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The impact of Central Nervous System Infections in Neurodevelopment

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The impact of Central Nervous System Infections in Neurodevelopment

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Abstract

Introduction: Central Nervous System (CNS) infections can have a negative impact in neurodevelopment. It is estimated that half of the survivors develop at least one long-term sequela. Neurodevelopmental and behavioral sequalae are the most commonly identified. The aim of this study was to characterize children with previous CNS infections and to identify possible neurodevelopmental outcome predictors.

Materials and Methods: We retrospectively analyzed data from 33 subjects followed-up at the Biological Risk Consultation in Coimbra Pediatric Hospital. Clinical, demographical and neurodevelopmental evaluation data was assessed. Age, gender, previous comorbidities, type of infection, pathogen involved, state at admission, duration of symptoms, glucose and protein concentration in cerebrospinal fluid (CSF) were chosen to be analyzed as possible neurodevelopmental outcome predictors. Correlation and subgroup statistical analysis were performed.

Results: A total of 33 children, 19 (57.6%) boys and 14 (42.4%) girls were identified. Median age of diagnosis was 4 months (IQR: 17) and 25 subjects (75.9%) had bacterial meningitis, 4 (12.1%) viral meningitis and 4 (12.1%) meningoencephalitis. From the children who completed evaluation with Griffiths Mental Development Scale, 11/28 (39%) showed one or more impaired areas of development (Development Quotient below 90). Hearing Loss was identified in 6 (18%) patients and Autism Spectrum Disorder was identified in 2 (6%). Age of diagnosis, protein concentration in CSF and gender were identified as neurodevelopmental outcome predictors.

Discussion: These results suggest that long-term neurodevelopmental outcome prediction following a CNS infection based only on clinical characteristics needs further studies. Therefore, in order to guarantee an early identification and intervention of neurodevelopmental disorders, children diagnosed with a CNS infection should be evaluated by a multidisciplinary neurodevelopment team.

Keywords: central nervous system infections, neurodevelopmental outcome, prognosis, meningitis

Resumo

Introdução: As infeções do Sistema Nervoso Central (ISNC) podem ter um efeito negativo no neurodesenvolvimento. Até metade das crianças sobreviventes desenvolve pelo menos uma sequela a longo prazo. As alterações do neurodesenvolvimento e do comportamento são a principal complicação. O objetivo deste estudo foi caracterizar as crianças com antecedentes de ISNC e identificar possíveis fatores preditores do impacto no neurodesenvolvimento.

Métodos: Análise retrospetiva dos processos de 33 crianças seguidas na consulta de Risco Biológico do Centro de Desenvolvimento da Criança do Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra. Foram analisadas as características clínicas, demográficas e do neurodesenvolvimento. Como possíveis preditoras do impacto no neurodesenvolvimento, foram selecionadas as seguintes variáveis: idade à data da ISNC, género, antecedentes pessoais prévios relevantes, características à admissão, tempo de evolução dos sintomas até ao diagnóstico de ISNC, características do liquido cefalorraquidiano e agente envolvido. A análise estatística incluiu a procura de correlação e a análise em subgrupos.

Resultados: Foram incluídas 33 crianças, 19 rapazes (57.6%) e 14 raparigas (42.4%). A mediana de idades no momento de diagnóstico foi de 4 meses (amplitude interquartil: 17). Identificaram-se 25 casos de meningite bacteriana (75.9%), 4 de meningite vírica (12.1%) e 4 de meningoencefalite (12.1%). Das crianças avaliadas pela Escala de Avaliação do Desenvolvimento de Ruth *Griffiths*, 11/28 (39%) apresentaram pelo menos uma área do neurodesenvolvimento afetada (Quociente de Desenvolvimento <90). A perda auditiva foi identificada em 6 crianças (18%) e uma Perturbação do Espectro do Autismo em 2 (6%). A idade de diagnóstico, o género e a proteinorráquia foram identificados como fatores preditores do prognóstico.

Conclusões: Estes resultados sugerem que a predição do impacto no neurodesenvolvimento com base em características clínicas necessita de mais estudos.
Assim, é necessário o acompanhamento das crianças com ISNC por uma equipa multidisciplinar do neurodesenvolvimento de forma a assegurar a identificação e intervenção precoce destas alterações.

Palavras-chave: infeções do sistema nervoso central, impacto no neurodesenvolvimento, prognóstico, meningite

Abbreviations

CNS	Central Nervous System
CSF	Cerebrospinal fluid
DQ	Development Quotient
Hib	*Haemophilus influenzae* serotype B
IQ	Intelligence Quotient
NM	*Neisseria meningitidis*
NM B	*Neisseria meningitidis* serotype B
NM C	*Neisseria meningitidis* serotype C
SP	*Streptococcus pneumoniae*
SPSS	Statistical Package for Social Sciences

1 Introduction

Neurodevelopment is a complex and delicate process of acquiring competences and establishing neuronal pathways in order to achieve independence skills. It is a continuum starting from the prenatal period and lasting until at least the second decade of life. During this process there is an intense cell proliferation, migration and differentiation defined by the genetic code, affected by epigenetics gene expression and molded by the environment [1]. It is clear that although there is a genetically defined basis, the stimuli provided by the environment are essential for neurodevelopment. This interaction between the intrinsic and extrinsic factors is usually described as "nature and nurture" [2].

Being such a complex process in which so many different variables play a role, the disruption of one single step or the interaction of several can lead to the an error that arise as a neurodevelopment disorder phenotype [3,4] . This can be explained by a genetic abnormality or caused by an insult: exposure to a teratogen or toxic, occurrence of trauma or infection [3,5].

Central Nervous System (CNS) Infections have been described as playing a pivotal role in the genesis of neurodevelopment disorders [1,3,6]. CNS Infections include meningitis, encephalitis and abscesses, which can be caused by bacteria and viruses [2]. Despite vaccination strategies and early establishment of antibiotic therapy, it is estimated that half of the survivors present at least one long-term sequela [7–9]. Sequelae include intellectual/behavioural deficits, hearing loss, visual impairment and neurological handicaps [10–12]. The most common long term sequelae are behavioral and intellectual disorders [7,8,13].

CNS Infections may originate from two main sources: (a) the birth canal and (b) the external environment. In neonatal CNS Infections, pathogens such as *Escherichia coli*, *Klebsiella pneumoniae* and *Streptococcus agalactieae* are usually involved [2,5,14]. After the third month of life, the most common pathogens involved are community acquired: *Streptococcus pneumoniae* (SP), *Neisseria meningitidis* (NM) and *Haemophilus influenzae* type B (Hib) [2]. SP seems to be the one resulting in more neurological complications and worst neurodevelopmental outcome when compared with Hib and NM [2,8,11,15–18]. When it comes to viral meningitis, the most common causing pathogens are enteroviruses [2,19,20]. Viral Meningitis has been described as presenting a good overall clinical outcome [19,21–24].

Clinical presentation is different depending on the age of diagnosis. In neonates, symptoms are usually unspecific, including fever, irritability, lethargy and feeding difficulties. Older children usually present fever, headaches, vomiting, confusion and meningeal signs. Petechial or purpuric exanthemas might also be present [2,6,23].

Several studies have been conducted in order to understand if clinical and laboratory features can be associated with different outcomes. Younger age [2,11,15,25–28], seizures [10,11,15,26,27,29,30] and severe illness at admission (coma, impaired consciousness and shock) [6,9–11,15,25,26,28,31] have been associated with poorer prognoses. It has also been described that delay from symptom onset to initiation of antibiotic therapy is associated with higher frequencies of long-term sequelae [9,11,26].

Regarding cerebrospinal fluid (CSF) analysis, lower glucose and higher protein concentrations have been considered markers of worst outcome [11,15,25,27–30].

Despite the definition of outcome predictors for neurological sequelae, overall survivor or long-term sequelae, to our knowledge, no previous study evaluated the possible prognostic factors involved in each area of neurodevelopment.

Therefore, the aim of this study is to describe the neurodevelopmental profile of children previously diagnosed with a CNS Infections and to identify potential prognostic factors associated with the neurodevelopment outcome in this population. The sample used was collected at the Coimbra Centro Hospitalar e Universitário - Pediatric Hospital - Centre for Development, analyzing the children followed-up at a specific and very high-specialized outpatient clinic for neurodevelopmental disorders - the Biological Risk Consultation.

2 Materials and Methods

2.1 Study Design, participants, inclusion and exclusion criteria

This was a retrospective cohort study including children evaluated in the Biological Risk Consultation, carried out in the Centre for Development of the Pediatric Hospital, Centro Hospitalar e Universitário of Coimbra, Portugal. From 2010 to 2019, 35 individuals with previous history of CNS Infections in this hospital were followed-up. Included and excluded subjects are presented on Figure 1. Two of the subjects were excluded for possible confounding factors: (1) had a cerebral abscess; (2) had a tumor under chemotherapy. Formal neurodevelopment evaluation was performed in 31 individuals, 2 of the subjects missed the evaluation appointment.

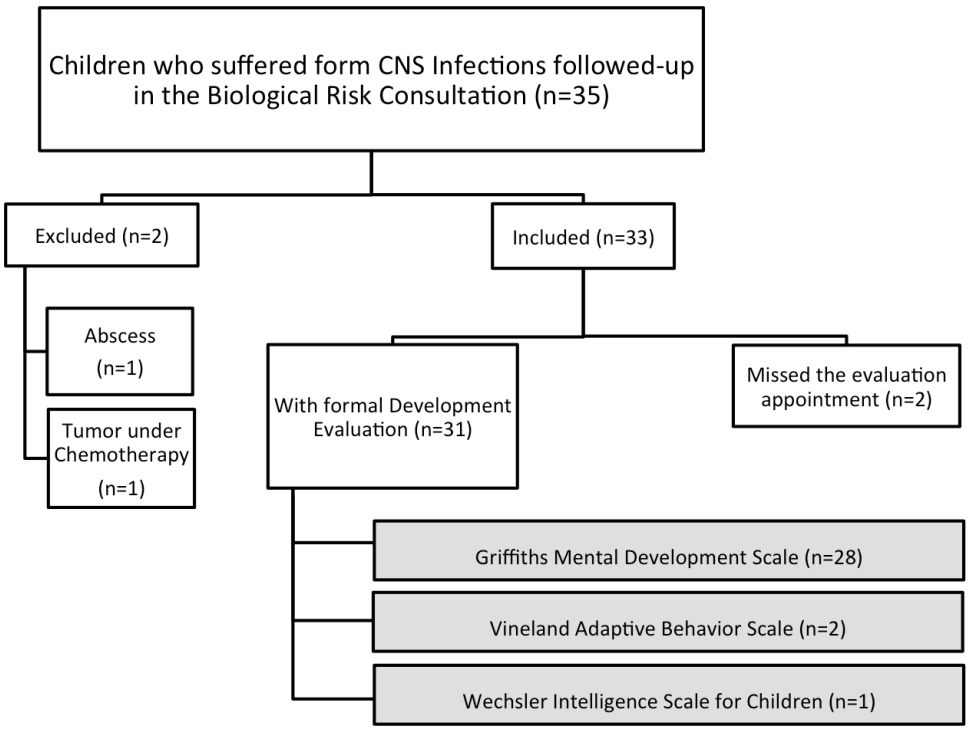


Figure 1: Included and Excluded Subjects. From an initial population of 33 subjects, 2 were excluded for possible confounding factors. From the 33 included subjects, 2 missed the neurodevelopment formal evaluation. Three different instruments were used: *Griffiths Mental Development Scale* [32] (n=28)*, Vineland Adaptive Behavior Scale* [33] (n=2) *and Wechsler Intelligence Scale for Children III* [34] (n=1).

2.2 Clinical and Demographical Data Collection from the hospital digital database

A comprehensive medical record review was performed from the patient history. The following clinical and demographical data were extracted: gender, age of diagnosis (in

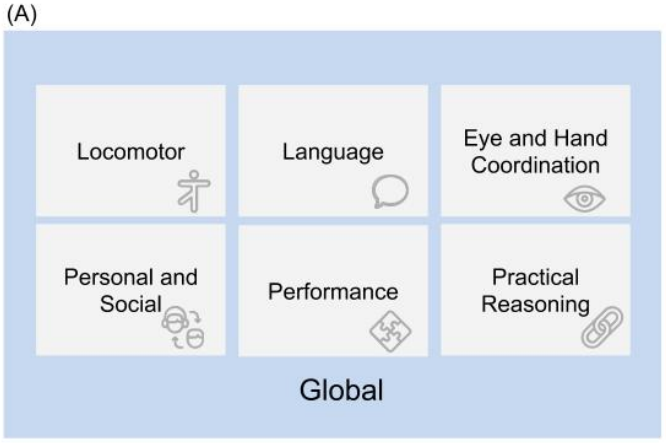
months) and previous medical history (other medical conditions and pregnancy history). Concerning the admission moment the following variables were analyzed: level of consciousness, seizures, meningeal signs, vomiting, cutaneous rash, fever (in degrees Celsius) and the duration of the symptoms before hospital admission (in hours). Cerebrospinal Fluid Parameters (CSF) – total protein concentration (in mmol/L) and glucose concentration (in mg/L) were reviewed. Diagnosis was established based on laboratory workup and clinical judgment.

2.3 Neurodevelopment Evaluation

A trained psychologist performed the formal neurodevelopment evaluation.

Griffiths Mental Development Scale [32] was used for 28 subjects. This evaluation can be applied from 1 to 8 years old, includes six different areas and can be classified in seven different ranges, as shown on Figure 2. Average values are 100±15 (mean ± SD). Practical Reasoning area can only be accessed after 36 months of age.

(A)



(B)

Range	DQ
Very Low	< 70
Low	70-79
Borderline	80-89
Average	90-109
High Average	110-119
High	120-129
Very High	> 129

Figure 2. (A) – *Griffiths Mental Development Scale* [32] includes 6 areas: Locomotor, Hearing and Language, Eye and Hand Coordination, Personal and Social, Performance and Practical Reasoning. The Global Development Quotient is calculated by the mean of the six areas.
(B) – Each Development Quotient (DQ) can be classified from Very Low to Very High in *Griffiths Mental Development Scale* [32]. Intelligence Quotients (IQ) in *Wechsler Intelligence Scale for Children* [34] follow the same distribution.

Two children under 24 months of age were evaluated using the *Vineland Adaptive Behavior Scale* [33] which includes four different domains: (1) Communication, (2) Socialization, (3) Daily living skills and (4) Motor skills. Average values are considered 100 ± 15 (mean ± SD).

Wechsler Intelligence Scale for Children [34] was used for one subject who was above 8 years old. This scale is applicable between the ages of 6 and 17 years old. Five different indexes are evaluated: (1) Verbal Comprehension; (2) Visual Spatial; (3) Fluid Reasoning; (4) Working Memory and (5) Processing Speed. Results are presented in Intelligence Quotients (IQ) and classified based on the range of Figure 2 (B).

2.4 Other complications and Interventions

Hearing impairment and other long-term complications of the infection were also analyzed. Hearing loss diagnosis was established by an otolaryngologist combining both clinical and audiometric data.

Interventional strategies used: Speech Therapy; Physiotherapy; Special needs education; National System for Early Intervention in Childhood were assessed.

Time of follow-up was calculated in months based on the first and last appointment dates.

2.5 Statistical Analysis

Statistical analysis was performed using SPSS Statistics 26.0 Software. *P-value < 0.05* was defined as statistically significant. The normality of quantitative variables was assessed using Shapiro-Wilk test.

A single sample T-Test was used to compare DQs (Global, Locomotor, Personal and Social, Eye and Hand Coordination and Performance) of the sample with reference values. A Wilcoxon Signed-Rank was used for Language and Hearing and Practical Reasoning DQs.

Predictors of adverse long-term outcome were selected based on previous literature. Subjects without *Griffiths Mental Development Scale* [32] evaluation were excluded (n=3) from this analysis. The existence of correlation between (1) Age of diagnosis (in months)*;* (2) glucose concentration in CSF; (3) protein concentration in CSF and (4) duration of symptoms prior to admission (in hours) and DQs was assessed by computing the corresponding Spearman coefficient. The same test was employed to identify a correlation between the age of diagnosis and duration of symptoms prior to admission.

A Mann-Whitney U test was employed to assess whether a difference existed between the DQs in subgroups defined by: (1) gender (male or female); (2) being a neonate (CNS infection before or after 28 days of life); (3) bacterial or viral infection; (4) Infection caused by SP or NM B; (5) altered consciousness and (6) seizures at admission; (7) previously identified comorbidities. This test was applied to understand if any of these characteristics could be classified as an outcome predictor. All age data was analyzed in months of age.

3 Results

3.1 Demographical Characteristics

The study included 33 children following a CNS Infection. Minimum age of diagnosis was 1 day of life, maximum age was 14 years old (median 4 months, IQR: 17). Table I presents the demographic characteristics of the sample.

Table I Demographic characteristics of 33 subjects following a CNS Infection.

		N	Mean	SD	Median	Interquartile Range
Age of CNS Infection		33	16	31	4	17
Age at First Appointment		33	27	30	14	37
Age of Neurodevelopment Evaluation		31	39	21	35	29
Follow-up Time		33	40	38	31	37
Gender	Male	19 (57.6%)				
	Female	14 (42.4%)				

All data is presented in months of age

IQR: Interquartile range

Symptoms at admission varied widely and are summarized on Table II. The most common symptoms at admission were vomiting and irritability (32.1%). Mean temperature at admission was 38.8ºC and symptoms lasted for an average of 20 hours before arrival to the hospital.

Table II Symptoms at admission, temperature and duration of symptoms prior to admission.

Symptoms at admission (N=28)		N (%)
Level of consciousness	Preserved	14 (50%)
	Declined	14 (50%)
Irritability		9 (32.1%)
Vomiting		9 (32.1%)
Meningeal Signs		6 (21.4%)
Seizures		4 (14.3%)
Exanthema	Petechiae	7 (25.0%)
	Purpuric exanthema	2 (7.1%)
	Macular exanthema	1 (3.6%)

Temperature (in ºC) (N=25)		
Mean ± SD	Maximum	Minimum
38.8 ±0.8	40.0	37.5
Duration of Symptoms (in hours) (N=27)		
Mean ± SD	Maximum	Minimum
20 ± 18	72	2

SD: Standard Deviation

Previous comorbidities were identified in five subjects (17.9%). Three were late preterm children, two born at 35 weeks of pregnancy and one at 34. Two individuals had hydrocephaly sonographically detected during pregnancy with placement of ventriculoperitoneal shunt.

3.2 Diagnosis

Lumbar Puncture was performed in 25 patients. Cerebrospinal fluid was collected from the ventriculoperitoneal shunt in two cases. Reports from three patients were not available. Two subjects did not undergo lumbar puncture because they were unstable at admission and later attempts were unsuccessful. These patients had a positive hemoculture and CNS symptoms. Antibiotic therapy was established empirically. Diagnosis was made combining both clinical and laboratory data.

One subject underwent lumbar puncture after the establishment of antibiotic therapy and values were therefore not taken into account.

Protein concentration (in mg/dL) and glucose (in mmol/L) concentration of the CSF were analyzed (Table III).

Table III Protein and Glucose concentrations in CSF of 27 patients diagnosed with CNS Infections.

Cerebrospinal fluid analysis (N=27)				
	Median	IQR	Maximum	Minimum
Protein concentration (mg/dL)	94	88	3235	35.2
Glucose concentration (mmol/L)	3	1	20	0.1

IQR: Interquartile Range

From the 33 children, 25 (75.8%) were diagnosed with bacterial meningitis. From these, the pathogen most commonly identified was NM B, accounting for 10/25 (40.0%) of the cases. The other involved bacteria were: SP (n=4), *Streptococcus agalactiae* (n=2), *Enterococcus faecalis* (n=1) and Escherichia coli (n=1). Five of the children did not have a positive culture for any of the investigated pathogens. These data are summarized on Figure 4..

In one of the cases caused by *Streptococcus agalactiae*, screening for genital infection of the mother was performed at the 37th week of pregnancy. Ampicillin therapy was established during labor. Prenatal data from the second patient was not available.

Four cases of meningoencephalitis and four of viral meningitis were found (Figure 3.). All cases of viral meningitis were caused by enteroviruses. Agents involved in meningoencephalitis were Herpes Simplex Virus type 2 (n=1), enterovirus (n=1), *Klebsiella Pneumoniae* (n=1) and the fourth case did not have an identified pathogen.

Involved pathogens throughout the years are presented in Figure 5..

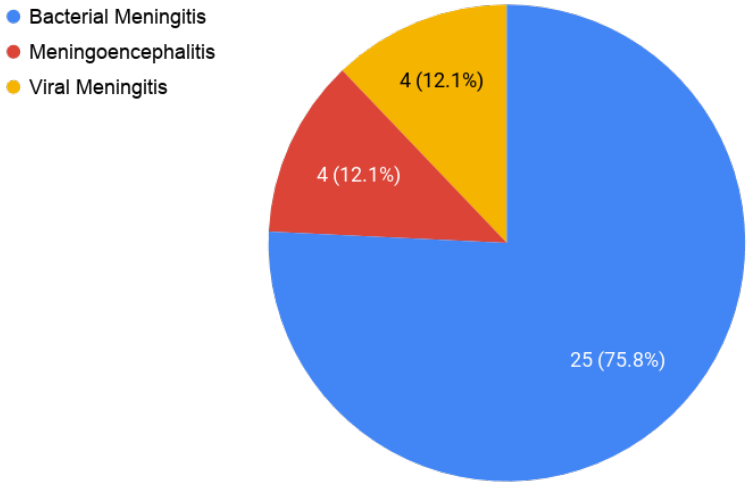


Figure 3. Types of CNS Infections among 33 affected subjects. N (%)

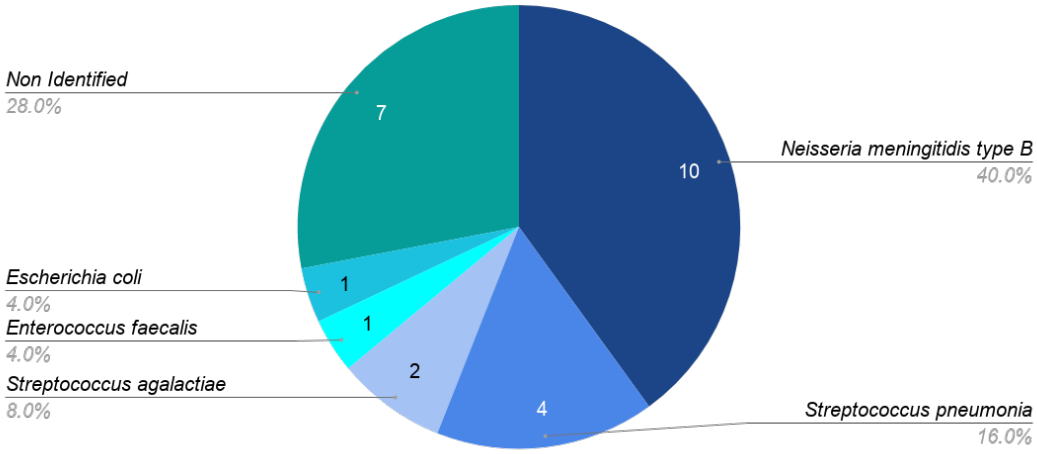


Figure 4. Etiology of Bacterial Meningitis among 25 affected subjects.

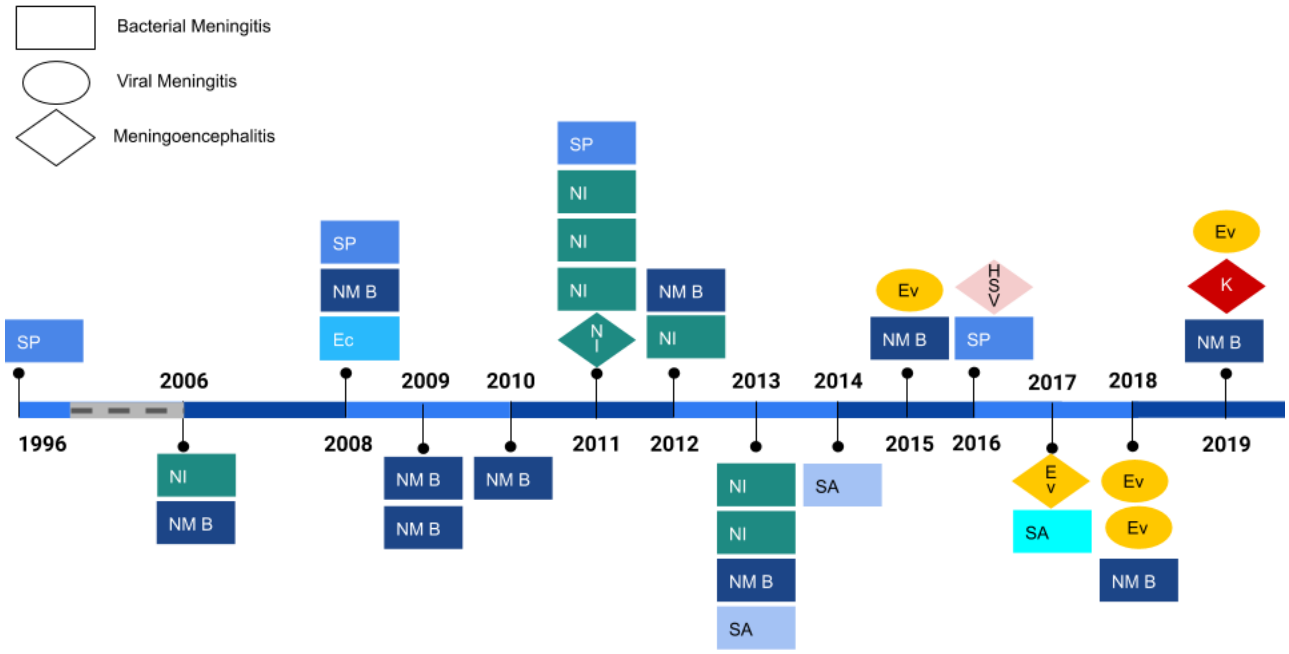


Figure 5. Involved pathogens throughout the years and diagnosis. Ec: *Escherichia coli; EF: Enterococcus faecalis;* Ev: Enterovirus; HSV: Herpes Simplex Virus; K: Klebsiella Pneumoniae; NI: non identified; NMB: *Neisseria meningitidis* serotype B; SA: *Streptococcus agalactiae;* SP: *Streptococcus pneumonia.*

From the four cases identified of Bacterial Meningitis by SP, three were before the introduction of the Vaccine in the Portuguese National Vaccination Program (in 2015). The fourth case was in 2016 and the child was 11 months old, having had two out of the three recommended doses. However, the serotype identified- 10A – is not covered by the immunization.

3.3 Neurodevelopmental Outcome

The subjects underwent formal neurodevelopmental evaluation based on *Griffiths Development Scale* [32] (n=28), *Vineland Adaptive Behavior Scale* [33] (n=2) and Wechsler *Intelligence* [34] (n=1) (Figure 1). Median age of evaluation was 35 months.

a) Griffiths Mental Development Scale

Developmental Quotients mean, SD, median, IQR, maximum and minimum for each area of development are summarized on Table IV. Practical Reasoning DQ was significantly lower than in normal population ($p<0.05$).

Table IV Development Quotients of *Griffiths Mental Development Scale* [32] – Descriptive Statistics of the 6 different Areas of Development.

Areas of Development (DQ)	N	Mean ± SD	Median	IQR	Maximum	Minimum	p-value
Locomotor	28	106 ±19	106	24	133	58	0.095
Personal and Social	28	101±19	104	22	128	51	0.854
Hearing and Language	28	94 ± 22	100	25	121	45	0.527
Eye-Hand Coordination	28	103 ± 19	102	17	134	51	0.467
Performance	28	101 ± 19	100	22	134	56	0.704
Practical Reasoning	**17**	**91 ± 18**	**100**	**18**	**105**	**39**	**0.041**
Global	28	99 ± 18	100	22	123	52	0.776

P-values were obtained using T-Student Test for one sample in Locomotor, Personal and Social, Eye-Hand Coordination and Performance. Wilcoxon Signed Rank Test was used for Hearing and Language and Practical Reasoning DQs because these two did not follow a normal distribution.
DQ: Development Quotient; IQR: Interquartile Range.

Each DQ can be classified from Very Low to Very High (Figure 2).

On Table V it is represented the number of subjects classified by range in each of the *Griffiths* Development Areas. In *Hearing and Language* 8 subjects showed DQ below 90. In *Personal and Social*, 8 were in this situation. Five subjects (18%) presented a Global DQ below 90.

Table V Number of subjects classified by range in each of the *Griffiths* Development Areas. N=28 except for Practical Reasoning, N=17.

	Very Low (<70)	Low (70-79)	Borderline (80-89)	Average (90-109)	High Average (110-119)	High (120-129)	Very High (>129)
Global	2	1	2	15	3	4	1
Locomotor	2	1	1	14	2	6	2
Personal and Social	3	2	1	14	3	0	0
Hearing and Language	5	1	2	15	4	1	0
Eye-Hand Coordination	2	1	1	15	3	4	2
Performance	2	2	1	15	2	4	2
Practical Reasoning	2	2	0	13	0	0	0

Table VI Number of patients with DQ < 90 in areas of Neurodevelopment. N=28

Number of areas affected (DQ < 90)	n (%)
At least one impaired area	11 (39%)
One impaired area	3 (11%)
Two impaired areas	3 (11%)
More than two impaired areas	5 (18%)
None	17 (61%)

b) Vineland Adaptive Behavior Scale

The two children under 24 months evaluated using the *Vineland Adaptive Behavior Scale* [33] presented the scores of Table VII.

Subject B presented Motor Skills below average values (<85). Both had an average Global Adaptive Score.

Table VII Scores of *Vineland Adaptive Behavior Scale* in two subjects following a CNS infection.

Domains	Subject A	Subject B
Communication	89	96
Socialization	104	98
Daily living skills	90	90
Motor skills	99	82
Global	93	88

c) Wechsler Intelligence Scale for Children

The subject evaluated with the *Wechsler Intelligence Scale for Children III* presented in Table VIII. A borderline Full Scale IQ was found (ranges classified as in Figure 2.)

Table VIII - Index Scores of *Wechsler Intelligence Scale for Children III* in one subject evaluated at 8 years old. IQ: Intelligence Quotient

Index (IQ)	Score
Verbal	91
Perceptual Reasoning	90
Full Scale	87
Verbal Comprehension	95
Perceptual Organization	93
Processing Speed	92

3.4 Hearing Loss and other complications

Six patients (18%) presented hearing loss after the CNS Infection. From these, three patients were infected with SP, two with NM B and one with *Klebsiella Pneumonia*. Two of the patients underwent cochlear implant.

Autism spectrum disorder was identified in two patients (6%).

Amputation of the first, second and third toes were recorded in one of the patients who suffered from meningococcal sepsis and meningitis.

3.5 Follow-up, social and interventional strategies

In Coimbra Pediatric Hospital, after the diagnosis of a Central Nervous System Infection and application of specific treatment and management, both a Biological Risk and a Otorhinolaryngology Consultations are scheduled.

In Biological Risk consultation, a multidisciplinary evaluation is done by a neurodevelopmental pediatrician, psychologist, speech therapist and nurse. Based on this evaluation, differentiated supports can be requested: *National system of early intervention in childhood*, speech therapy, physiotherapy and/or special needs education (Table IX).

Table IX Number of subjects beneficiating from different interventional support strategies.

Interventional Support Strategy	Number of subjects
Speech therapy	13 (39%)
National system of early intervention in childhood	7 (21%)
Special needs education	4 (12%)
Physiotherapy	2 (6%)

Mean follow-up time was 40 months (Table I).

From the 33 subjects, 15 (45%) are still on follow-up, 10 (30%) were already discharged (mean age of discharge: 89 months) and 8 (25%) missed the follow-up appointment.

3.6 Predictors of Neurodevelopmental Outcome following a Central Nervous System Infection

a) Association of clinical findings with neurodevelopmental outcome

In order to understand possible risk factors for worse neurodevelopmental outcome in children following CNSI, the correlation between the DQ (for each area- Figure 2A) and four variables was analyzed: (1) age of diagnosis; (2) glucose concentration in CSF (in mmol/L); (3) protein concentration in CSF (in mg/L) and (4) duration of symptoms prior to admission (in hours). Correlations were assessed by calculating the Spearman coefficient (r).

A negative moderate correlation was found between the variables age of diagnosis and Performance DQ (r=-0.397, p=0.037). Between protein concentration and Eye-Hand Coordination, a negative moderate correlation was found (r=-0.499, p=0.18).

No other statistically meaningful correlations were found between the other variables and DQs– Global, Locomotor, Personal and Social, Language, Eye and Hand Coordination and Practical Reasoning. P-values and r are presented on Table X.

Table X Correlation between (1) Age of Diagnosis (in months), (2) Glucose concentration in CSF (mmol/L), (3) Protein concentration in CSF (mg/dL), (4) duration of the symptoms prior to admission (in hours) and Development Quotients analyzed with Spearman Correlation Test.

		Areas of Griffiths Mental Development Scale (in Development Quotients)						
		Global	Locomotor	Personal and Social	Language	Eye-Hand	Performance	Practical Reasoning
Age	r	-.223	-.295	-.340	-.060	-.036	**-.397***	-.063
	p	.254	.128	.077	.763	.856	**.037***	.812
	N	28	28	28	28	28	28	17
Glucose	r	-.077	-.159	-.224	-.082	.155	-.160	.147
	p	.735	.479	.317	.715	.492	.477	.633
	N	22	22	22	22	22	22	13
Protein	r	-.342	-.145	-.128	-.327	**-.499***	-.090	-.214
	p	.120	.519	.571	.138	**.018***	.690	.483
	N	22	22	22	22	22	22	13
Duration of symptoms	r	-.141	-.152	-.213	-.129	-.087	-.192	.557
	p	.541	.510	.354	.576	.709	.404	.060
	N	21	21	21	21	21	21	12

r: Spearman coefficient

*p < 0.05 considered statistically significant

To understand if there was a correlation between the age of diagnosis and the duration of symptoms, the Spearman Correlation coefficient was calculated. A moderate positive correlation was found (r=0.578, p=0.02;).

b) Subgroup Analysis

In order to evaluate if different clinical characteristics were associated with different outcomes, subgroups were defined. A Mann-Whitney U test was employed to assess whether a difference exists between *Development Quotients* in different subgroups:

(A) Male *versus* Female

(B) Neonatal (< 28 days) *versus* non-neonatal (> 28 days) infection

(C) Bacterial *versus* Viral Infection

(D) Infection caused by SP *versus* NM B

(E) Declined *versus* preserved consciousness at admission

(F) With *versus* without seizures at admission

(G) With *versus* without previous comorbidities

Table XI illustrates the obtained p-values.

Statistically significant differences in Personal and Social (p=0.009) and Performance DQs (p=0.048) were found between neonates (infection before 28 days of life) and non-neonates. In Personal and Social Area, mean DQ in neonates was 110 and to in non-neonates 96. Comparing the Performance Scores, mean value was 110 for neonates and 98 for non-neonates.

Between male and female subjects, a statistically significant difference was in the areas of: Eye-Hand Coordination (p=0.008), Practical Reasoning (p=0.048) and Global Development (0.037). Mean values for each gender are represented on Table XII.

No statistically significant differences were found for any of the other development areas or clinical characteristics.

Table XI Are there different outcomes in different subgroups? *P-values* of the application of the Mann-Whitney U test to compare different subgroups based on clinical characteristics.

	Areas of Griffiths Mental Development Scale						
Clinical Subgroups	Global	Locomotor	Personal-Social	Language	Eye-Hand	Performance	Practical Reasoning
Gender (Male *vs* Female) (n=16; n=12)	**.037***	.209	.546	.125	**.008***	.066	**.048***
Neonatal (Neonatal *vs* Non-neonatal) (n=10; n=18)	.068	.076	**.041***	.080	.388	**.035***	.646
Type of infection (Bacterial *vs* Viral) (n=22; n=6)	.741	.674	.631	.857	.400	.307	-
Identified Pathogen (SP vs NM B) (n=3 ;n=8)	.919	.081	1.000	1.000	.539	.151	.638
Level of consciousness (Declined vs Preserved) (n=11; n=12)	.295	.229	.139	.060	.781	.459	.061
Seizures at admission (Present vs Absent) (n=19 ;n=4)	.273	.350	.180	.256	.776	.329	.106
Previous Comorbidities (Present ¶ vs Absent) (n=5; n=23)	.254	.367	.674	.568	.186	.952	.702

NM B- *Neisseria meningitidis* type B; SP: *Streptococcus pneumoniae*; vs: *versus*
- None of the patients of Viral Infections had Practical Reasoning evaluation
¶ preterms (n=3) and hydrocephaly with ventriculoperitoneal shunt (n=2)
*p < 0.05 considered statistically significant

Table XII Mean values of Global, Eye and Hand Coordination and Practical Reasoning DQs between male and female subjects.

	Global	Eye and Hand	Practical Reasoning
Female	107	113	100
Male	93	95	93

5 Discussion

Long-term outcome and neurodevelopmental sequelae of survivors of CNS Infections tend to be underestimated and under-recognized. The two aims of this study were to (1) characterize the neurodevelopmental profile of 33 children who survived a CNS Infection and (2) to identify possible predictors of neurodevelopmental outcome.

Median age of diagnosis was 4 months (IQR: 17). The large range of ages (1 day old to 14 years old) explains the variety of symptoms at admission (Table II). It is known that in newborns, CNS Infections mainly present with lethargy, irritability or refusal to feeding while in older children confusion, vomiting and meningeal signs are usually present [2,6,23].

The frequency of the pathogens involved (Figures 4,5 and 6) illustrates the demographic changes of the past two decades: there are no cases identified of NM C and Hib. The most frequent pathogen involved in bacterial meningitis was NM B, accounting for 40% of cases (n=10). These events are explained by the introduction of the vaccines in the Portuguese Immunization Program: 2000 for Hib, 2006 for NM C and 2015 for SP [2] . It has recently been announced that in 2020 NM B vaccine will also be part of the Program [35], promising the reduction of even more cases of meningitis and, therefore, long-term complications. Although a total of 24% (n=8) of the cases did not have an identified pathogen, it follows the tendency from previous studies, with values ranging from 17% to 59% [15,16,19,20].

All the development areas (of *Griffiths Mental Development Scale* [32]) showed a mean value over 90 (average or superior). Only Practical Reasoning evidenced statistically lower value then normal populations (p=0.041). In our sample, 11 subjects (39%) presented at least one area with DQ borderline or inferior. The most commonly affected areas were *Hearing and Language* and *Personal and Social*. Hearing loss was identified in 6 (18%) patients and Autism Spectrum Disorder in 2 (6%). This frequency is consistent with previous studies, where long-term sequelae ranged from 9.4% to 50% [7–9,12,15,16] . However, conclusions should be taken carefully since the evaluated outcomes of previous studies are heterogeneous, ranging from mild disabilities to major neurological sequelae. On the other hand, different pathogens were taken in to account and different clinical settings were implied, making the results difficult to compare.

The age of diagnosis showed a negative moderate correlation (r=-0.397, p=0.037) with the DQs in the area of Performance. When comparing the groups of neonatal with non-neonates survivors, a difference was found in the areas of Personal-Social and Performance. This data suggests that earlier infections had a less prominent effect in these neurodevelopment areas

then later ones, which does not corroborate with the literature. Either early age as been described as a risk factor for worst outcome [2,11,15,25,27,28] or no correlation with age was found [8,10,31]. Several reasons can be found to justify this difference. First, considered cut-offs were different; second, different outcome instruments were compared (neurological sequelae, sequelae free survival, good or bad clinical outcome). Third, neonates' parents tend to consult healthcare facilities earlier in the evolution of the disease, probably guaranteeing an earlier onset of treatment. In our sample, a moderate correlation (r=0.578) was found between the duration of symptoms and age. However, conclusions should be taken carefully. The small size of the sample is no doubt one of the major limitations.

A negative moderate correlation was found between the protein concentration in the CSF and Eye and Hand coordination (r=-0.499, p=0.018). This finding corroborates with previous data, suggesting that higher protein concentrations are associated with worst outcomes [11,15,27,29]. Protein concentration in CSF has been described as a marker of acute severe disease, which can contribute to the impact in neurodevelopment at long-term [11].

Regarding glucose concentration in the CSF, no correlation was found with DQs. Although it has been described as a possible outcome predictors in previous studies [11,25,28,30], these evaluated bacterial meningitis individually while ours analyzed viral and bacterial infections as whole, resulting on a possible confounding factor.

When it comes to duration of symptoms prior to admission, literature seems to be ambivalent [9,11,18,25,26]. In our sample, no correlation was found between duration of symptoms and development outcome.

Differences were found between male and female gender in the areas of Global, Eye and Hand Coordination and Practical Reasoning (Table XI). Female gender seems to be a predictive factor for better neurodevelopment outcome in these areas. This fact is coincident with previous studies, where male gender as been described as a predictor of worse outcome [11,13,30] .

Although altered consciousness and convulsions at admission were previously cited as definers of worse outcome [6,9–11,15,18,25–29], our sample did not present any difference between subgroups with or without these symptoms. One of the limitations found during our study was that information regarding hospital admission is found hard to synthetize. Emergency Room records are written on text notes, differing from clinician to clinician. In order to be able to compare the different symptoms present at hospital admission (seizures, vomits, meningeal and signs) a checklist could be developed. Glasgow Coma Scale below 8 has been described as a good predictor of long-term neurological sequelae of bacterial meningitis [6,9,10].

Due to lack of register of this scale in medical records it was not possible to investigate its relation.

Comparing viral with bacterial infections, no statistically meaningful difference was found. Although viral infections have been described as benign and limited, with less recorded sequelae then bacterial ones [2,19,21,23,24], the same was not found in our sample. The inclusion of different bacterial and viral agents in each group can explain this incongruence. For example Herpes Simplex Virus, despite being a viral infection has a much higher documented morbidity [2,20,21,23], than enteroviruses. In order to overcome this problem in the bacterial group, a direct comparison between the groups infected with NM and SP was done but no statistically significant difference between the two groups was found.

To overcome the fact that previous comorbidities could consist possible confounding factors, the groups with and without previously diagnosed comorbidities were compared. Our sample included three late preterms and two subjects with hydrocephalus diagnosed before birth (with placement of ventriculoperitoneal shunt). No differences were found between the groups with or without previous comorbidities, sustaining the fact that these were probably not confounding factors.

To our knowledge, this is the first study that investigates the role of possible prognostic factors with each of the neurodevelopmental areas. One of the advantages includes the variety of pathogens involved, bringing a general overview of the CNS Infections outcome. The small size of the sample and the retrospective analysis are no doubt some of the limitations and further studies should be conducted with larger populations.

Our study enlightens the need to establish formal follow-up protocols for children who suffered from CNS Infections. Both Neurodevelopmental and Hearing examinations should be preformed. The identification of possible deficits guarantees early intervention from which both the children and their families can beneficiate.

6 Conclusion

This study described the clinical, demographical and neurodevelopmental characteristics of 33 survivors of CNS infections. Impairment of at least on area of development was found in 39% of the subjects (DQ < 90). Age, gender and protein concentration in CSF were identified as prognostic factors. Survivors from CNS Infections should be followed-up closely to guaranty early identification and intervention of possible neurodevelopmental problems.

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