight (9.6%) of a neurogenic disease, of which four affected by motor neuron disease (spinal muscular atrophy) and four by peripheral neuropathy; ten (12.2%) were affected by metabolic disease; the remaining eighteen included non-neuromuscular diseases, namely: eight patients with genetically determined central nervous system disorders or genetic syndromes, one with paraneoplastic encephalopathy, others with unspecified or undefined neurological disorders.

Within the myopathic group there were nineteen congenital myopathies (41.3%), nine (19.6%) muscular dystrophies, thirteen (28.2%) inflammatory myopathies, and five (10.9%) patients with hyperCKemia.

In the group of myopathic disorders, the final genetic diagnosis was present in 12/19 patients (63.2%) in congenital myopathies and 8/9 (88.9%) in congenital muscular dystrophies; in the group of hyperCKemia, one patient had RYR1 mutation and one was a DMD mutation carrier.

In the neurogenic disorders group, four patients were diagnosed with SMA; of the four patients with neuropathy three had a genetically confirmed diagnosis, one patient had iatrogenic neuropathy.

No patients with neuromuscular junction disease were present in the sample.

In the group of patients with metabolic disorders, one patient had ADPRHL2 metabolic encephalopathy, one had ATAD3A mutation, one had Zellweger syndrome, PDH deficiency was found in one patient, all were diagnosed with mitochondrial encephalopathy including MELAS and Leigh .

Final diagnoses are summarised in Table 19.

Etiological diagnosis	
Myopathic disorders	46 (56.1%)
Congenital myopathies	19 (41.3%)
Congenital muscular Dystrophies	9 (19.6%)
Inflammatory myopathies	13 (28.2%)
hyperCKemia	5 (10.9%%)
Neurogenic disorders	8 (9.7%)
Peripheral neuropathy	4
Spinal muscular atrophy	4
Metabolic disorders	10 (12.2%)
Others	18 (22%)
Total	82

Table 19: Aetiological diagnosis in study patients

EMG, NCS, muscle biopsy results and accuracy

EMG, NCS AND MUSCLE BIOPSY RESULTS

EMG was performed in 63 patients; for one patient, only the final result is given. Twelve patients (19%) had normal EMG, another 12 patients had myogenic EMG, neurogenic EMG was present in 15 patients (23.7%) and 24 (38.1%) had non-specific changes.

NCSs were performed in 79 patients, of which only 20 (25.3%) were altered.

Finally, normal muscle biopsy was found in 10 patients (12.1%), 30 patients (36.6%) showed myopathic changes, 13 patients (15.9%) had neurogenic changes on biopsy and 29 patients (35.4%) had non-specific changes.

These results are summarised in Table 20.

	EMG, (N and %) Total patients= 63					NCS (N and %) Total patients=79			Biopsy, (N and %) Total patients= 82				
	Norm	Myo	Neur	Asp	**Tot**	Norm	Alt	**Tot**	Norm	Myo	Neur	Asp	**Tot**
Myopathic disorders	9 14.3%	11 17.5%	3 4.8%	19 30.2%	**42** **66.8%**	34 43%	9 11.4%	**43** **54.4%**	2 2.4%	30 36.6%	4 4.9%	10 12.2%	**46** **56.1%**
Neurogenic disorders	0	0	4 6.3%	0	**4** **6.3%**	4 5.1%	4 5.1%	**8** **10.2%**	1 1.2%	0	5 6.1%	2 2.4%	**8** **9.7%**
Metabolic disorders	1 1.6%	0	4 6.3%	0	**5** **7.9%**	6 7.6%	4 5%	**10** **12.6%**	1 1.2%	0	3 3.7%	6 7.3%	**10** **12.2%**
Others	2 3.2%	1 1.6%	4 6.3%	5 7.9%	**12** **19%**	15 19%	3 3.8%	**18** **22.8%**	6 7.3%	0	1 1.2%	11 13.5%	**18** **22%**
Tot	12 19.1%	12 19.1%	15 23.7%	24 38.1%	**63** **100%**	59 74.7%	20 25.3%	**79** **100%**	10 12.1%	30 36.6%	13 15.9%	29 35.4%	**82** **100%**

Table 20: Electromyography (EMG), nerve conduction studies (NCS) and muscle biopsy results in patients by aetiological diagnosis. Norm= normal; Myo= myopathic; Neur= neurogenic; Asp=aspecific; Alt= alterated

DIAGNOSTIC VALUE OF EMG AND MUSCLE BIOPSY

EMG was 26% sensitive and 95% specific in regards to final diagnosis of definitive myopathy; muscle biopsy was high specific (100%) and moderate sensitive (65%) for detection of myopathy. Furthermore, EMG had 100% sensitivity and 81% specificity for a neurogenic disorder; in contrast, muscle biopsy had 62% sensitivity and was highly specific for a neurogenic disorder.

Diagnostic accuracy of muscle biopsy was 80.5% for myophaties and 86.6% for neurogenic disorders; diagnostic accuracy of needle EMG was 49.2% for myopathies and 82.5% for neurogenic disorders.

Agreement between EMG and muscle biopsy agreement was 42.9% (27/63); agreement between EMG and final diagnosis was 31.7% (20/63).

The sensitivity, specificity, positive and negative Predictive values for EMG and muscle biopsy predicting neurogenic disorder are described in Table 21.

Diagnosis	method	Sensitivity	Specificity	PPV	NPV
Myopathy	Muscle biopsy	65%	100%	100%	69%
	EMG	26%	95%	92%	39%
Neurogenic disorder	Muscle biopsy	62%	89%	38%	96%
	EMG	100%	81%	27%	100%

Table 21: sensitivity, specificity, Positive Predictive value (PPV) and Negative Predictive value (NPV) of muscle biopsy and electromyography (EMG) according to final diagnosis

EMG, NCS, muscle biopsy findings and correlation between EMG and histopathological findings

NCS and EMG FINDINGS

Specific NCS and needle EMG findings are described in Table 22.

Motor axonal polyneuropathy/alterations (with low CMAP amplitudes) was present in twenty patients (25.3%), of which nine patients (11.4%) associated low SNAP amplitudes.

Axonal and demyelinating motor polyneuropathy was described in four patients (5%), while slowing of SCV (sensory conduction velocity) associated with reduced SNAP amplitude was found in three patients (3.8%).

Eighteen patients (29%) had fibrillation potentials on EMG. Increased phases are described in 67.7% of patients. Alterations in the recruitment pattern were found in twenty-seven patients (45.8%), as well as alterations in the MUAP duration (43.5%). Of the 39 patients in whom TAA (Turns/Amplitude Analysis) was performed, ten (25.6%) presented increased T/A and twelve (30.8%) decreased T/A. No patients in our sample had myotonic discharges.

NCS findings (tot=79)	**N (%)**
Low CMAP amplitudes (79)	20 (25.3%)
Slowing of the MCV (79)	4 (5%)
Low SNAP amplitudes (79)	9 (11.4%)
Slowing of the SCV (79)	3 (3.8%)
EMG Findings (tot=62)	
Pathological insertional activity	2 (3.2%)
Fibrillation potentials (62)	18 (29%)
Positive sharp waves (PSW)	5 (8%)
MUAP's amplitude (62)	
Reduced amplitude	13 (21%)
Increased amplitude	13 (21%)
MUAP's duration (62)	
Short duration	3 (4.8%)
Long duration	24 (38.7%)
Increased phases (62)	42 (67.7%)
Recruitment pattern (59)	
Reduced recruitment	24 (40.7%)
Rapid recruitment	3 (5.1%)
Turns (39)	
Increased T/A	10 (25.6%)
Decreased T/A	12 (30.8%)

Table 22: Specific NCS and needle EMG findings and their frequency. T/A: Turns/Amplitude Analysis.

The presence of specific needle EMG findings was assessed relative to the final clinical diagnosis (Table 23). The following should be highlighted.

Fibrillations potentials are most frequent in muscular dystrophies.

Moreover, inflammatory myopathies are those in which the EMG is most altered in a myopathic sense: amplitude of the MUPs is altered in 5 out of 12, never increased; polyphasia occurs in 10 out of 12, in 5 out of 7 patients the T/A is decreased; the duration of the MUAP's does not appear to be significantly different. Finally, all patients with neurogenic disorders who performed EMG showed increased T/A.

Patients with isolated hyperCKemia showed few alterations at EMG.

Final diagnosis (N/total)	Low CMAP amplitude	Fibs	Reduce amplitude MUAP	Increas eamplitude MUAP	Short duration MUAP	Long duration MUAP	Increase phases	Reduce rectruitment	Rapid recruitment	Increased T/A	Decreased T/A
Congenital myopathies (19/19)	4/17 (23.5%)	2 (10.5%)	3 (15.8%)	3 (15.8%)	1 (5.3%)	6 (31.6%)	10 (52.6%)	3 (15.8%)	1/17 (5.9%)	0	2/9 (22.2%)
Congenital muscular dystrophies (8/9)	3/9 (33.3%)	6 (75%)	2 (25%)	2 (25%)	1 (12.5%)	1 (12.5%)	6 (75%)	3 (37.5%)	1 (12.5%)	1 (12.5%)	2/7 (28.6%)
Inflammatory myopathies (12/13)	2 (16.7%)	2 (16.7%)	5 (41.7%)	0	1 (8.3%)	2 (16.7%)	10 (83.3%)	5/10 (50%)	1/10 (10%)	0	5/7 (71.4%)
HyperCKemia (2/5)	0	0	0	0	0	0	1 (50%)	0	0	0	0
Peripheral neuropathy (1/4)	2 /4 (50%)	0	0	1 (100%)	0	1 (100%)	1 (100%)	1 (100%)	0	1 (100%)	0
SMA (3/4)	2/4 (50%)	0	0	3 (100%)	0	3 (100%)	3 (100%)	1 (100%)	0	2/2 (100%)	0
Metabolic disorders (5/10)	4/10 (40%)	1 (20%)	0	2 (40%)	0	4 (80%)	4 (80%)	3/3 (100%)	0	4/4 (100%)	0
Others (12/18)	3/18 (16.7%)	7 (58.3%)	3 (25%)	2 (16.6%)	0	7 (58.3%)	7 (58.3%)	8/11 (72.2%)	0	2 (16.6%)	3 (25%)
Total	**20**	**18**	**13**	**13**	**3**	**24**	**42**	**24**	**3**	**10**	**12**

Table 23: Frequency of specific needle EMG findings by etiology. The number of patients who performed EMGs compared to the number of patients in total are reported in the first column In the case of missing data, the number of total examinations is given directly in the table. Fibs: fibrillations. T/A: Turns/Amplitude Analysis.

MUSCLE BIOPSY FINDINGS:

Regarding histopathological features, the majority of the sample presented increased variability of fibre size (82.9%), presence of hypo/atrophic fibres (76.8%), followed by fibrosis (62.2%) and COX deficiency (62.2%).

All muscle biopsy findings are described in Table 24.

Muscle biopsy findings	N (%)
Hypo/atrophic fibres	63 (76.8%)
Increased variability fibre size	68 (82.9%)
Fibre type grouping	24 (29.3%)
Fibrosis	51 (62.2%)
Necrotic fibres	15 (18.3%)
Fibre type predominance	
Type 1	22 (26.8%)
Type 2	6 (7.3%)
COX deficient fibres	51 (62.2%)

Table 24: Histopathological findings and their frequency. COX: Cytocrome C oxidase.

The presence of specific needle histopathological findings was assessed relative to the final clinical diagnosis (Table 25).

Among the neurogenic disorders, increased variability of fibre size was present in 100% of the sample and fibrosis in 87.5%. Fibre type grouping was present in 75%, with 100% of patients with motor neuron disease. Predominance type 2 fibres was only present in patients with neuropathy (50%); finally, 62.5% of patients had COX deficiency at muscle biopsy.

With regard to children with myopathic disease, again the most frequent histopathological alteration was an increased variability fibre size; this was followed by the presence of hypo/atrophic fibres (89.5% in congenital myopathies, 77.8% in congenital muscular dystrophies and 69% in inflammatory myopathies). Fibrosis and necrosis were more frequent in congenital muscular dystrophies (100% and 66.7%) and in inflammatory myopathies (84.6% and 69.2% respectively).

COX deficiency was most frequent in patients with inflammatory myopathies (84.5%) and in patients with metabolic disorders (90%). They also frequently presented increased variability of fibre size (90%) and hypo/atrophic fibres (80%).

Finally, decreased CMAP amplitude at the NCS was present in 23.5% of patients with congenital myopathy, 33.3% of patients with congenital muscular dystrophy, and 16.7% of patients with inflammatory myopathy. Of our 3 patients with merosine dystrophy, one had demyelinating peripheral neuropathy, one had an axonal form, one patient had normal NCS (33%). Of our two patients with nemaline myopathy, one had decreased CMAP amplitude.

	Hypo/ atrophic fibres	Increased variability fibre size	Fibre type grouping	Fibro Sis	Necrotic fibres	Predomina nce 1	Predomi nance 2	COX deficient fibres
Congenital myopathies (19/19)	17 (89.5%)	16 (84.2%)	3 (15.8%)	13 (68.4%)	0	11 (57.9%)	1 (5.3%)	11 (57.9%)
Congenital muscular dystrophies (9/9)	7 (77.8%)	8 (88.9%)	3 (33.3%)	9 (100%)	6 (66.7%)	2 (22.2%)	1 (11.1%)	3 (33.3%)
Inflammatory myopathies (13/13)	9 (69.2%)	11 (84.6%)	5 (38.5%)	11 (84.6%)	9 (69.2%)	4 (30.8%)	0	11 (84.6%)
HyperCKemia (5/5)	4 (80%)	4 (80%)	0	1 (20%)	0	1 (20%)	1 (20%)	1 (20%)
Peripheral neuropathy (4/4)	3 (75%)	4 (100%)	2 (50%)	4 (100%)	0	0	2 (50%)	2 (50%)
SMA (4/4)	3 (75%)	4 (100%)	4 (100%)	3 (75%)	0	0	0	3 (75%)
Metabolic disorders (10/10)	8 (80%)	9 (90%)	4 (40%)	5 (50%)	0	3 (30%)	0	9 (90%)
Others (18/18)	11 (61.1%)	12 (66.7%)	3 (16.7%)	5 (27.8%)	0	1 (5.6%)	1 (5.6%)	11 (61.1%)
Total (82/82)	**62**	**68**	**24**	**51**	**15**	**22**	**6**	**51**

Table 25: *Frequency of specific muscle biopsy findings by etiology. COX: Cytochrome c oxidase*

CORRELATION BETWEEN EMG AND HISTOPATHOLOGICAL FINDINGS

The percentages of patients with specific EMG findings compared with histopathological findings are described in the table 26. The following should be highlighted.

No patients who showed fibre type grouping on muscle biopsy had reduced MUAP duration; among patients with fiber type grouping, patients with increased MUAP amplitude were more than twice as many as those with reduced MUAP amplitude. Necrosis was more frequent in patients with reduced MUP amplitude than in those with increased MUAP amplitude, as well as being more frequent in patients with decreased T/A and absent in those with increased T/A.

Findings	Hypo/atrophic fibres	Increased variability fibre size	Fibre type grouping	Fibrosis	Necrotic fibres	COX deficient fibres
Fibrillation potentials (18/62)	14 22.6%	14 22.6%	5 8.1%	13 21%	5 8.1%	8 12.9%
MUAP's amplitude (62)						
Reduced amplitude (13)	9 14.5%	12 19.4%	4 6.5%	12 19.4%	6 9.7%	7 11.3%
Increased amplitude (13)	10 16.1%	12 19.4%	9 14.5%	10 16.1%	1 1.6%	6 9.7%
MUAP's duration (62)						
Short duration (3/62)	3 4.8%	3 4.8%	0	3 4.8%	2 3.2%	1 1.6%
Long duration (24)	19 30.6%	22 35.5%	10 16.1%	16 25.8%	1 1.6%	14 22.6%
Increased phases (42/62)	33 53.2%	36 58.1%	16 25.8%	31 50%	11 17.7%	23 37.1%
Recruitment pattern (59)						
Reduced recruitment (24)	20 33.9%	19 32.2%	9 15.2%	15 25.4%	4 6.7%	14 23.7%
Rapid rectruitment (3)	3 5.1%	3 5.1%	1 1.7%	3 5.1%	2 3.4%	2 3.4%
Turns (39)						
Increased T/A (10)	7 17.9%	8 20.5%	6 15.4%	6 15.4%	0	6 15.4%
Decreased T/A (12)	7 17.9%	10 25.6%	4 10.3%	10 25.6%	6 15.4%	7 17.9%
Reduced CMAP amplitude (79)	18 22.8%	17 21.5	9 11.4%	14 17.7%	2 2.5%	16 20.5%

Table 26: Patients with specific EMG findings compared with histopathological findings. MUAP: motor unit action potential. COX: Cytochrome c oxidase. T/A: Turns/Amplitude Analysis.

There was a correlation between increased amplitude and presence of fibre type grouping on muscle biopsy (p =0.002) and between reduced amplitude on EMG and the presence of fibrosis (p =0 0.045) and necrosis (p=0.021) on muscle biopsy.

A statistically significant correlation was noted between long duration of MUAPs and necrosis (p = 0.011).

Finally, a reduction of T/A at EMG correlated statistically with the presence of necrosis on muscle biopsy (p = 0.014).

The correlations between needle EMG results and histopathological findings are reported in Table 27. Pathological insertional activity and positive sharp waves (PSW) on EMG were not evaluated because there were too few cases such as Fibre type predominance for muscle biopsy.

We did not find any correlations between the presence of fibrillation and alterations on muscle biopsy; a statistically non-significant correlation (trend) was noted between fibrillation and cytochrome C oxidase (COX) deficient fibres (p = 0.094).

A statistically non-significant correlation (trend) was also noted between increased phases and fibre type grouping (p = 0.08) and fibrosis (p = 0.87) and between the presence of Increased T/A at EMG and the presence of fibre type grouping and necrosis at biopsy (p= 0.056). Finally a statistically non-significant correlation (trend) was noted between reduced CMAP amplitude of nerve conduction study of inferior legs and cytochrome C oxidase (COX) deficient fibres (p = 0.066).

No correlation was also found between muscle biopsy parameters and the presence of early recruitment or reduced duration of MUAP at EMG.

	Hypo/ atrophic fibres	Increased variability fibre size	Fibre type grouping	Fibrosis	Necrotic fibres	COX deficient fibres
Fibrillation potentials						0.094
Increased amplitude			0.002			
Reduced amplitude				0.045	0.021	
Long duration					0.011	
Short duration						
Increased phases			0.082	0.087		
Rapid rectruitment						
Reduced recruitment						
Reduced T/A					0.014	
Increased T/A			0.056		0.079	
Reduced CMAP amplitude (NCS)						0.066

Table 27: Neurophysiological vs histopathologic findings; statistically significant correlations are marked with a star. COX: Cytochrome c oxidase. T/A: Turns/Amplitude Analysis. CMAP: motor action potential.

We also assessed the sensitivity and specificity of neurophysiological findings in identifying histopathologic findings (Table 28).

Increased phases at EMG had a high sensitivity for pathological changes of fibre atrophy (72%), increased variability fibre size (71%), fibre type grouping (84)%, fibrosis (76%), and necrosis 85%. The other parameters had low or moderate sensitivity.

Nevertheless, the EMG parameters had moderate or high specificity. More particularly, the presence of fibrillation potentials was specific for atrophy (75%), fibrosis (76%), necrosis (73%). The presence of reduced amplitude of MUAPs was specific for atrophy (75%), increased fibre size variability (91%), fibrosis (95%), necrosis (86%). The presence of increased amplitude of MUAPs was specific for atrophy (81%), increased fibre size variability (91%), fibre type grouping (91%), fibrosis (86%), necrosis (75%).

Moreover, short MUAPs duration was high specific for increased fibre size variability (100%), fibre type grouping (93%), fibrosis (100%), necrosis (98%) and the presence of COX deficient fibres (92%). Long duration of MUAPs instead was high specific only for increased fibre size variability (82%) and the presence of COX deficient fibres (71%).

Similar to this, rapid recruitment was high specific for presence of hypo/atrophic fibres (100%), increased fibre size variability (100%), fibre type grouping (94%), fibrosis (100%), necrosis (98%) and the presence of COX deficient fibres (95%).

Reduced T/A had high specificity for the presence of fibrosis (85%), necrosis (80%) and increased variability fibre size (71%), while increased T/A for fibre type grouping (85%), hypo/atrophic fibres (77%), increased variability fibre size (71%) and for the presence of COX deficient fibres (73%).

Finally, reduced CMAP amplitude on NCS had high specificity for the presence of hypo/atrophic fibres (89%), COX deficient fibres (87%), fibrosis (81%), fibre type grouping (80%), necrosis (72%) and increased variability fibre size (79%).

	Hypo/atrophic fibres		Increased variability fibre size		Fibre type grouping		Fibrosis		Necrotic fibres		COX deficient fibres	
	Se %	Sp %	Se %	Sp %	Se%	Sp %	Se %	Sp %	Se %	Sp %	Se %	Sp %
Fibrillation potentials	30	75	27	64	26	70	32	76	38	73	21	58
Reduced amplitude	20	75	23	91	21	21	29	95	46	86	67	47
Increased amplitude	22	81	23	91	47	91	24	86	8	75	16	71
Short duration	41	69	59	100	0	93	7	100	15	98	3	92
Long duration	48	69	43	82	53	67	39	62	8	52	16	71
Increased phases	72	44	71	45	84	39	76	48	85	37	60	21
Rapid rectruitment	7	100	7	100	6	94	8	100	18	98	6	95
Reduced recruitment	59	69	44	50	53	58	42	47	36	52	42	50
Reduced T/A	27	61	31	71	31	69	38	85	67	80	29	67
Increased T/A	27	77	31	71	46	85	23	69	0	67	25	73
Reduced CMAP amplitude (NCS)	30	89	26	79	37	80	29	81	14	72	33	87

Table 28: sensitivity and specificity of neurophysiological findings in identifying histopathologic findings.

DISCUSSION

Rationale:

Electrodiagnosis and histopathological investigations are an essential part of the evaluation of patients with suspected neuromuscular disorder.

In particular, neurophysiological tests have a role to evaluate the involvement of the neuromuscular system, identifying an ongoing myopathy, differentiating a neuropathy from a myopathy (Naddaf et al, 2018), and help to recognize both as the main manifestation of the disease, and as a clinical sign of systemic pathologies involving other organs and systems.

This applies to both acquired and genetically determined forms; indeed, although imaging and genetic testing are increasingly gaining importance (Arnold et al, 2012) (Kassardjian et al, 2016), (Fischer et al, 2016), (Hafner et al, 2019), needle electromyography and muscle biopsy retain an important role in the evaluation of patients with suspected NMD, especially for patients with more heterogeneous phenotype, for which recognizing the early and subtle changes is essential (Hafner et al, 2019).

Furthermore electrodiagnostic tests assist in the detection of a pattern of abnormalities that can point to a specific myopathy and to select the appropriate muscle for biopsy. Finally, they have a role not only for the initial diagnosis but also for evaluating the clinical course and response or side effects to pharmacological therapies. This response does not only concern acquired forms but also genetic forms, for which new therapies are being developed, such as for spinal muscular atrophy (Finkel et, 2016) (Finkel et, 2021), (Day et al, 2022), metabolic diseases, other genetic disorders (Mendell et al, 2021) (Nitschke et al, 2018) (Sevin C, Deiva K., 2021) and Duchenne muscular dystrophy (Elangkovan et al, 2021).

Neurophysiological evaluation offers recognized advantages: low cost, execution to bedside, possibility of execution from day one of patient's life and monitoring over time. Furthermore, they do not require sedation and are the only system for functional evaluation.

In children, however, these techniques are often avoided (Chang et al, 2011), (David et al, 1994). The reason is because on one hand they are considered invasive and on the other because they are considered by the neurophysiologists themselves to be difficult to perform due to poor compliance and even more difficult to interpret (Pitt et al, 2012). Indeed, in addition to an immaturity of the nervous system and technical errors due to the small size of the subjects (Chang et al, 2011), the same diseases are different in different age groups and occur in a different way depending on age, particularly in younger children (under two years of

age) (Pitt, 2012), and accurate normative data is very difficult to find in the paediatric age group.

Finally, at times, the diagnosis ultimately requires a muscle biopsy regardless of the EDX study results (Paganoni et al, 2013).

Muscle biopsy has historically been considered to be the gold standard in the diagnosis of muscle disease (Hafner et al, 2019) allowing for the precise characterisation through histological, biochemical, immunocytochemical and ultrastructural analyses (Joyce et al, 2012; Thavorntanaburt et al, 2018).

Muscle biopsy is still central for the definite diagnosis of inherited neuromuscular disorders to target genetic analysis and for acquired conditions affecting the muscle, such as inflammatory myopathies. Regardless the NMD condition, muscle biopsy can also provide information on the severity of the underlying disease and the extent of muscle damage and/or fibrotic substitution.

In NMD with a neurogenic or NMJ pathogenesis the use of muscle biopsy is limited albeit typical lesions appear on the histopathological studies (such as type grouping or neuromuscular junction alterations).

On the other hand, congenital myopathies, caused by genetically determined defects in structural proteins of the muscle are classified on the basis of muscle biopsy findings (Cassandrini et al, 2017; North et al, 2014).

However, muscle biopsy is an expensive, invasive, time-consuming, and resource-dependent procedure: the clinician needs to plan ahead, determine how much tissue will be needed for processing, and then, supervise the collection and delivery of the tissues to prevent artifact and ensure high quality results (Joyce et al, 2012). A successful muscle biopsy requires optimal cryo-processing of the fresh specimen in order to preserve viable macromolecules for enzyme histochemistry and metabolic assays (Joyce et al, 2012). Furthermore, pathological characterisation of disease presents a number of challenges, including overlapping morphological signatures between different diseases, the absence of specific pathological features, and the small sample size available for evaluation (Hafner 2019), whereby tissue biopsies can't determine which muscles are affected (Chang et al, 2011). Finally, the need for general anaesthesia in children also increases the risks associated with this procedure (Thavorntanaburt et al, 2018).

In this retrospective study the first aim was to ascertain normal values for paediatric NCS and EMG. The second aim was to describe the diagnostic accuracy of electrodiagnostic tests (NCSs and EMG) and muscle biopsy in children with suspected neuromuscular disorders, to compare sensitivity, specificity, positive and negative predictive value of the two tests and demonstrate in a systematic way if correlations exist between electrophysiological parameters and histopathologic findings in the same paediatric population.

Paediatric normative values

With regard to the first aim, it is important to address some methodological issues as they could represent a limitation.

First of all, regarding the electroneurographic study, our sample size of 715 paediatric electroneurographic studies in the age range 0-3 years, represents the largest paediatric study we are aware of to date in this age range.

Nor are there any studies in the literature that stratify the sample with such narrow intervals. In particular, there are no studies that divide the sample into weeks in the first month of life, in the interval of one month up to 3 months of life, in the interval of 3 months up to 12 months, in a range from 6 months up to 24 months and then in a range of 12 months. For this reason, the data collected in our study, although in part comparable to those currently present in the literature, may partly depart from it.

As regards the electromyographic study, we evaluated the semiquantitative and quantitative data in a range of ages from 0 to 78 months of life.

In the literature there are also no studies that stratify the sample with such narrow ranges, in particular there are no studies that divide the sample in the range of one month up to 3 months, in the range of three months up to twelve months, in a range of 6 months up to 24 months and then in a range of 12 months; in the literature there are normative values divided into ranges of at least 1-2 years for the paediatric age.

Furthermore, in the literature there are currently no studies reporting the normative values of Quantitative EMG in children, so the Turn/sec and Turn/Amplitude values reported in this study represent a novelty in the international neurophysiological field.

Another methodological element worthy of consideration is related to technical factors.

Indeed, the differences between our data and those of previous studies may be due to sample sizes or patient populations, but may also be related to technique. It is widely known that testing for NCS is heavily reliant on proper techniques, and this is a problem in determining normative data.

Information is available on the different factors influencing measurements in nerve conduction studies. The influence of the distance between the recording electrodes, the distance between the stimulating electrode and the recording electrode, temperature, skin impedance, height, handedness are just a few examples (Hlavova et al., 1970; Bolton and Carter, 1980; Winkler et al., 1991; Stetson et al., 1992; Rivner et al, 2001; Hasanzadeh et al, 2008; Werner et al., 2012; Morris, 2013; Cinar et al., 2013; Sajadi et al., 2014). Electrode size is also known to influence the amplitude of the responses and there is an inverse correlation between the surface area and the amplitude of the evoked response (Ven et al., 2004).

Technical factors and technique are rigorously controlled in our EMG laboratory. Our lab has clear protocols regarding electrode set-up, distance measurements, avoidance of overstimulation of adjacent nerves, minimum acceptable limb temperature, low- and high-frequency filter settings, and managing local skin factors for every NCS we perform.

The examinations performed in intensive care or in an operating room were not included in this study.

Temperature is an essential parameter to note when performing NCS, as low temperatures can slow conduction velocity and increase amplitude (Ryan et al, 2019). Although a strict protocol was imposed in our Institution, temperature regulation may not have been as rigorous and could potentially affect the range of normal values. This issue has been previously addressed in the Methods section.

Regarding the use of electrodes in children, this was addressed using electrodes with a surface equal to half of the standard ones in children under the age of two.

Therefore, in order to obtain such standard values we investigated evolution of both motor and sensory nerves in children using a non-invasive technique, i.e. using surface electrodes.

Some previous studies used needle electrodes or a combination of needle and surface electrodes (Garcia et al, 2000), but now is clear that surface electrodes are more comfortable and offer reliable results. Furthermore, using surface recording electrodes, CMAP morphology and amplitude are less variable than with needle electrodes (Falck and Stalberg, 1995). However, despite SNAPs obtained using surface electrodes being of lower amplitudes, this allows potentials to be obtained with less variability in serial studies (Wilbourn, 1994).

Another factor to be considered in the electroneurographic study is that due to the age of the sample the limbs have not grown completely. As is our laboratory practice, anatomical landmarks were used to determine the proper site of nerve stimulation.
For this reason, the distance from stimulation site to recording site is not uniform, and thus the distal latency is less standardized and comparable; this could be a limit for our data. For this reason, we think conduction velocity is more standardized and comparable as it is determined using the measured distance between two sites of stimulation (Ryan et al, 2019).
Finally, the methodological rigor of our study is also supported by the fact that these children were followed by trained neurophysiologists.

In addition to the aforementioned technical considerations, it is important to consider that in this paper we used a relatively recent statistical method, called E-norms (see Methods).
In fact, normal ranges of many biomedical markers are unavailable for some subpopulations such as infants and children for a variety of reasons. First of all, it is not possible to study normal children with a procedure which is perceived as being uncomfortable. Secondly, many of the normative data, usually obtained in adults, requires that there be standard conditions for the measurement of both nerve conduction and EMG parameters trying to limit the effect of all the known factors that can influence the results. Finally, under two years of age there are very significant changes in the range of normal data for most measurements made during paediatric EMG and VCS. For example, a particular problem occurs in EMG as the muscle fibre diameter being a much smaller in neonates and infants than in their older siblings, so the normal interference pattern can appear myopathic (Pitt, 2012).
The E-norms technique exploits another property of variables derived from normal individuals that distinguishes them from variables derived from patients with pathology, a property called ‘‘normal clustering” (Jabre, 2018), that is a range with low first-order difference.
So, this method offers many advantages, one of the most compelling arguments for it is the fact that it would be a true reflection of the patient population in the practitioner’s own referral pool. An additional advantage is the speed of this technique.
This statistical analysis has already been used in the neurophysiological field, especially in the paediatric field, to find jitter reference values (Jabre and Pitt, 2017), and for paediatric NCS normal values (Jabre et al, 2020).
Possible limitations of this technique are related to the sample size. In most instances, good results have been obtained when the number of data points is equal or above100 (Jabre et al,

2015). Recently, the E-norms method was used to calculate Jitter MCD e-norms for a dataset consisting of only 38 stimulated single-fiber EMG, and it was found that e-norms data were close to the MCD e-norms derived from a much larger dataset (Pitt et al, 2017).

NCS correlates

Moving on to analyse the characteristics of the sample, our sample size of 715 paediatric electroneurographic studies in the age range 0-3 years represents the largest paediatric study we are aware of to date and provides robust data for determining normal values for paediatric NCS. Overall, our findings are largely comparable to those of previous studies but with some notable differences and findings.

As already reported in the literature, in our sample it was also found that in the neonatal group MCV and SCV mean values are about one-half of those of normal young adults, and that most rapid increase occurs during the first year of life (Thomas and Lambert, 1960; Gamstorp, 1963; Baer and Johnson, 1965; Gamstorp and Shelburne, 1965; Wagner and Buchthal, 1972; Cruz Martinez et al., 1977, 1978; Vecchierini-Blineau and Guiheneuc, 1984; Miller and Kuntz, 1986; Duron and Khater-Boidin, 1992; Parano et al., 1993; Kwast, 1995; Cai and Zhang, 1997; Garcia et al 2000). These features are consistent with histological changes due to conduction velocity in myelinated fibres (Waxman, 1980).
In our sample, adult values for both MCV and SCV also seem to be reached during the first 5 years.
In previous studies it was found that adult MCV values were reached earlier in the lower limb nerves, probably due to the different motor milestones between legs and arms (Gamstorp, 1963; Cruz Martinez et al., 1978a; Cai et al, 1997).
In our sample, however, we did not find a difference in MCV of the lower limbs compared to the upper limbs, and the p-value was not significant. This could be due to the different methodology used for statistical analysis, as the E-Norms could cancel some disparities found in the past. However, it cannot be excluded that the achievement of adult values in our sample, especially for the median nerve, is not linear.

Regarding amplitude, it was also found in our sample that amplitudes of the motor and sensory responses show a less linear pattern. This may be due to higher dispersion for

different myelinic fiber maturation at various gestational ages. This result was also reported in the study by Jabre (Jabre et al, 2020) where the same statistical method was used.
As in Jabre et al, in our sample we also found a difference in the mean values of the tibial nerve amplitudes with respect to the peroneal nerve, with a greater amplitude for the tibial nerve. A difference in maturation in the amplitude of the tibial nerve with respect to the peroneal nerve was also found, as already reported in the literature: the amplitude of AH being the first to reach adult values (Cai and Zhang, 1997) (Garcia et al, 2000).
Even in our sample the adult amplitude values seem to be reached around 24 months, in particular for sensory nerve (median nerve and medial plantar nerve), as already described (Gamstorp and Shelburne, 1965; Cruz Martinez et al., 1978b; Garcia et al, 2000) earlier than observed for most CMAP.

However, we found some differences in the average values of amplitude and conduction velocity, both for motor nerves and sensory nerves.
We note first of all that compared to previous studies (Ryan et al, 2019), the mean conduction velocity of the peroneal nerve studied in the first period of life is lower in our sample, while it is similar to 3 years of age. This is because the two initial values are not completely superimposable; in fact in the previous study the patients are grouped from 0 to 1 month of life, while in our study they are subdivided in the first month of life in subgroups every 7 days, therefore the first period of life studied is between 0-7 days.
Even compared to Parano et al (1993), the initial value of conduction velocity is not completely comparable; in fact also in this study the average of the conduction velocities from 7 to 28 days of life is considered, therefore resulting slightly higher than the average of our series.
It cannot be compared with Jabre et al (2020), because MCV of the peroneal nerve is not reported.
Also with regard to the mean values of the peroneal nerve amplitude, they seem slightly lower than those in the literature, in particular they are lower than the values reported by Parano et al, while they closely approximate those reported by Ryan and Jabre (Ryan et al, 2019) (Jabre et al, 2020).
The same results are obtained with regard to the amplitude values and the conduction velocities of the other motor nerves studied (median nerve and posterior tibial nerve); for example, the amplitude of the tibial nerve in our sample is slightly higher than that reported by Jabre (Jabre et al, 2020).

As for the sensory nerves, the average values of the conduction velocities of the median nerve appear to be superimposable to those reported by Parano et al, slightly lower than those reported by Ryan et al; the mean values of the median sensory nerve amplitudes of our sample are lower than those already present in the literature (Parano et al, 1993) (Ryan et al,2019) (Duron et al, 1992)(Garcia et al 2000).
Data on the medial plantar nerve and sural nerve are very similar to the normative values reported by Jabre et al (2020). The SCV and the sural nerve amplitude are superimposable in the two studies. The SCV of the medial plantar nerve is lower in our sample while the amplitudes are similar. The difficulty in finding the sural in the baby of a few months is known, so if it is not found it should not be considered pathological and the medial plantar should be chosen as an AAII sensory nerve. Our sample confirms this fact perhaps also due to the fact that being expert centres the medial plantar is favoured in the first months and the sural one after (therefore not homogeneous sample).

These differences found could be partly explained by the difference in stratification of our sample compared to previous studies; in particular, unfortunately, in literature there are no studies describing values of amplitude and conduction velocity divided into ranges of one week in the first month of life and divided into monthly ranges up to 6 months of age. This is therefore also one of the strengths of our study.
In addition to this, the previous studies were based on a retrospective evaluation of the sample, so that in retrospect all patients with electroneurographic values or with dubious symptoms were excluded; with the exception of Jabre et al, (Jabre et al, 2020) where the same statistical methodology was used as ours, data was collected only by normal patients. So often the sample was reduced, and in this way it is possible that all the values set at the normal limits have been eliminated from the analysis. This would explain why both the conduction velocity and the amplitude the data of our study have a slightly lower average than that already reported in the studies present in the literature.
All this underlines the importance of having not only international paediatric normative data to compare, but also the fact that every Neurophysiology laboratory should have its own normative data.

Electromyographic correlates

Semiquantitative EMG:

Also for the electromyographic study, as mentioned above, our study is the first to divide the sample into relatively narrow ranges: range of one month up to three months of age, range of three months up to twelve months of age, range of six months up to two years of age, range of one year up to six years of age. There are no studies in the literature with such a stratified paediatric sample; in the literature normative values are reported divided into a range of one to two years (Buchthal et al, 1954) (Sacco et al, 1962) (Scandinavian University Group, 1975).
For this reason, the data obtained are not completely comparable with those already present in the literature.
It is however possible to how the MUPs amplitude and duration of our sample change.
The technique of MUAP quantification was pioneered by Buchthal and coworkers beginning in the 1950s. Those investigators measured the amplitude between the maximum negative to maximum positive peaks, i.e., peak-peak. The MUAP onset occurs when it first deviates from the baseline; MUAP terminates when the potential returns to the baseline and remains at that level until the next MUAP discharge. The time interval between these events is the total MUAP duration; the mean duration was computed after excluding the polyphasic MUAPs.
Therefore, normative values for the main characteristics of the MUP have been published.

Regarding MUAP amplitude, in our sample the mean value of MUPs amplitude reported in other studies for adults (see Figure 49 -50), are reached around 12 months of life.

Table 9.3 Reference values for individual and mean values of MUAP features are tabulated. These investigators did not find age-dependent variation in normal subjects until the sixth decade of age

	Amplitude (μV)			Duration (ms)			Thickness		
Muscle	Mean ± SD	Minimum	Maximum	Mean ± SD	Minimum	Maximum	Mean ± SD	Minimum	Maximum
Deltoid	550 ± 110	162	1,531	10.4 ± 1.3	4.2	18.4	1.56 ± 0.22	0.65	2.94
Biceps	436 ± 115	1,788	1,414	9.9 ± 1.4	4.2	16.4	1.46 ± 0.2	0.56	2.09
First dorsal interos	752 ± 247	188	2,301	9.4 ± 1.3	4.0	18.0	1.38 ± 0.22	0.49	2.61
Vastus lat	687 ± 239	172	1,954	11.7 ± 1.9	4.6	21.6	1.72 ± 0.23	0.60	3.11
Anterior tibialis	666 ± 254	194	1,572	11.4 ± 1.2	4.6	18.4	1.67 ± 0.23	0.58	2.81

Data from Bischoff et al. [17]

Figure 49 (from Nandedkar S.D and Barkhaus P.E)

Table 9.2 MUAP amplitude (μV) measurements in muscles of normal adult subjects are summarized

Muscle	Mean	SD	Range
Deltoid	212	147	150–304
Triceps	340		
Biceps	180		120–390
Ext dig comm	210	115	
Abd dig quinti	350		
Abd poll brev	260		
Vast med	230		150–360
Vast lat	260		210–370
Rect fem	170		130–215
Gastrocnemius	160	95	
Tib ant	220		
Ext dig brev	460		

Data from Buchthal [30]

Figure 50 (from Nandedkar S.D and Barkhaus P.E).

The MUPs amplitude in our sample is fluctuating. This can be partly due to the characteristics of the sample, and to the characteristics of this parameter. Indeed, a slight difference due to maturation of motor unit is well definable; therefore, it is indeed known that the same motor unit can give rise to many different profiles depending on the position of the needle tip; the amplitude normally varies from several hundred microvolts to few millivolts with the use of a concentric needle (King et al, 1996).

The MUPs duration parameter is much more reliable and is of fundamental importance in the electromyographic evaluation to discriminate between neurogenic and myogenic forms fundamental in the differentiation of neurogenic and myogenic forms.
In previous studies (Sacco et al, 1962), has been found a significant increase in the duration of motor unit potentials at 20 years of age, as compared with at 3 months of age.
In our sample, there is a linear increase in duration starting from the first months of life reaching the values of adult by 18 months (comparison with the data of the adult, from Howard et al, 1988 and from Nandedkar et al, 1991- Figure 51); the mean value of MUPs duration at 18 months in our sample is in fact 12.28 ms.

Table 9.1 Mean values of MUAP duration (ms) in normal muscles are tabulated

Age (years)	Deltoid	Triceps	Biceps	Ext dig comm	Abd dig quinti	Abd poll brev	Vast med	Vast lat	Rect fem	Gastroc	Tib ant	EDB
0	7.8	9.0	7.7	7.1	6.2	6.2	7.9	9.7	8.7	7.2	9.5	7.2
3	8.3	9.6	8.2	7.6	6.8	6.8	8.4	10.3	9.2	7.7	10.1	7.7
5	8.6	9.9	8.5	7.8	7.3	7.3	8.7	10.7	9.6	8.0	10.5	8.0
8	9.0	10.3	8.9	8.2	7.9	7.9	9.1	11.2	10.0	8.4	11.0	8.4
10	9.3	10.6	9.1	8.4	8.3	8.3	9.3	11.5	10.3	8.6	11.2	8.6
13	9.6	11.0	9.4	8.7	8.7	8.7	9.6	11.8	10.6	8.8	11.6	8.8
15	9.8	11.2	9.6	8.8	9.0	9.0	9.8	12.1	10.7	8.9	11.7	8.9
18	10.0	11.4	9.8	9.0	9.2	9.2	10.0	12.3	11.1	9.2	12.1	9.2
20	10.2	11.6	10.0	9.2	9.2	9.2	10.2	12.6	11.3	9.4	12.3	9.4
25	10.5	11.9	10.3	9.5	9.2	9.2	10.5	13.0	11.6	9.7	12.7	9.7
30	10.7	12.0	10.6	9.8	9.3	9.3	10.8	13.4	12.0	10.0	13.1	10.0
35	11.1	12.1	10.9	10.0	9.3	9.3	11.1	13.7	12.3	10.2	13.4	10.2
40	11.3	12.2	11.1	10.2	9.3	9.3	11.3	14.0	12.6	10.4	13.6	10.4
45	11.4	12.3	11.2	10.3	9.4	9.4	11.4	14.1	12.7	10.5	13.8	10.5
50	11.6	12.4	11.4	10.5	9.4	9.4	11.6	14.4	12.9	10.7	14.0	10.7
55	11.8	12.5	11.6	10.7	9.4	9.4	11.8	14.6	13.1	10.9	14.3	10.9
60	12.1	12.6	11.9	11.0	9.5	9.5	12.1	15.0	13.5	11.2	14.7	11.2
65	12.1	12.7	12.2	11.2	9.5	9.5	12.4	15.4	13.7	11.5	15.0	11.5
70	12.4	12.8	12.4	11.4	9.5	9.5	12.6	15.6	14.0	11.7	15.3	11.7
75	12.8	12.8	12.6	11.6	9.5	9.5	12.8	15.9	14.2	11.8	15.5	11.8
80	13.0	12.8	12.8	11.8	9.5	9.5	13.0	16.1	14.4	12.0	15.7	12.0

Data from Buchthal [30]
Note the increase in duration with age and identical values for different muscles

Figure 51 (from Nandedkar S.D and Barkhaus P.E)

As is known, the duration of the MUP is a diagnostic index to differentiate between myogenic and neurogenic forms. Buchthal and coworkers defined reference values for the mean amplitude, mean duration, and percentage of polyphasic MUAPs in normal subjects (Buchthal et al, 1991). He considered abnormal durations that differ by more than 20% from the normal mean. Many investigators have used a similar approach and described upper and lower normal limits of amplitude and duration (Dorfman et al, 1989) (Stewart et al, 1989).

The debate is still open in the literature, on the range with which a diagnosis can be made, neither in adulthood nor even in the paediatric age.

In conclusion, in our sample we found that from birth to six years old MUAP amplitude and duration showed increase with age reaching the values of adult (from Howard et al, 1988) by 9-12 months and 18 months, respectively.

Quantitative EMG:

A technique for manual turns–amplitude analysis was introduced by Willison (1964) in the 1960s and a few years later this was automised (Fitch, 1967).

In the manual method, a 500 ms EMG epoch is “frozen” on the display for visual assessment. A waveform occurring in a recurrent fashion is a MUAP. The MUAP waveform is quantified manually. As computers and software have become more powerful, many have developed

algorithms to decompose the EMG signal into the discharges of its constituent MUAPs; algorithms have been generated to make automated measurements of the MUAP.

Since several MUs are simultaneously acquired and analyzed, the method may also be called the "multi-motor unit analysis (MMA)" or "decomposition". Because of minimal analysis time and a yield of one to four different MUAPs from each tested site, it is possible to analyze 20 MUAPs within only a few minutes, including muscles that are considered difficult to study. (Nandedkar et al, 2014).

Hence the development of the QEMG, that can measure many different features of the EMG signal, e.g., amplitude, duration, and phases.

QEMG includes numerous parameters, of which the most used are: quantitative analysis of MUP parameters, multi-MUP analysis, "upper centile amplitude"; quantitative analysis of motor unit firing rate, quantitative analysis of the interference pattern, e.g TAA (Turns/amplitude analysis), clouds, "number of small segments" (NSS), Motor unit number estimation (MUNE).

In our analysis we evaluated two main values, Turns/sec and Amplitude/Turn.

MUAP Assessment at High Force: Analysis of Interference Pattern

The number of turns (NT) is used to quantify the Interference Pattern. The mean amplitude change between successive turns (MA) is used to assess the amplitude changes.

The NT and MA values depend on the force of contraction. NT increases with force until it is about 50 % of maximum. With further increase in force, the NT increases mildly or may even decrease slightly. The MA increases with force at all levels of activation. Since the IP measurements depend on the force, the recordings are performed when the subject exerts a standard force.

Analysis of the interference pattern at a given force of 2 kg showed increased number of turns/second in most patients with myopathy, in some patients associated with decreased mean amplitude per turn (Fuglsang-Frederiksen and Mansson, 1975; Fuglsang-Frederiksen et al., 1976; Willison, 1964). However, at a given force (e.g. 2 kg) the number of turns/second increases with decreasing strength of the muscle of control subjects (Fuglsang-Frederiksen et al, 1975), i.e. the weaker the subject, the higher the number of turns/second. An increased number of turns/second at a force of 2 kg in a weak subject may therefore, suggest a false-positive finding of myopathy (Fuglsang-Frederiksen et al., 1976).

Analysis of turns–amplitude at a force fixed relative to maximum (e.g. 30% of MVC) in muscles from patients with myopathy or neurogenic disorders indicated that this method was better at discriminating between myopathic, neurogenic, and normal muscle than analysis at a force of, e.g. 2 kg (Fuglsang-Frederiksen et al., 1976; Haridasan et al., 1979). The number of turns/second was increased and mean amplitude per turn decreased in myopathic muscles, whereas the opposite was the case in neurogenic muscles. The ratio of turns to mean amplitude was increased in patients with myopathy and decreased in patients with neurogenic disorders (Cruz Martinez et al., 1984; Fuglsang-Frederiksen et al., 1976; Smyth and Willison, 1982); and the number of small time intervals between turns (e.g. less than 1.5 ms per time unit) was increased in patients with myopathy and decreased in patients with neurogenic disorders (Fuglsang-Frederiksen et al., 1976, 1977).

This approach to IP analysis has several limitations: it requires force monitoring, and requires patient cooperation.

Stålberg and coworkers ignored the force of contraction and used a novel way to assess the abnormalities: their analysis, now called turns and amplitude (TA), is performed as follows: The IP signal is recorded from six to ten different sites. At each site, the signal is analyzed at three to four different force levels that range from minimal to maximum. The force is varied when the patient matches the resistance offered by the clinician. A plot of MA versus NT is created. An area on this plot is defined which contains more than 90 % of data points in normal subjects. The boundary of this area is called the normal cloud. A typical investigation acquires 20 data points. Hence, the study will be abnormal when three data points are outside the normal cloud. In patients with neuropathy, the data points fall on the upper side of the normal cloud. In contrast, some data points occur below or to the right of the normal cloud in patients with myopathy.

So the TA analysis allows excluding the force variable, and this is particularly important in the paediatric age, where it is difficult to obtain complete cooperation, especially in the 0-6 age group. Therefore, TA allows to obtain an indirect evaluation of the interferential pattern without the need for a full collaboration of the patient in achieving a gradually maximal force, as is instead necessary in the standard EMG.

Normal clouds for four group of muscles (tibialis anterior, extensor digitorum communis, biceps, quadriceps) for men and women aged above and below 60 years were collected by Stalberg (Stalberg et al, 1983); normal values for biceps, tibialis anterior and first dorsal interosseous for men and women over 60 years of aged were published by Nandedkar et al

(1991), and for brachial biceps, the abductor pollicis brevis, medial vastus, and anterior tibial in adults (Liguori, 1992).

Unfortunately, in the literature there are currently no studies reporting the normative values of Quantitative EMG in children, also for Turn/sec (T/S) and Amplitude/Turn (A/T) values. Therefore our values are difficult to compare with the normative values present in the literature, which only concern adulthood.

Therefore, the Turn/sec and Amplitude/Turn values reported in this study represent the first normative values for QEMG in the neurophysiological study, and a novelty in the international neurophysiological field.

Neurophysiological and hystopathological correlation

Diagnostic value of EMG and muscle biopsy

The aim of this work was not to provide a study with epidemiological value, but to be a pilot study on the usefulness of neurophysiological studies compared to muscle biopsy in children with suspected neuromuscular pathology.

A strength of our study is that electrophysiologists, pathologist, and clinicians all worked independently and did not alter the documented reports. All the professionals involved were experts in their field thus, in almost all cases, we are able to obtain an adequate EMG and histopathological study since performed under these controlled conditions.

Additionally, in our cases the etiological diagnoses of definitive myopathies (congenital myopathy, genetically confirmed myopathy, inflammatory myopathy, metabolic myopathy, and muscular dystrophy) were established in a higher proportion compared with the previously published series (Ghosh et al, 214) (Constantinides et al, 2018) (Hafner et al, 2019). Moreover, the cohort did not include myopathic and neurogenic cases which were so clinically obvious such as to prompt focused genetic screening possibly without either EMG and muscle biopsy. Two previous studies reported a higher proportion of patients with Duchenne Muscular Dystrophy (DMD) (25% to 50%) (Hellmann et al, 2005) (Rabie et al, 2007). However, as also reported by Ghosh (Ghosh et al, 2014), due to the widespread availability of genetic tests for DMD, clinicians rarely request EMG in cases with suspected DMD in children any longer, and none of our patients had DMD. This can create a problem of selection bias; the patients selected tended to be those with more complex clinical pictures, the referral bias inherent to a tertiary care centre.

Considering the characteristics of the sample, we specify that the studies in the literature with which we compared the data are those evaluating the accuracy of EMG relative to the final diagnosis in children with suspected NMD (Hellmann et al, 2005) (Chang et al, 2011) (Rabie et al, 2007) (Ghosh et al, 2014) (Constantinides et al, 2018) (Hafner et al, 2019) (Sener 2019) (Naddaf et al, 2018) (Dardiotis et al, 2011). Previous studies (Brusa et al., 1963) (Humphrey and Shy, 1962) (Schwartz et al., 1966) (Hausmanowa-Petrusewicz and Karwanska, 1971) (Black et al, 1974) (Micaglio et al., 1980) (Buchthal and Kamieniecka, 1982) (Packer et al., 1982) (Gibertoni et al, 1987) (Werneck et al, 1988) (David and Jones, 1990, 1994) (Russell et al., 1992) on the other hand have investigated the accuracy of neurophysiological tests compared to muscle biopsy in NMD patients, and not with respect to the final diagnosis. This created an important bias, particularly for genetic disorders, in which a genetic diagnosis was still unavailable in the majority of cases.

The retrospective studies on the diagnostic value of EMG and muscle biopsy in children are summarised in Table 29.

	Hellman n et al, 2005	Rabie et al, 2007	Cetin et al, 2009	Dardiotis et al, 2011*	Chang et al, 2011	Ghosh et al, 2014	Constanti nides, 2018	Hafner et al, 2019	Sener et al, 2018*	Current study
Patient group	NMD	NMD	NMD	NMD	NMD	Myopa thies	NMD	NMD	Myopat hies	NMD
Number of patients	498	27	37	39	62	72	123	171	218	62
Age range	<16y	6m-16y	<2y	adults	1m-18 y	6d-16y	NI (adults?)	childre n	adults	childre n
N. muscles tested-EMG	2	4	4	NI	2	>4	≥1	≥1	≥1	≥1
EMG analysis	quantita tive	qualitati ve	quantitati ve	quantitat ive	quantit ative	Semiqu antitati ve	NI	quantit ative	NI	quantit ative
NCS included	yes	yes	yes	no	yes	yes	no	yes	NI	yes
Myopathic cases (N)	49	11	24	31	51	35	89	98	150	46
NCS characteristic s	50-91% compati ble with EMG	9% low CMAP amplitu de	1 abolished MCV, 23 normal	no	100% normal	NI	NI	NI	NI	21% low CMAP amplitu de
EMG sensitivity	80%	36.4%	21%	24-69% (with reference to biopsy)	98% (96% EMG, 100% TAA)	91%	76.4%	87.8%	95.3%	26%
EMG specificity	NI	NI	NI	NI	NI	67.6%	58.8%	67.1%	48.5%	95%
EMG PPV	NI	NI	NI	NI	NI	NI	82.9%	NI	NI	92%
EMG NPV	NI	NI	NI	NI	NI	NI	48.7%	NI	NI	39%
Biopsy sensitivity	NI	100%	NI	NI	NI	NI	86.5%	84.5%	NI	65%;
Biopsy specificity	NI	NI	NI	NI	NI	NI	97.1%	75.7%	NI	100%
Biopsy PPV	NI	NI	NI	NI	NI	NI	98.7%	NI	NI	100%
Biopsy NPV	NI	NI	NI	NI	NI	NI	73%	NI	NI	69%
Neurogenic cases (N)	195	4	13	4	3	NI	10	18	11	8
NCS characteristic s	96-100% compati ble with EMG	NI	53.8% low CMAP amplitud e	No	100% normal	NI	NI	NI	NI	50% low CMAP amplitu de
EMG sensitivity	99.5%	100%	100%	NI	66% (100% TAA)	NI	100%	94.4%	NI	100%;
EMG specificity	NI	NI	NI	NI	NI	NI	92.9%	96.1%	NI	81%
EMG PPV	NI	NI	NI	NI	NI	NI	55.6%	NI	NI	27%
EMG NPV	NI	NI	NI	NI	NI	NI	100%	NI	NI	100%
Biopsy sensitivity	NI	25%	NI	NI	NI	NI	100%	33.3%	NI	62%;
Biopsy specificity	NI	NI	NI	NI	NI	NI	94.7%	99.3%	NI	89%
Biopsy PPV	NI	NI	NI	NI	NI	NI	62.5%	NI	NI	38%
Biopsy NPV	NI	NI	NI	NI	NI	NI	100%	NI	NI	96%

*Table 29: Retrospective studies on the diagnostic value of EMG and muscle biopsy in children. NMD: neuromuscular disorders; NI: not indicated; N: number; m: months; y: years; TAA: Turn Amplitude Analysis. * these studies analysed the correlation of individual electromyographic and histopathologic findings.*

For neurogenic disorders, EMG was particularly sensitive and specific (100% sensitivity, 81% specificity). These results are comparable with other studies (see Table 29) (Hellmann, Rabie, Cetin, Constantinides).

Hafner (Hafner et al, 2019) found a sensitivity only slightly lower (94.4%); only Chang (Chang et al, 2011) found a lower sensitivity with conventional EMG (66%), rising to 100% using qEMG (Turns/Amplitude analysis). EMG specificity for neurogenic disorders is slightly lower when compared to the only two other articles that evaluated it (Constantinides et al, 2018) (Hafner et al, 2019), but still remains high (81%). The positive predictive value (PPV) is low (26%), while the negative predictive value (NPV) is 100%, overlapping with the literature (Constantinides et al, 2018). This can be explained by the incidence of neurogenic disorders in our sample. As the incidence increases, so does the positive predictive value (in a population where a certain disease is widespread, a positive test is very likely to correctly indicate the presence of the disease). In our sample, only 8 out of 82 patients had diagnoses of neurogenic disorders and this could explain the low PPV.

Therefore, EMG is an accurate screening test in recognising neurogenic disorders in children with a suspected neuromuscular disorder according to previous studies (Rabie et al, 2007) (Hafner et al, 2018).

Only a few studies compared muscle biopsy sensitivity in recognising neurogenic disorders. In our sample muscle biopsy had a modest sensitivity (62%), but higher than that described in previous studies (Hafner et al, 2019; Hellmann et al, 2005), while only Constantinides et al (Constantinides et al, 2018) found a sensitivity of 100%.

As the aim of this study was to compare the accuracy of EMG and muscle biopsy in detecting disease in the same cohort, patients who had performed both tests as part of their clinical investigations were selected. This introduced a recruitment bias, as it would not have been necessary to perform muscle biopsy in patients with neurogenic clinical features and obvious NCS and EMG findings. Consequently, the neurogenic cases observed were often those with unusual and difficult presentations. This fact as well as the small number of patients can explain the reduced biopsy sensitivity for a neurogenic diagnosis in this series.

Moreover, as emphasized by Hafner et al, several factors precluded confident distinction between a neurogenic and myopathic process, particularly in needle biopsies from a proximal muscle such as the quadriceps (standard biopsy site in our series). These include large swathes of neurogenic fibre type grouping mimicking myopathic slow fibre predominance/uniformity,

non-specific fibre size variation (pre-pathological SMA) and a constellation of pseudomyopathic architectural changes including unevenness of staining, moth-eaten fibres, mini-cores and larger cores, internal nuclei, split fibres, whorled fibres and fibro-fatty infiltration, seen in milder 5q- SMA I and SMA-LED (Sewry et al, 2015) . We tried to avoid this by adding a group of diagnosis which is mixed or non-specific alteration that include most of the above mentioned alterations. However this could affect biopsy sensitivity for a neurogenic diagnosis.

The specificity of muscle biopsy, on the other hand, remains high (89%) and is not far from what was reported in previous studies (94.7% by Constantinides, and 99.3% by Hafner) (Constantinides et al, 2018) (Hafner et al, 2019). PPV and NPV also compare favourably with the results described by Constantinides et al (Constantinides et al, 2018).

However, in our cohort, EMG lacked sensitivity in myopathic disorders; in our sample the sensitivity of EMG in detecting myopathies is very low (26%), with a specificity of 95%. This is despite the fact that quantitative EMG was used.

This finding is partly comparable to that of EMG protocols examining at least 2 muscles with qualitative (Rabie et al, 2007) or quantitative EMG (Cetin et al, 2009; Dardiotis et al, 2011), but lower than more recent studies using quantitative EMG (Chang et al, 2011; Hellmann et a, 2005; Ghosh et al, 2014; Constantinides et al, 2018; Sener et al, 2018; Hafner et al, 2019) (See table 29). This confirms that EMG should therefore not be considered as a screening test for myogenic disorders.

In contrast, the specificity was 95%, higher than Ghosh, Constantinides, Hafner, Sener (48.5%-67.6%), with a PPV of 92%.

These results can have various explanations. In part, it can be explained by the composition of the sample. As already noted by Hellmann (Hellmann et al, 2005), when more patients with congenital myopathies were included in studies, the overall diagnostic yield of the method decreased. With reference to the other studies with children with suspected NMD, in our group of myopathic patients as many as 41.3% were congenital myopathies.

In the reference studies that described a higher sensitivity of the EMG in recognising myopathic disorders than ours, the highest percentage of myopathies was described in Hafner (Hafner et al, 2019) where they accounted for 49%; Hellmann (Hellmann et al, 2005) described 18/49 (36. 7%) congenital myopathies, with an EMG sensitivity of 50%; in Ghosh (Ghosh et al, 2014) congenital myopathies accounted for 22%; in Constantinides (Constantinides et al, 2018)

only 7/89 (7.9%) had congenital myopathy. In Chang (Chang et al, 2011) no patients had congenital myopathy (43 muscular dystrophies, 4 dermatomyositis, 4 patients had an unidentified muscle disease) and the final diagnosis was often based on muscle biopsy alone. Finally, the sample described by Sener (Sener et al, 2018) only includes adult patients.
Thus, the only study with a comparable percentage is that of Hafner et al; in their sample, however, 27.1% of congenital myopathies and 71.4% of those with dystrophy had a genetic diagnosis; in our sample, 12/19 patients (63.2%) in myopathies and 8/9 (88.9%) in congenital muscular dystrophies had a genetic diagnosis.
It should be noted that EMG can provide exactly similar patterns in cases that differ widely in histopathological features. On the other hand, exactly similar patterns of pathological change in muscle can be observed in very advanced stages of both myogenic and neurogenic atrophy (Hausmanowa-Petrusewicz et al, 1971). The differential diagnosis between primary muscular and neurogenic lesions is additionally complicated by the fact that changes most probably corresponding to those of denervation may be found in typical muscular dystrophy as well as aggressive and chronic myopathies will often lead to denervation of the muscle fibre and therefore neurogenic findings superimposed on a myopathic pattern on biopsy (DeGirolani et al, 1997).
It should be emphasised that in the group of patients with myopathic disorders who underwent EMG, only 26.2% had EMG with myogenic features, but 45.2% still had alterations on EMG even if they were non-specific.

The study showed that muscle biopsy had a very high diagnostic yield in myopathic disorders, with 100% specificity, higher even than values found in the literature (Hafner et al, 2019). Nevertheless, in our sample muscle biopsy had a sensitivity of 62%, lower than the studies cited above (Rabie, et al, 2007; Constantinides et al, 2018; Hafner et al, 2019).
However, it must be noted that the sample described by Constantinides (Constantinides et al, 2018) was made up of adults, whereas in Rabie's study (Rabie et al, 2007) of the 11 children with suspected myopathic disorder (age <9 y), final diagnosis was uncertain in 54.5% of the myopathies with diagnosis based precisely on the muscle biopsy results (four probable myopathies and two definitive myopathies on muscle biopsy), while the other 5 patients were diagnosed with congenital myopathies (45.4%).
Therefore, the only study with a higher sensitivity of muscle biopsy in recognising myopathic disorders than ours is that of Hafner et al (Hafner et al, 2019).

In this context, however, it must be considered that in our study only two patients out of forty-six had normal biopsies, four had neurogenic biopsies (three congenital and one inflammatory myopathy), and ten had an altered biopsy that was not considered sufficiently specific for myopathy (90% with grouping and polydimensionality). Of these three were hyperCKemias. Thus a further reason could also be the composition of the sample: in our sample, unlike Hafner et al, there were also hyperCKemias of which only 1/5 (20%) had a myogenic biopsy. In contrast, however, our sample had a higher incidence of inflammatory myopathies (28.2%), unlike Hafner et al (4%) or Rabie et al (0%), and usually inflammatory diseases are those in which muscle biopsy is most unequivocal.

Finally, an interesting point can be made about the results of neurophysiological investigations in patients with metabolic pathology.

The 80% of the patients undergoing EMG showed neurogenic alterations; in two of these patients the biopsy was concordant (neurogenic) while in the other two it was non-specifically altered. The diagnoses of these patients varied from one patient with PDH deficiency, one patient with ATAD3A mutation, one patient with Melas, and one patient with Leigh syndrome. The only patient with normal EMG and normal biopsy had a clinical and neuroradiological picture compatible with mitochondrial encephalopathy but no genetic characterisation. This highlights the need for more studies.

Correlation between EMG and histopathological findings

As mentioned above, it should be noted that to the best of our knowledge few studies have assessed the correlation between EMG findings and muscle biopsy results; all studies involved adult patient samples.

Furthermore, it should be noted that Naddaf (Naddaf et al, 2018) analysed possible correlations between EMG and hystopathologic findings, while Sener (Sener et al, 2018) and Dardiotis (Dardiotis et al, 2011) evaluated the sensitivity of EMG results in identifying certain histopathologic abnormalities.

However, it should be observed that while the EMG parameters are more standardised, the muscle biopsy parameters chosen are different in each study. Sener (Sener et al, 2018) focused on inflammation, fibre splitting, fibre necrosis, and vacuoles. Dardiotis (Dardiotis et al, 2011) divided muscle biopsy findings into four categories: increased variability in muscle fibre size involving both fibre types, the presence of necrosis and/or regeneration, the presence of

endomysial fibrosis indicating chronicity and fibre loss, alterations in the fibre architecture without significant fibre loss or variability in fibre size. Finally, in Naddaf (Naddaf et al, 2018) histopathologic findings included: atrophic fibres, necrotic fibres, regenerating fibres, fiber splitting, fibres harboring vacuoles, ragged-red fibres, COX negative fibres, fibres with target formations, fibres with increased glycogen content, fibres reacting for NSE, fibres with congophilic inclusions, amount of endomysial connective tissue, perimysial inflammation, endomysial inflammation, fiber type grouping, type 1 and type 2 fiber atrophy.

We have only reported those alterations for which a correlation could be calculated as they were numerically sufficiently represented.

We now examine electrodiagnostic and hystopathological findings in our sample and their possible correlations.

Fibrillations potentials

Fibrillation potentials in our sample are most frequent in muscular dystrophies. While Sener (Sener et al, 2018) and Naddaf (Naddaf et al, 2018) described the fibrillation potentials as most frequent in inflammatory myopathies, in Ghosh et al cases (Ghosh et al, 2014), fibrillations were always present in children with inflammatory myopathies and in a high proportion of cases with dystrophic muscle biopsies.

However, in our sample inflammatory myopathies are those in which the EMG is most altered in a myopathic sense and this confirms what has already been described in the literature. Indeed, fibrillation in neurogenic disorders suggest active denervation; in myopathies, fibrillation suggests partial denervation of the muscle fibres by necrosis, inflammation or fibres splitting (Wilbourn et al., 1993). The identification of fibrillation potentials in patients with a myopathic EMG provides an aetiological clue to clinicians (Ghosh et al, 2014).

In our sample, fibrillation had no statistically significant correlation with any parameter of the muscle biopsy. This differs from what has been found in the literature. In the study by Naddaf (Naddaf et al, 2018) there was a correlation between the presence of fibrillation potentials on EMG and atrophic fibres, necrotic fibres, regenerating fibres, fibre splitting, fibres harbouring vacuoles, fibres reacting for NSE, fibres with congophilic inclusions, increased endomysial connective tissue and inflammation on muscle biopsy.

In Sener (Sener et al, 2018), fibres also appeared sensitive to inflammation (73%), fibre splitting (74%), and necrosis (71%), against which it also had high specificity (73.3%).

However, as already anticipated by previous studies (Naddaf et al, 2018), the presence of fibrillation potentials must be interpreted with caution and should not be considered a surrogate for inflammation or disease activity alone. Indeed, the true correlation of fibrillation potentials with these histopathological findings may not be reflected, in part due to the fact that a clinically involved muscle with fibrillation potentials on EMG is usually a preferred target for biopsy (Naddaf et al, 2018).

Interestingly, in our sample, a statistically non-significant correlation (trend) was observed between fibrillation and cytochrome C oxidase (COX) deficient fibres ($p = 0.094$). The reason is unclear. One explanation could be that denervation can also give COX deficiency especially in the acute phase (Dubowitz, Sewry, 2007).

Duration of the motor unit potentials

The duration of the motor unit potentials is a key component to determine the nature of an underlying process (Joyce et al, 2012). As expected, short duration MUAPs were present only in patients with myopathies; with regard to long duration of MUAPs, this was described in all patients with neurogenic disorders and in 80% of patients with metabolic disorders, but were also found in 22% of patients with myopathies.

In our sample an increase in MUAP duration was present in 30.6% of the sample, while few patients showed short duration MUAPs on EMG (3/62, 4.8%). This was in contrast to Naddaf in whom 85% of patients had short duration MUAPs, which showed correlation with the following histopathologic findings: atrophic fibres, necrotic fibres, regenerating fibres, increased endomysial connective tissue, and perimysial inflammation (Naddaf et al, 2018); long duration of MUAPs in their sample was present in 19% of patients, and showed a statistically significant correlation with the presence of grouping on muscle biopsy.

Thus, this difference in frequency could explain the lack of correlation of short duration of MUAPs in our sample. Another difference to consider is that childhood myopathies differ in frequency and characteristics from those present in adulthood, in which the histopathological picture is different.

Even for Sener (Sener et al, 2018) short duration MUAPs were sensitive for certain findings more typical of myopathic forms than of neurogenic ones (inflammation, necrosis, fibre splitting, and vacuoles).

We want to underline that, unlike in Sener where single fiber alterations were mainly described, we have mainly considered the presence of histopathological alterations as a whole.

In our sample, however, we found a statistically significant correlation between long duration of MUAPs and necrosis. The presence of long-duration polyphasic potentials (LDPPs) had already been described in patients with myopathy (Uncini et al, 1990), and in particular they were found most often in chronic polymyositis and more rarely in muscular dystrophy (Becker Muscular Dystrophy); LDPPs are attributed to desynchronisation of single-fiber potentials within the MUAP and may be due to slow conduction in regenerating muscle fibres (Uncini et al, 1990). The study by Uncini et al (1990) also emphasised the need to exclude LDPPs when calculating the mean duration of MUAPs for diagnosis. In fact, the mean duration of all potentials was reduced in only 64% of patients because LDPPs increased the mean, when only simple potentials were considered, however, the mean duration was decreased in 95% of patients. Thus the presence of long-lasting motor unit potentials should not be regarded as the only sign of neurogenic disease in the absence of other neurogenic features on EMG. This is because it can be interpreted as a sign of chronicity of the myopathy since histopathologically with its presence it suggests necrosis of the fibers. Therefore, our result could be explained by the known fact that aggressive and chronic myopathies often lead to denervation of muscle fibers and therefore to neurogenic findings superimposed on a myopathic pattern on biopsy (DeGirolani et al, 1997).

<u>Amplitude of motor unit potentials</u>

Despite duration is considered the most sensitive and specific parameter of EMG (Joyce et al.), in our sample there was a correlation between increased amplitude of MUAPs, typical of neurogenic forms, and presence of fibre type grouping on muscle biopsy. In a normal muscle, there is random distribution of histochemical fibre types; in reinnervated skeletal muscles, the checkerboard appearance is disrupted and replaced by grouping of fibres of the same histochemical type, as fibres innervated by a single motor unit become of the same histochemical type (Warmolts et al, 1972).

A correlation between reduced amplitude on EMG and the presence of fibrosis and necrosis, all common features of myopathic forms (Paganoni et al, 2013), was also noted.

Naddaf (Naddaf et al, 2018) and Sener (Sener et al, 2018) did not assess the amplitude of MUAP, so we are the first to describe it, especially in the paediatric population.

A possible confirmation of our data can be found in Dardiotis (Dardiotis et al, 2011); in his article the amplitude outlier method was significantly more sensitive than the duration outlier.
These data also seem to be confirmed by evaluating how the presence of increased amplitude of MUAPs was high specific for increased fibre size variability (91%) and fibre type grouping (91%), whereas reduced amplitude of MUAPs was high specific for increased fibre size variability (91%), fibrosis (95%)and necrosis (86%). Therefore, altered amplitude at EMG should always be carefully assessed when performing EMG and not considered secondary to the MUAP duration parameter.

Increased phases:

Similar to previous reports, (Werneck et al, 1988) increased phases were highly sensitive for fibre type grouping, fibrosis and necrosis. A statistically non-significant correlation (trend) was also noted between increased phases and fibre type grouping ($p = 0.08$) and fibrosis ($p = 0.87$). Naddaf (Naddaf et al, 2018) also described a correlation between increased phases and fibrosis, as well as a correlation with atrophic fibres that we did not find. As expected, increased phases, which can be present in both neurogenic and myogenic EMG, are sensitive for histopathological changes both typical of neurogenic (grouping) and myopathic (i.e. necrosis) pathologies and is therefore one of the less specific parameters.
It should be emphasised that the increased variability in muscle fibre size, which is considered one of the most sensitive histological features of myopathy (Kokotis et al, 2016), shows no statistically significant correlation with the EMG findings, but only increased phases appear to be sufficiently sensitive in identifying it

T/A Analysis:

T/A analysis, one of the quantitative analysis of interference pattern analysis, reflects the motor units which are induced in an entire range of force (Chang et al, 2011).
There are no other studies evaluating possible correlations between this parameter and histopathological findings.
In our sample, all patients with neurogenic disorders who performed EMG showed increased T/A; TAA is highly specific in recognising fibre type grouping, and a statistically non-significant correlation (trend) was noted between Increased T/A at EMG and the presence of fibre type grouping and necrosis at biopsy. A presence of increased T/A could suggest a denervation of muscle fibres (DeGirolani et al, 1997).

A statistically significant correlation was noted between a reduction of T/A at EMG and the presence of necrosis on muscle biopsy; the presence of reduced T/A could therefore suggest a myopathy with fibre necrosis.

Correlation between NCS and histopathological findings

Peripheral nerve involvement in paediatric myopathies has already been described by various authors. Advanced cases of nemaline myopathy may have neurogenic alterations on ENMG (Wallgren-Petterson et al, 1989).

Several authors found peripheral nerve involvement in congenital muscular dystrophy, especially in primary merosin deficiency (Packer et al, 1982) (Di Muzio et al, 2003) (Matsumura et al, 1997) (Ferreiro et al, 2002). However, as emphasized by Quijano-Roy et al., a demyelinating peripheral neuropathy may be found in a merosine positive congenital muscular dystrophy (Quijano-Roy et al, 2004).

Cetin et al performed a study of NCV measure in 60% of their cases with congenital muscular dystrophies and we never found any demyelinating neuropathy (Cetin et al, 2009) .

In our sample, decreased CMAP amplitude at the NCS was present in 23.5% of patients with congenital myopathy, 33.3% of patients with congenital muscular dystrophy, and 16.7% of patients with inflammatory myopathy.

Of our patients with merosine dystrophy, 33% had demyelinating peripheral neuropathy and 33% had an axonal form, Of our two patients with nemaline myopathy, one had decreased CMAP amplitude. These data seem to confirm what was reported by previous studies.

As far as we know, no studies have been reported in the literature that have evaluated the correlation between EMG results and muscle biopsy results.

In this study, no statistically significant correlations were found; a statistically non-significant correlation (trend) was noted between reduced CMAP amplitude of nerve conduction study of inferior legs and cytochrome C oxidase (COX) deficient fibres ($p = 0.066$), but the pathogenetic mechanism underlying this trend remains unclear. It should be underlined that effectively 80% of patients with reduced CMAP amplitude presented COX deficient fibers on muscle biopsy, despite the variability of the etiological diagnosis in these patients.

Limits:

About the first aim, a first limitation of our study is the fact that our sample was stratified in relatively narrow ranges. Although this was done to obtain data as precise as possible in relation to age, on the one hand it reduced the number of patients for each range, on the other it partially invalidated the comparison with the studies already present in the literature, in which the sample is usually stratified in wider ranges. Another limitation of our study can be considered to have used the E-Norm method: although this method is rich in advantages (see above), on the other hand it is a relatively new validated method, so few normative data have been obtained with this method in children population; furthermore it is possible that the diversity of this method is responsible for some differences found in the mean values of and NCV.

Regarding the second aim, there were a number of study limitations, some of which have already been noted. Our sample size of 82 subjects was small, and the data were from a single muscular disease center, so our results should be generalized with caution.

Because the above data was collected from a muscular disease center at a university hospital, the proportion of patients with severe forms of muscular disease, was high; there was the problem of selection bias.

However it was based on material acquired on a pragmatic approach in the investigation of patients. Others limitations of our study include the retrospective design. Additionally, our study has a small percentage of children <2 years of age; sometimes, it may be difficult to perform a satisfactory and adequate study in an awake or struggling infant.

Furthermore, we only studied one muscle; however, it is true that in children, neuromuscular disorders tend to be generalised and focal findings are unusual, and as Hafner has already shown, it is sufficient to examine one muscle provided this is done comprehensively (Hafner et al, 2019).

The ideal study would have been prospective and should have included patients in which the EMG and biopsy are performed sequentially in the same muscle.

Last no formal morphometry on the biopsies was carried out in our study, such as deriving average diameters and hypotrophy or hypertrophy values, since this is not routinely practiced in our laboratory . In addition the median muscle fibre diameter dramatically increase with age in the very first years of life.

CONCLUSION

Neurophysiological investigations are an essential part in the diagnostic algorithm of neuromuscular pathologies, and this applies to both acquired and genetically determined forms, especially for patients with more heterogeneous phenotype for which recognizing the early and subtle changes is essential.

In children however, these techniques are often avoided on one hand because they are considered invasive and on the other because they are considered by the neurophysiologists themselves to be difficult to perform due to poor compliance, but even more because they are considered difficult to interpret. Currently, in the literature, normative data of the electrophysiological studies (NCS and EMG) for the study of neuromuscular diseases in paediatric age, from newborn to adolescent, are lacking. This greatly limits their clinical and research use.

Finally, at times, the diagnosis ultimately requires a muscle biopsy, regardless of the electrodiagnostic study results (Paganoni et al, 2013).

Muscle biopsy has historically been considered to be the gold standard in the diagnosis of muscle disease (Hafner et al, 2019), allowing for the precise characterisation through histological, biochemical, immunocytochemical and ultrastructural analyses.

However, muscle biopsy is an expensive, invasive, time-consuming, and resource-dependent procedure. Furthermore, pathological characterisation of disease presents a number of challenges, including overlapping morphological signatures between different diseases, the absence of specific pathological features, and the small sample size available for evaluation.

The first aim of this study was to help bridge the gap of normal values for paediatric NCS and EMG. The secondary aim was to describe the diagnostic accuracy of electrodiagnostic tests (NCSs and EMG) and muscle biopsy in children with suspected neuromuscular disorders, to compare sensitivity, specificity, positive and negative predictive value of the two tests, and demonstrate in a systematic way if correlations between electrophysiological parameters and histopathologic findings in the same paediatric population exist.

Our sample size of 715 paediatric electroneurographic studies in the age range 0-3 years, represents the largest paediatric study we are aware of to date in this age group and provides robust data for determining normal values for paediatric NCS.

Many findings already described in previous studies have been confirmed: in our sample it was also found that in the neonatal group MCV and SCV mean values are about one-half of those of normal young adults, and that most rapid increase occurs during the first year of life; also in

our sample, adult values for both MCV and SCV seems to be reached during the first years of life.

Regarding amplitude, it was also found in our sample that amplitudes of the motor and sensory responses show a less linear pattern and this may be due to higher dispersion for different myelinic fiber maturation at various gestational ages. We also found a difference in the mean values of the tibial nerve amplitudes with respect to the peroneal nerve, with a greater amplitude for the tibial nerve, and a difference in maturation in the amplitude of the tibial nerve with respect to the peroneal nerve. Even in our sample the adult amplitude values seem to be reached around 24 months, in particular for sensory nerve.

However, we found some differences in the average values of amplitude and conduction velocity, both for motor nerves and sensory nerves.

In particular, it seems that our median values of amplitude and nerve conduction velocity are slightly lower than those reported in the literature, especially in the first months of life.

This can be partly explained by the different stratification of the sample, but also by the use of a different statistical analysis, called E-norms, that exploits another property of variables derived from normal individuals that distinguishes them from variables derived from patients with pathology, a property called ‘‘normal clustering”.

In conclusion, this collection of normal paediatric cases reliably establishes normative data for paediatric NCS across several commonly tested motor and sensory nerves. The cut-off values described can be used clinically to determine normal and abnormal NCS, which are helpful in the evaluation of children with known or suspected neuromuscular disorders. In paediatric field, it’s good practice to control during the time both NCS or EMG doubt parameters to be sure of normal or pathological range within a few days/weeks for the speed of maturational processes.

Therefore, this study underlines the importance of this new method of statistical analysis. Finally this work underlines the importance of having not only international paediatric normative data to compare, but also the fact that every neurophysiology laboratory should have its own normative data. When using published reference values, the examiner should perform studies in a few healthy subjects to confirm their suitability and reproducibility for their particular laboratory.

Our future intent is to be able to expand the sample, in order to obtain reliable data for other motor and sensory nerves and for the study of CMAP and SAP distal latencies and F-waves.

Regarding electromyographic investigations, this study represents a novelty in the international field.

First of all, our study is the first to divide the sample into relatively narrow ranges: range of one month up to three months of age, range of three months up to twelve months of age, range of six months up to two years of age, range of one year up to six years of age. There are no studies in the literature with such a stratified paediatric sample.

Although the data obtained are not completely comparable with those already reported in the literature, it is confirmed, as also reported by previous studies, that from birth to 6 years, MUAP amplitude and duration showed increase with age reaching the values of adult (from Howard et al, 1988) by 9-12 months and 18 months, respectively.

The MUAPs amplitude in our sample is fluctuating. This can be partly due to the characteristics of the sample, and to the characteristics of this parameter. In fact, a slight difference due to maturation of motor unit is well definable.

The MUAPs duration parameter is much more reliable and is of fundamental importance in the electromyographic evaluation to discriminate between neurogenic and myogenic forms.

As reported in previous studies, also in our sample there is a linear increase in duration starting from the first months of life reaching the values of adult by 18 months.

QEMG is a study approach that is finding more and more space in the pediatric field; it was demonstrated that QEMG could be potentially very useful in not cooperative children, because compliance is relatively less important factor for QEMG (and in particular for TAA) than for traditional interference pattern analysis; it allows the examination of mild to moderate neuromuscular pathologies in which the electrophysiological abnormalities reveal only slight abnormalities; it allows serial re-examination in which the results need to be compared with previous studies to assess the progression of the disease. QEMG allows a rapid characterization of the properties of MUAPs and the degree of maturation. Sedation is not required for a successful EMG recording in children at all ages.

Unfortunately, in the literature there are currently no studies reporting the normative values of Quantitative EMG in children, then our values are difficult to compare with the normative values present in the literature, which only concern adulthood.

Therefore, the Turn/sec and TAA values reported in this study represent the first normative values for QEMG in the neurophysiological study, and a novelty in the international neurophysiological field.

Our intention is to collect more data, in order to expand the sample, obtain more standardized data, and to obtain regulatory values even for other semiquantitative and quantitative electromyography parameters.

Regarding diagnostic value of neurophysiological test and muscle biopsy in patients with suspected NMD disorders, the present study aimed to highlight the diagnostic yield and the possible discrepancies of two classical and well-established diagnostic tools for neuromuscular diseases.

For neurogenic disorders, EMG was particularly sensitive and specific in recognising neurogenic disorders, while muscle biopsy had a modest sensitivity. EMG is accurate as a screening test in recognising neurogenic disorders in children with a suspected neuromuscular disorder, according to previous studies. In NMD with a neurogenic or NMJ pathogenesis the use of muscle biopsy is limited albeit typical lesions appear on the histopathological studies (such as type grouping or neuromuscular junction alterations).

However, as for myopathic diseases, in our cohort EMG lacked sensitivity in myopathic disorders. In our sample the sensitivity of EMG in detecting myopathies is very low, while the specificity remained high. On the other hand, muscle biopsy had a very high diagnostic yield in myopathic disorders, with discrete sensitivity, lower than published studies, but very high specificity, higher even than values found in the literature.

In conclusion, focused neurophysiological tests and muscle biopsy remain in children of all ages an indispensable diagnostic tool guiding clinicians in their decision-making and helping in a prompt diagnosis.

Since neurophysiological investigations can be achieved without anaesthesia, in forms not clearly diagnosable by clinical and genetic-molecular investigations, they remain one of the investigations of choice.

EMG still remains the more accurate test when determining neurogenic abnormality but not so much in case of myopathic forms where the muscle biopsy has a better diagnostic accuracy.

EMG and muscle biopsy complement each other and therefore this could translate into a confirmation and indication for clinical practice, precisely in the case of differential diagnosis between myopathy and neurogenic pathology, if the EMG is normal it is necessary to proceed with a muscle biopsy.

We then tried to evaluate whether some neurophysiological parameters could somehow indicate the presence of specific alterations on muscle biopsy. EMG and muscle biopsy are complementary investigations, however, associating specific EMG findings with histopathologic correlates remains challenging.

This could be considered a pilot study as to the best of our knowledge few studies have assessed the correlation between EMG findings and muscle biopsy results and none with a sample composed of paediatric patients. Some interesting correlations have been found.

As expected, increased phases, which can be present in both neurogenic and myogenic EMG, are sensitive for histopathological changes both typical of neurogenic (grouping) and myopathic (i.e. necrosis) pathologies.

Interestingly, we unexpectedly found a statistically significant correlation between the long duration of MUAPs and necrosis. Our result could be explained by the known fact that aggressive and chronic myopathies often lead to denervation of muscle fibres and therefore to neurogenic findings superimposed on a myopathic pattern on biopsy (De Girolani et al, 1997).

Moreover, we investigated some EMG parameters that previous studies had not evaluated in correlation to histopathologic findings, in particular alterations in MUAP amplitude and variations in T/A analysis.

Our study, to the best of our knowledge, is the first in which the amplitude of the MUAP is investigated as a possible marker of histopathological changes in neuromuscular diseases, especially in the paediatric population. In our sample there was a correlation between increased amplitude of MUAPs, typical of neurogenic forms, and presence of fibre type grouping on muscle biopsy, typical of reinnervated skeletal muscles; a correlation between reduced amplitude on EMG and the presence of fibrosis and necrosis, all common features of myopathic forms, was also noted.

A presence of increased T/A could suggest a denervation of muscle fibres (De Girolani et al, 1997), as there seemed to be a non-statistically significant correlation (trend) with the presence of grouping. A statistically significant correlation was also noted between a reduction of T/A at EMG and the presence of necrosis on muscle biopsy; the presence of reduced T/A could therefore suggest a myopathy with fibre necrosis.

Finally, a statistically non-significant correlation (trend) was noted between reduced CMAP amplitude of nerve conduction study of inferior legs and cytochrome C oxidase (COX) deficient fibres, but the pathogenetic mechanism underlying this trend remains unclear. It should be underlined that effectively 80% of patients with reduced CMAP amplitude presented COX

deficient fibres on muscle biopsy, despite the variability of the etiological diagnosis in these patients.

Therefore, the findings and the methodology of this study could serve as a pilot study for future studies to better understand the muscle biopsy-neurophysiological tests correlation in individual NMD disorders affecting developmental age.

APPENDIX

Main abbreviations used in this work.

Sigla	*Significate*
CNS	Central Nervous System
PNS	Peripheral Nervous System
EDX	Electrodiagnostic study
M-NCV	Motor nerve conduction velocity
S-NCV	Sensory nerve conduction velocity
NCS	nerve conduction study
EMG	Electromyography
SFEMG	Single fibre Electromyography
QEMG	Quantitative EMG
RNS	Repetitive nerve stimulation
cMAP	Motor action potential
SNAP	Sensitive action potential
TAA or T/A	Turns/amplitude analysis
NSS	"number of small segments"
MUNE	Motor unit number estimation
MUP/MUAP	Motor Unit Potential
NMJ	Neuromuscolar Junction
NMD	Neuromuscolar Disease
SMA	Spinal muscular atrophy
DMD	Duchenne's Muscular Dystrophy
COX	cytochrome C oxidase

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