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Neurodegenerative Langerhans cell histiocytosis: long-term follow-up of 63 patients from the Italian Registry

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ES, IT and CB contributed to study conception and design; MC, AC and LB contributed to biological analysis; CB and KR contributed to neurological and neurophysiological examinations; MM and AP contributed to radiological examinations; MT contributed to data analysis and interpretation; IT and ES retrospectively reviewed patient records and contributed to data collection; ES, IT, FP and CB wrote the paper. All authors revised the paper and approved the submitted version

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Abstract

Neurodegenerative Langerhans cell histiocytosis (ND-LCH) is a potentially devastating complication of LCH. We analyzed the natural history and the long-term outcome of patients with ND-LCH enrolled in the Italian LCH registry.

ND-LCH was diagnosed in 63/637 patients with LCH (10%). Overall, at ND-LCH diagnosis 60% (38/63) patients were asymptomatic, 24% (15/63) had mild clinical manifestations including abnormal neurological examination and/or evoked potentials, and 16% (10/63) had overt symptoms. Brain MRI showed progressive structural changes in 13/63 (21%) patients over a median time of 1.5 years. Clinical ND-LCH developed after a median of 2.5 years since ND-LCH diagnosis. 30/63 patients (17 pauci-symptomatic, 11 symptomatic, two asymptomatic but with severe brain MRI) received treatment, and 17/30 (57%) were stable or improved at the last follow-up). 33/63 patients (mostly asymptomatic) were not treated and 31/33 (94%) remained stable through follow-up. At univariable analysis, the risk of developing overt clinical symptoms increased with LCH reactivations (OR 6.40, p=0.018), severe brain MRI abnormalities at ND-LCH diagnosis (OR 10.40, p<0.001), and MRI findings worsening during follow-up (OR 10.25, p=0.001). The association of overt ND with reactivations and MRI findings worsening was confirmed at multivariable analysis (ORs of 8.15, p=0.040, and 7.31, p=0.034, respectively).

In conclusion, asymptomatic patients presenting with mild radioneuroimaging lesions at ND-LCH onset remained stable during follow-up; conversely, a history of LCH reactivation and worsening of brain MRI findings were associated with the appearance of overt clinical symptoms. These results may lay the basis for patients selection for treatment and different monitoring strategies.

Introduction

Langerhans cell histiocytosis (LCH) is an inflammatory myeloid disorder driven by activating mutations in the MAPK pathway (e.g., *BRAF*^{V600E}). The clinical presentation is highly heterogeneous ranging from self-healing single lesions to life-threatening multisystem disease. Some patients develop a debilitating, slowly progressive neurodegenerative complication (ND-LCH). Based on histopathological findings, ND-LCH appears to be driven by inflammatory signals[1]. However, mutations of the MAPK pathway seem to be involved in disease pathogenesis, although the specific mechanisms are still debated[2-4].

In historic cohorts, ND-LCH prevalence ranged from 1% to 24%[5-7] and typically affected patients with DI and CNS-risk bone lesions[7]. Recently, lesional *BRAF*^{V600E} status was suggested as an additional risk factor[7].

Imaging and/or clinical findings consistent with ND-LCH can arise at LCH diagnosis but more commonly occur years after LCH onset[5,8,9]. ND-LCH is characterized by a typical magnetic resonance imaging (MRI) pattern of bilateral and symmetric T2 and FLAIR hyperintense lesions in the cerebellar grey matter, sometimes extending to the underlying white matter, in the basal ganglia and brainstem[1,5,8,9]. Despite homogeneous MRI findings, the clinical presentation is widely heterogeneous. Symptoms may include ataxia, dysarthria, tremors, behavioral changes, learning impairment, or psychiatric disorders. The disease can remain stable over many years or progress and cause severe disability[5,10]. However, patients have been rarely evaluated over a long-term period to analyze natural history and long-term outcome.

An effective treatment is still lacking. Several approaches were proposed for the treatment of symptomatic ND-LCH obtaining, at best, a stabilization of neurological symptoms. There is, thus, an urgent need to prevent clinical neurodegeneration, by earlier detection and improved methods to monitor the disease course and treatment response. A standardized

diagnostic and monitoring approach based on neurologic assessment and evoked potentials was proposed by our group to early detect and monitor the disease in pauci-symptomatic or asymptomatic patients[10,11]. More recently, cerebrospinal fluid (CSF) neurofilament light have been proposed as possible biomarker for ND-LCH[12].

Herein, analyze the natural history of ND-LCH in a large cohort enrolled in the Italian LCH registry. We describe their clinical presentation over a long-term period and identify predictors of ND-LCH progression to overt clinical manifestations.

Methods

Study population

Patients diagnosed with ND-LCH between 2000 and 2023 in nine centers of the AIEOP network and enrolled in the Italian LCH Registry (RICLa) coordinated at the Meyer Children's Hospital IRCCS in Florence (Italy) were considered for study inclusion.

ND-LCH is defined based on the typical MR pattern. As part of a prospective diagnostic study ongoing in Italy since 2010[10], all patients with ND-LCH or risk factors for ND-LCH underwent baseline evaluation including structural 3T MRI and MR spectroscopy (MRS), neurological examination (NE) with the Scale for ataxia (SARA) and neurophysiologic assessments with somatosensory evoked potentials (SEPs) and brainstem evoked potentials (BAEPs). Patients were subsequently followed using the same multidisciplinary diagnostic protocol every 6-12 months[11].

Patients were considered eligible for inclusion in the study whether they had brain MRI findings consistent with ND-LCH and a minimum follow-up of 6 months since diagnosis. Although most patients included in the registry were children, young adults (<25 years at ND-LCH diagnosis) were also considered for study inclusion.

Outcome definition

Patients were defined as “asymptomatic” if they had MRI findings consistent with ND-LCH and a normal neurological and neurophysiological (SEPs and BEAPS). Patients with evoked potential abnormalities and/or an abnormal neurological examination but without overt symptoms were defined as having “mild” impairment; patients who had neurological or psychiatric symptoms were defined as having “overt clinical” impairment. Throughout the manuscript, the term ND-LCH encompasses these three categories.

MRI grading was defined as previously described[10]. Here, we defined MRI abnormalities as “mild” if the grading was 1-2 and “severe” if the grading was 3-4. Any increase in MRI grading or in the number and/or extensions of ND lesions was considered as worsening of MRI findings. Neurological examination was considered as showing clinical worsening if new neurological signs or symptoms appeared or the SARA score increased by at least three points, and clinically improved if any neurological signs or symptoms disappeared or the SARA score was decreased by at least three points. BAEPs findings were considered to have worsened if they became abnormal (*i.e.,* low amplitude, delayed latency, or absence of III or IV potentials according to reference values) or if there was increased abnormality of previously abnormal responses. SEPs were deemed as worsened if worsening of at least one response (*i.e.,* latency, amplitude) could be demonstrated. BAEPs and SEPs were considered as improved in case of improvements up to normalization of latency and/or amplitude.

The result of each follow-up assessment, in both treated and not treated patients, was defined as a composite end-point, combining imaging, clinical, and neurophysiological response criteria, and was categorized as improved, stable, or worsened in comparison to previous results. Changes in at least one criterion were requested for response evaluation.

Data collection and statistical analysis are reported in the supplementary file

Ethics statement

The study was approved by the Pediatric Ethics Committee of the Tuscany Region, Italy, and was carried out following the ethical standards of the 1964 Declaration of Helsinki and its later amendments.

The study was conducted within the Italian LCH Registry (RICLa).

Written informed consent was obtained from the parents of affected children or directly from the patients if they were older than 18 years of age

Results

Study population

Of the 637 patients enrolled in the Italian LCH Registry, 63 were diagnosed with ND-LCH. Their baseline characteristics are detailed in ***Table 1***. Thirty-nine patients were male (62%). The median age at LCH diagnosis was 22 months (IQR 10-39). Twenty patients (32%) had single-system LCH and forty-three (68%) multisystem LCH, including thirteen (20%) with risk-organ involvement. Organ involvement at LCH diagnosis included lytic bone lesions (n=54, 86%), skin (n=29, 46%), liver (n=11, 17%), bone marrow (n=8, 13%), spleen (n=7, 11%), tumor-like central nervous system (n=1, 2%), intracranial bone lesions (n=4, 6%), lymph nodes (n=4, 6%), bowel (n=2, 3%), and lungs (n=1, 2%). Three patients (5%) had clinical and pathological features consistent with mixed Erdheim-Chester disease (ECD)-LCH. Out of the 55 tested patients 44 (80%) harbored the $BRAF^{V600E}$ mutation in their biopsy. Reactivations were reported in 41 patients (65%).

First-line treatment for LCH included chemotherapy according to LCH-III (NCT00488605) and LCH-IV (NCT02205762) protocols in 30 (48%) and 29 (46%) patients, respectively. Among the remaining patients, three (5%) received local therapy (curettage/topical steroid) and one (2%) did not receive any treatment. Most patients (n=39, 62%) required more

than one line of treatment due to disease progression or reactivation during follow-up. Second- or third-line therapies consisted of chemotherapy (n=27), BRAFi (n=15), indomethacin (n=3), and bone marrow transplant (n=1).

ND-LCH was diagnosed at a median age of 59 months (IQR 44-108), with a delay of 35 months (IQR 17-62) from LCH diagnosis. Of note, nine patients developed neurodegeneration within six months of LCH diagnosis. Fifty-one patients (81%) had known risk factors for ND-LCH (*i.e.*, diabetes insipidus and/or craniofacial bone lesions). Out of the twelve remaining patients, the *BRAF*^{V600E} mutation was found in the 11/11 who were evaluable. However, we could not find significant differences in the timing of ND-LCH onset depending on lesional *BRAF*^{V600E} status, disease extension, and the presence of known ND-LCH risk factors (***Figure S1***). As previously reported, seven patients (11%) developed ND-LCH during vemurafenib treatment for LCH or immediately after its discontinuation[13].

Clinical presentation at ND-LCH diagnosis

At ND-LCH diagnosis, thirty-eight patients (60%) had only imaging lesions consistent with ND-LCH (***Figure 1***). MRI abnormalities were mild (grading 1-2) in twenty-six patients (68%) and severe (grading 3) in the remaining twelve (32%), while none had an MRI grading of 4. All but one patient showed radiological cerebellar involvement, while 11 patients (29%) had severe brainstem abnormalities at brain MRI. At ND-LCH diagnosis, no patients had concomitant active brain tumorous lesions.

Fifteen patients (24%) had mild clinical manifestations, consisting of abnormal evoked potentials in all patients: SEPs in seven patients (47%), BAEPs in two (14%), and both EP modalities in six (40%). Abnormalities included delayed responses or reduced to absent amplitudes. In addition, 10 patients had an abnormal neurological assessment. In 12 patients (80%) low-grade MRI abnormalities were observed (***Figure 1***).

Ten patients (16%) had overt clinical symptoms at ND-LCH diagnosis. Cerebellar symptoms were reported in six patients and included ataxia, dysmetria, tremor, and dysarthria. Cognitive impairment was reported in four patients and behavioural/psychiatric manifestations in three. Finally, two patients had brainstem involvement with VI and VII cranial nerves palsy, dysarthria, dysphagia, and pyramidal signs. Brain MRI showed severe abnormalities in seven patients (***Figure 1***).

Natural history

Patients were followed for a median of eight years since ND-LCH diagnosis (IQR, 3.5-10.5). Of the 38 patients who were asymptomatic at ND-LCH diagnosis, 27 (71%) remained asymptomatic during follow-up. One of them had severe MRI abnormalities at baseline, that and was treated with BRAFi for ND-LCH after worsening of MRI findings was noticed. Six of the 38 (16%) patients developed abnormal evoked potentials; four of them were treated with IVIG and two did not receive any treatment. Both untreated patients experienced deterioration of neurological findings and EP responses (***Figure 1A***). The remaining five patients (14%) developed overt clinical symptoms (cerebellar in all, cognitive in two, and severe brainstem involvement in one). Of note, all these patients had severe MRI abnormalities at diagnosis. Three patients were started IVIG, chemotherapy, and BRAFi, respectively at development of clinical signs. In contrast, the remaining two patients experienced further clinical and imaging deterioration and later received IVIG or BRAFi.

Out of the 15 patients with mild ND-LCH at diagnosis, two (13%) rapidly worsened to overt clinical symptoms and received IVIG. Ten patients received IVIG to prevent clinical progression. The remaining three patients received no treatment and remained stable through follow-up (***Figure 1B***).

Of the 10 patients who were symptomatic at ND-LCH diagnosis, three remained stable without therapy. The remaining seven received IVIG (n=5), BRAFi (n=1), or a combined regimen (IVIG+BRAFi, n=1) (***Figure 1C***).

Overall, 33/63 patients did not receive any treatment. In this group, brain MRI abnormalities were mild in 26 patients (79%). Out of the 33 untreated patients, 27 were asymptomatic (82%), three had mild ND (4%), and three (4%) had overt clinical symptoms. At a median time of six years (IQR, 1-9.5) from ND-LCH diagnosis, 30 of these 33 untreated patients (90%) remained stable (***Figure 2***). The three patients who worsened had severe brain MRI abnormalities at ND-LCH diagnosis.

Treatment

Thirty patients (48%) received treatment for ND-LCH, which was started at a median time of 30 months (IQR, 3-72) from ND-LCH diagnosis (***Figure 2***). Indications for treatment, according to the referral centers, included abnormal evoked potentials in 14 patients, overt clinical symptoms in 14, and severe MRI findings in two asymptomatic patients. First-line treatment included IVIG in 23 patients and chemotherapy in three, while the remaining four patients received BRAFi alone or combined with IVIG (three and one patient, respectively) (***Table 2***).

Of the 23 patients treated with IVIG, 12 remained stable (n=5) or improved (n=7) at the last follow-up. Responses consisted of improved EP responses (n=2), neurological improvement (n=1), or both (n=4). The remaining 11 patients experienced deterioration of clinical (n=3) or MRI findings (isolated in one patient and associated with worsening of neurological or neurophysiological findings in seven). Six of these 11 patients were already symptomatic at treatment initiation. Second-line treatments were used in six patients (BRAFi in three and IVIG+BRAFi, BRAFi+MEKi, and chemotherapy in one each). One patient treated with BRAFi and the patient who received chemotherapy showed further

worsening and received third-line treatment with BRAFi+MEKi, both achieving stabilization (***Figure 2***).

Three patients received BRAFi as first-line treatment, and two of them achieved disease stabilisation; the third patient, who had mild abnormalities at diagnosis, experienced progression of abnormal imaging findings. Finally, three patients were treated with first-line LCH-directed chemotherapy: one of them was asymptomatic at the beginning of treatment and remained stable through follow-up; the remaining two patients experienced further deterioration and thus received combined MAPKi therapeutic regimens, achieving clinical and radiological stabilization (***Figure 2***).

Time to event analysis and predictors of overt clinical symptoms

Brain MRI remained stable in 50 patients (79%) and worsened in 13 (21%) at a median time from diagnosis of 1.5 years (IQR, 1-5) (**Figure S2**). Representative MRI images of ND-LCH at diagnosis and during follow-up are shown in ***Figure 3***.

Out of the 63 included patients, 18 developed overt ND-LCH (28%). Ten patients were already symptomatic at diagnosis, while the remaining eight were asymptomatic (n=5) or had mild abnormalities (n=3). They developed clinical ND-LCH after a median of 2 years (IQR 1-4) from the ND-LCH diagnosis. We identified significant differences in the development of overt clinical ND-LCH in patients with or without reactivations (log-rank p=0.032), in patients with and without severe MRI brain abnormalities at ND-LCH diagnosis (log-rank p<0.001), and with and without MRI worsening during follow-up (logrank p<0.001) (***Figure 4***).

At univariable Cox regression analysis, the risk of development of overt clinical symptoms increased with disease reactivations (OR 6.40, 95%CI 1.31-31.16, p=0.018), severe brain MRI abnormalities at ND-LCH diagnosis (OR 10.40, 95%CI 2.94-36.81, p<0.001) and MRI worsening during follow-up (OR 10.25, 95%CI 2.58-40.79, p=0.001) (***Table 3***). The

predictive value of disease reactivation and MRI worsening was confirmed at multivariable analysis, with an OR of 8.15 (CI 1.38-87.6; p=0.040) and of 7.31 (CI 1.29-59.3; p=0.034), respectively (***Table 3***).

Discussion

We report a cohort of 63 patients with ND-LCH followed through a standardized approach over a long-term period and describe the natural history of this condition, which ranges from clinically asymptomatic to severe and progressive neurological impairment. We also analyze the response to different treatments and identified novel predictors of overt clinical ND-LCH.

ND-LCH is a challenging consequence of LCH that can develop many years after disease onset. In our cohort of 637 patients with LCH enrolled in the Italian Registry, we identified 63 patients (10%) with an imaging diagnosis of ND-LCH, made at a median delay of 2.9 years from LCH. Some patients developed ND-LCH within a few months from LCH diagnosis, while up to 25% even after five years. These findings are consistent with previous studies in which imaging findings of ND-LCH were identified at a median of 2.6 after LCH diagnosis[5]. The frequency of ND-LCH in our cohort is in line with previous studies (4-24%), which however were mostly conducted at single centers and in small LCH cohorts[8,9]. Moreover, it is challenging to identify the real incidence of ND-LCH, as brain MRI was usually performed only in patients with known risk factors for ND or with neurological or psychiatric symptoms.

ND-LCH typically affects patients with DI and/or CNS risk lesions but *BRAF*^{V600E} was proposed as an additional risk factor[7]. In our cohort, 80% of patients had known risk factors for ND-LCH. Of the remaining 12 patients, most had a multisystem disease and experienced multiple reactivations, and all the tested patients harbored the *BRAF*^{V600E} mutation. Of note, in some patients, ND-LCH developed despite vemurafenib treatment[11].

Therefore, we believe that the indication for ND screening by brain MRI should be extended to patients with *BRAF*^{V600E} mutation, particularly if with multisystem or reactivating disease. According to our findings, these patients should be seen at least annually for 10 years after LCH is diagnosed.

The clinical presentation of ND-LCH in our cohort was heterogeneous. Most patients (60%) were completely asymptomatic at diagnosis, and they typically presented with mild abnormalities on brain MRI[10]. In an area where it is difficult to parse into clear categories, the use of a standardized multidisciplinary protocol allowed us to clearly differentiate patients with only MRI abnormalities (defined as asymptomatic) from those with mild to overt clinical manifestations. The group of patients defined as mild presented at diagnosis with EP abnormalities variably associated with subclinical neurological impairment. This group accounted for about 25% of our cohort and usually presented with mild MRI findings. Finally, 15% of patients had overt clinical symptoms at diagnosis, ranging from subtle deficits such as gait and behavioural disturbances to severe cerebellar impairment, spastic quadriparesis or overt psychiatric symptoms. Compared to previous studies[14,15], we report a lower rate of psychiatric symptoms, accounting for around 5% of patients in our cohort. This proportion could be underestimated because neuropsychiatric evaluation was not routinely applied in our cohort. Most of these patients had severe MRI abnormalities at ND-LCH diagnosis. At the last follow-up, the prevalence of overt clinical ND-LCH in the Italian LCH cohort was 2.8% (18/637), representing 28% of ND-LCH, in range with the largest available cohorts[5,7].

In most instances, asymptomatic patients remained stable through follow-up, but a few of those with severe MRI grading at diagnosis later progressed and developed mild or overt clinical ND. Most patients with mild ND received monthly IVIG to prevent progression to overt clinical manifestations, which was effective in most. However, we were not able to infer whether EP abnormalities could predict the development of clinical symptoms.

Eight patients developed overt clinical symptoms during follow-up, at a median time of 2.5 years from ND-LCH diagnosis. As mentioned, five of them were asymptomatic but had severe brain MRI abnormalities, while three had mild ND-LCH at diagnosis. Although most of them developed overt clinical symptoms early during follow-up, late events seldom occurred. This observation reinforces the need for a long-term periodic assessment of these patients for at least 10 years after ND-LCH diagnosis.

The development of overt signs and symptoms is the main concern in ND-LCH, considering their impact on the quality of life and refractoriness to treatment. It is commonly agreed that treatment should be initiated before the onset of clinical ND-LCH[10,16,17] and thus we sought to identify factors associated with overt clinical manifestations. We observed that the risk of developing overt clinical symptoms increased with disease reactivations, severe brain MRI abnormalities at ND-LCH diagnosis, and MRI worsening during follow-up. However, as most patients with MRI worsening already had severe brain MRI abnormalities, only disease reactivations and worsening of brain MRI findings were confirmed at multivariable analysis. Indeed, our data confirm the clinical relevance of LCH reactivation in the context of permanent consequences as previously reported[18]. Thus, patients who have a history of disease reactivations, severe abnormalities of brain MRI at diagnosis and/or MRI worsening during follow-up should be carefully monitored and could benefit most from early treatment. Conversely, asymptomatic patients with mild brain MRI findings at diagnosis are less likely to progress and require therapeutic interventions and can be followed less frequently. Neurophysiological abnormalities are a valuable monitoring tool in ND-LCH, but we could not evaluate their role as predictors of clinical ND as most patients with EP abnormalities (mild ND-LCH) received treatment that could have affected the clinical evolution.

The efficacy of treatment for ND-LCH is a very challenging and poorly known subject and was not a primary aim of the present study. However, although no definitive conclusion

can be drawn, we sought to report individual responses that may be of support for clinician decisions. Our findings confirm that IVIG can be effective, if started early, to prevent disease progression in patients with mild brain MRI abnormalities and neurophysiological impairment, as previoulsy reported[11]. Conversely, IVIG monotherapy was ineffective in most symptomatic patients, while targeted therapies could be beneficial, despite, in our experience, this strategy was mostly used in patients with severe ND. Prospective trials will be necessary to validate the efficacy of targeted treatments both in the prevention and treatment of ND-LCH. In this regard, the identification of early clinical and biological predictors is essential. Recently, cerebrospinal fluid biomarkers including osteopontin and neurofilament light (NFL) have been associated with ND-LCH[12,19], and NFL was proposed as a promising biomarker to monitor the response to MAPK inhibitors[12]. In addition, *BRAF*^{V600E} positivity in cell-free DNA or in mononuclear peripheral blood cells has been associated to poor prognosis in patients with LCH and its role in ND has been suggested[19]. Overall, these findings support an integrated clinical, neuroimaging, neurophysiological, and biological assessment in patients with ND-LCH, which should be modulated based on known and novel risk factors and predictors of clinical deterioration.

The study has limitations, which include the heterogeneous follow-up duration and the introduction of a standardized monitoring protocol only in 2010, which limits the neurophysiological data in older patients. In addition, biological data were limited to *BRAF*^{V600E} lesional mutation because of the old study population. Finally, we were unable to properly analyze the response to treatment, due to heterogeneous indications and therapeutic strategies.

In conclusion, ND-LCH is not rare and can develop in patients without classical risk factors. Patients presenting with mild MRI findings and no EP abnormalities showed a smoldering disease course, while patients with a history of LCH reactivations and severe MRI abnormalities were prone to imaging and clinical progression. These findings should

be integrated with novel biomarkers to help identify and effectively treat patients at risk of developing clinical ND-LCH**.**

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Male, n (%)	39 (62)
Age at LCH diagnosis (months), median (IQR)	22 (10-39)
Age at ND-LCH diagnosis (months), median (IQR)	59 (44-108)
Difference between LCH and ND-LCH diagnosis (months)	35 (17-62)
Risk factors for ND-LCH, n (%) only DI only CBL DI and/or CBL	 8 (13) 14 (22) 51 (81)
BRAFV600E, n (%)	44/55 (80)
Disease classification, n (%) SS MS-RO- MS-RO+	 20 (32) 30 (48) 13 (20)
Disease reactivation during follow-up, n (%)	41 (65)

Table 1 - **Baseline clinical characteristics of the 63 patients.** LCH: Langerhans Cell Histiocytosis; ND-LCH: neurodegenerative Langerhans Cell Histiocytosis; IQR: interquartile range; DI: diabetes insipidus; CBL: craniofacial bone lesion; SS: single-system; MS: multi-system; RO: risk organ.

	First-line		Second-line		Third-line	
	n (%)	Re or St (%)	n (%)	Re or St	n (%)	Re or St
IVIG	23 (77)	12 (52)	-	-	-	-
CT	3 (10)	1 (33)	1 (12.5)			
cladribina	2			-	-	-
ARA-C+VCR	1	1				
6-MP+MTX			1			
MAPKi	4 (13)	3 (23)	7 (87.5)	5 (71)	3 (100)	3 (100)
vemurafenib	2	2	4	2		
dabrafenib	1		1	1		
cobimetinib					1	1
trametinib						
vemu+IVIG	1	1	1	1		
dabra+trame			1	1	2	2

Table 2 - Treatment and response. IVIG: intravenous immunoglobulin; CT: chemotherapy; MAPKi: MAPK inhibitors; ARA-C: cytarabine; VCR: vincristine; 6-MP: 6 mercaptopurine; MTX: methotrexate; Re: remission; St: stabile disease.

	Univariable		Multivariable	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Male	0.36 (0.10-1.26)	0.152		
Risk factors for ND-LCH only DI only CBL DI and/or CBL	 1.15 (0.38-3.51) 1.30 (0.39-4.33) 0.76 (0.20-2.92)	 1.00 0.770 0.720		
BRAFV600E	0.66 (0.16-2.65)	0.907		
Disease classification SS MS-RO- MS-RO+	 2.67 (0.71-9.95) 0.73 (0.11-4.68)	 0.204		
Disease reactivation	5.40(1.31-31.16)	**0.018**	8.15(1.38-87.6)	**0.040**
Brain MRI grading 3-4	10.40(2.94 - 36.81)	**<0.001**	4.18(0.97-19.0)	0.056
Brain MRI findings worsening	10.25(2.58 – 40.79)	**0.001**	7.31(1.29-59.3)	**0.034**
Mild ND-LCH	1.75(0.56 - 5.48)	0.405		

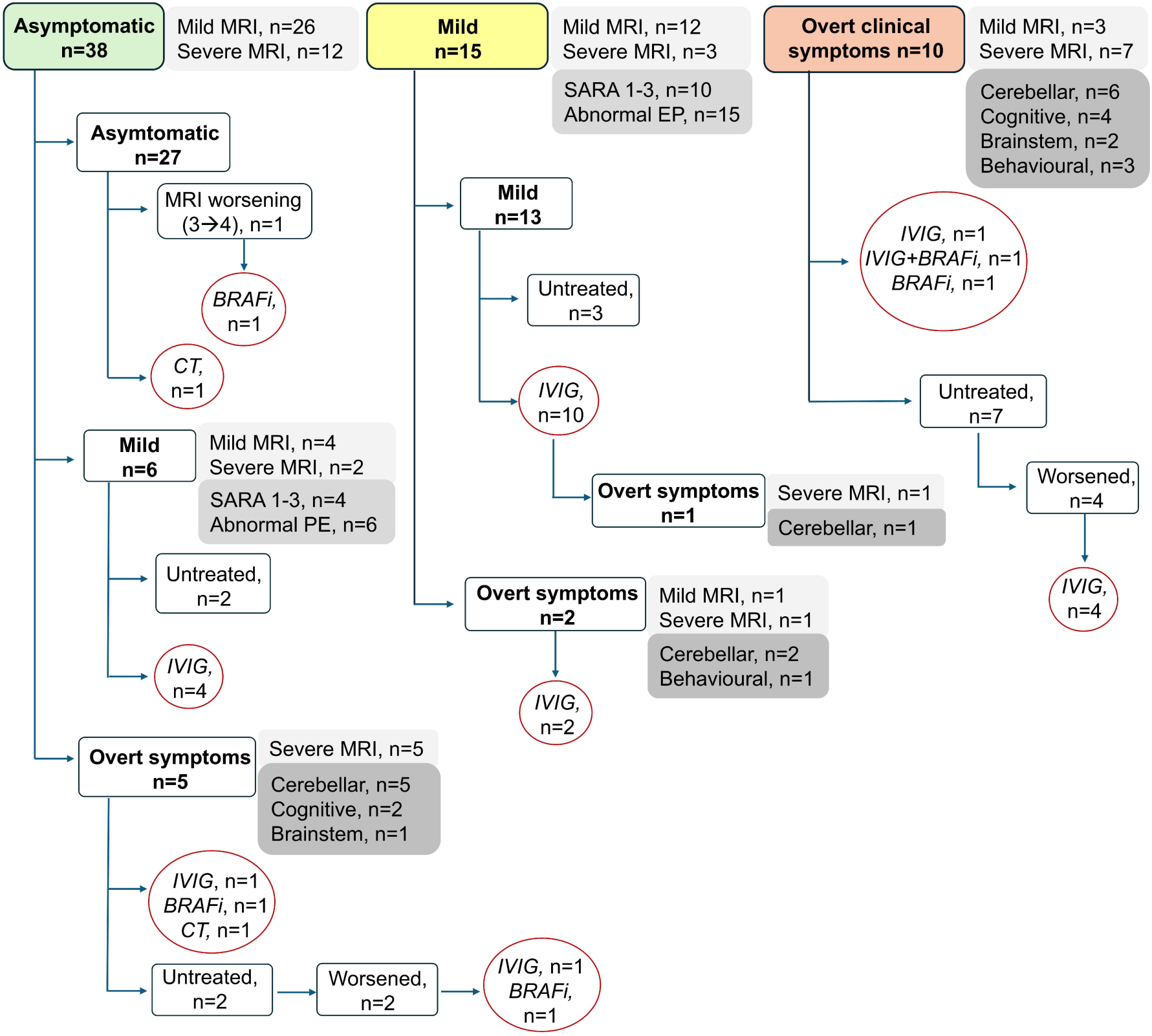
Table 3 - Predictors of clinical ND-LCH. LCH: Langerhans Cell Histiocytosis; ND-LCH: neurodegenerative Langerhans Cell Histiocytosis; IQR: interquartile range; DI: diabetes insipidus; CBL: craniofacial bone lesion; SS: single-system; MS: multi-system; RO: risk organ; MRI: magnetic resonance imaging; OR: odd ratio; CI: confidence interval

Figure 1 - Flow Chart of natural history of ND-LCH patients. A: asymptomatic patients; B: patients with mild impairment; C: patients with overt clinical symptoms. MRI: magnetic resonance imaging; CT: chemotherapy; IVIG: immunoglobulin; BRAFi: BRAF inhibitors; SARA: scale for ataxia; EP: evoked potentials.

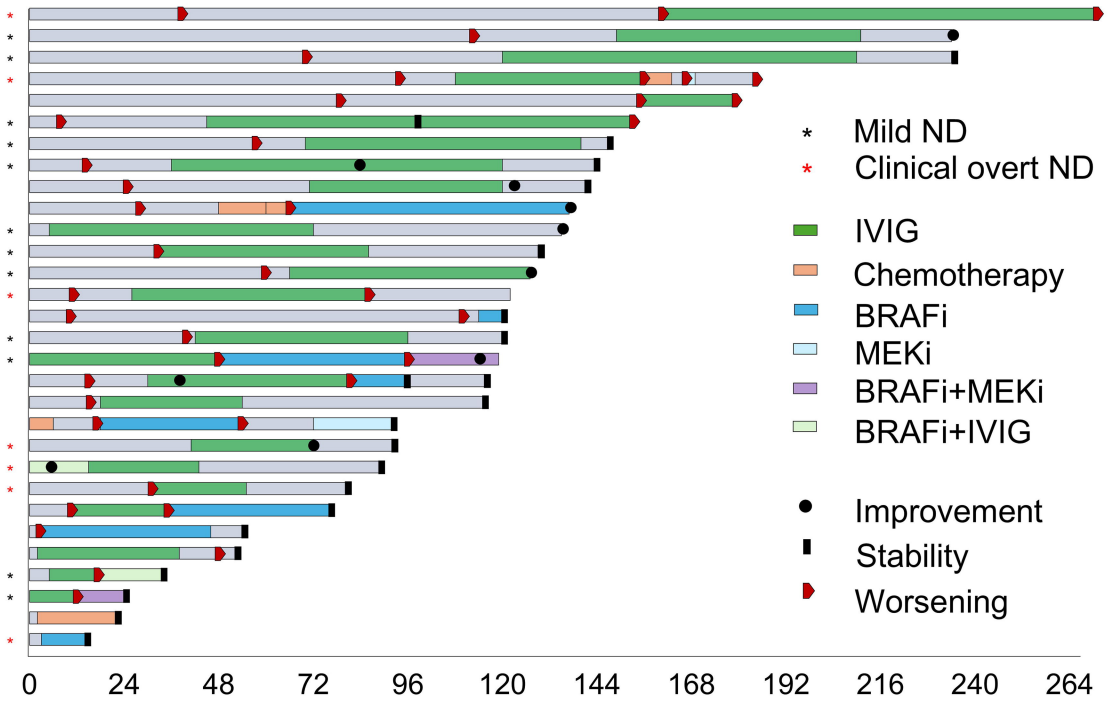
Figure 2 - Swimmer plot of treated (A) and untreated (B) patients. IVIG: immunoglobulin; BRAFi: BRAF inhibitors; MEKi: MEK inhibitors.

Figure 3 - Typical brain magnetic resonance imaging findings. A–C: progressive worsening of brainstem abnormalities observed over 10 years follow-up; D: cerebellar hemispheres and dentate nuclei; E: dentate nuclei; F: hippocampi; G: dentate nuclei; H: caudate nuclei; I: brainstem.

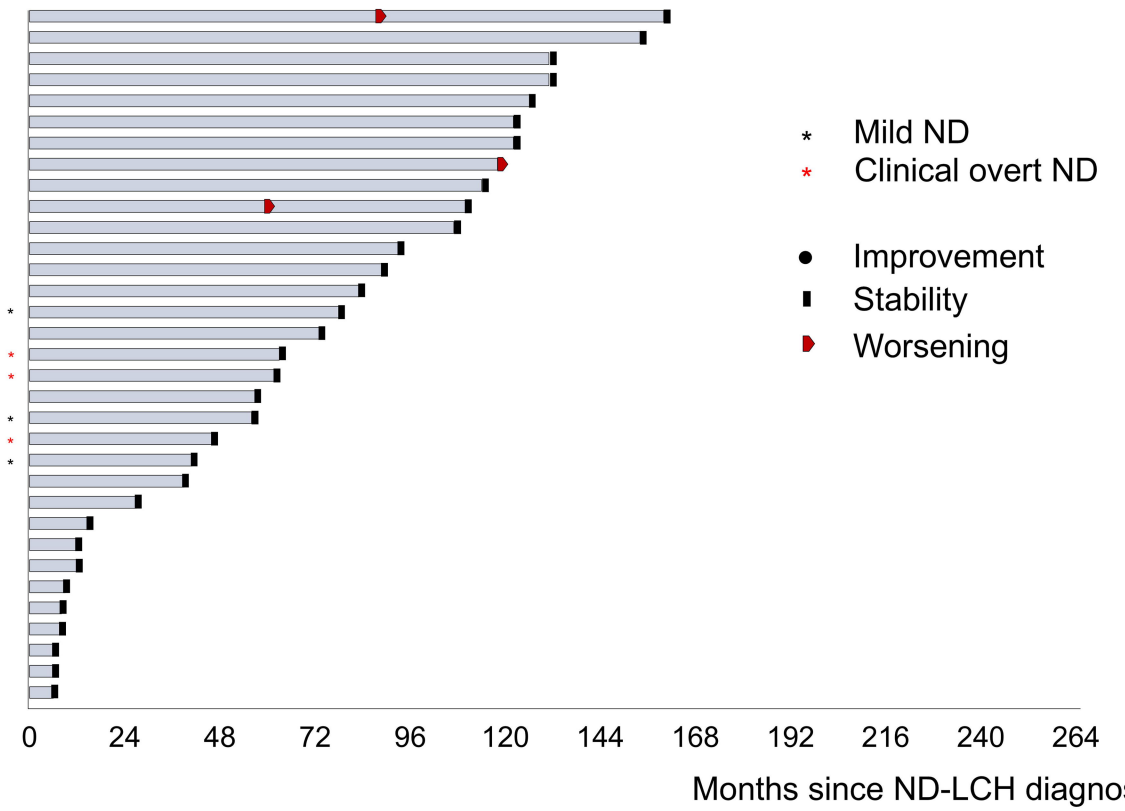
Figure 4 - Kaplan-Meier time to overt ND-LCH. MRI: magnetic resonance imaging

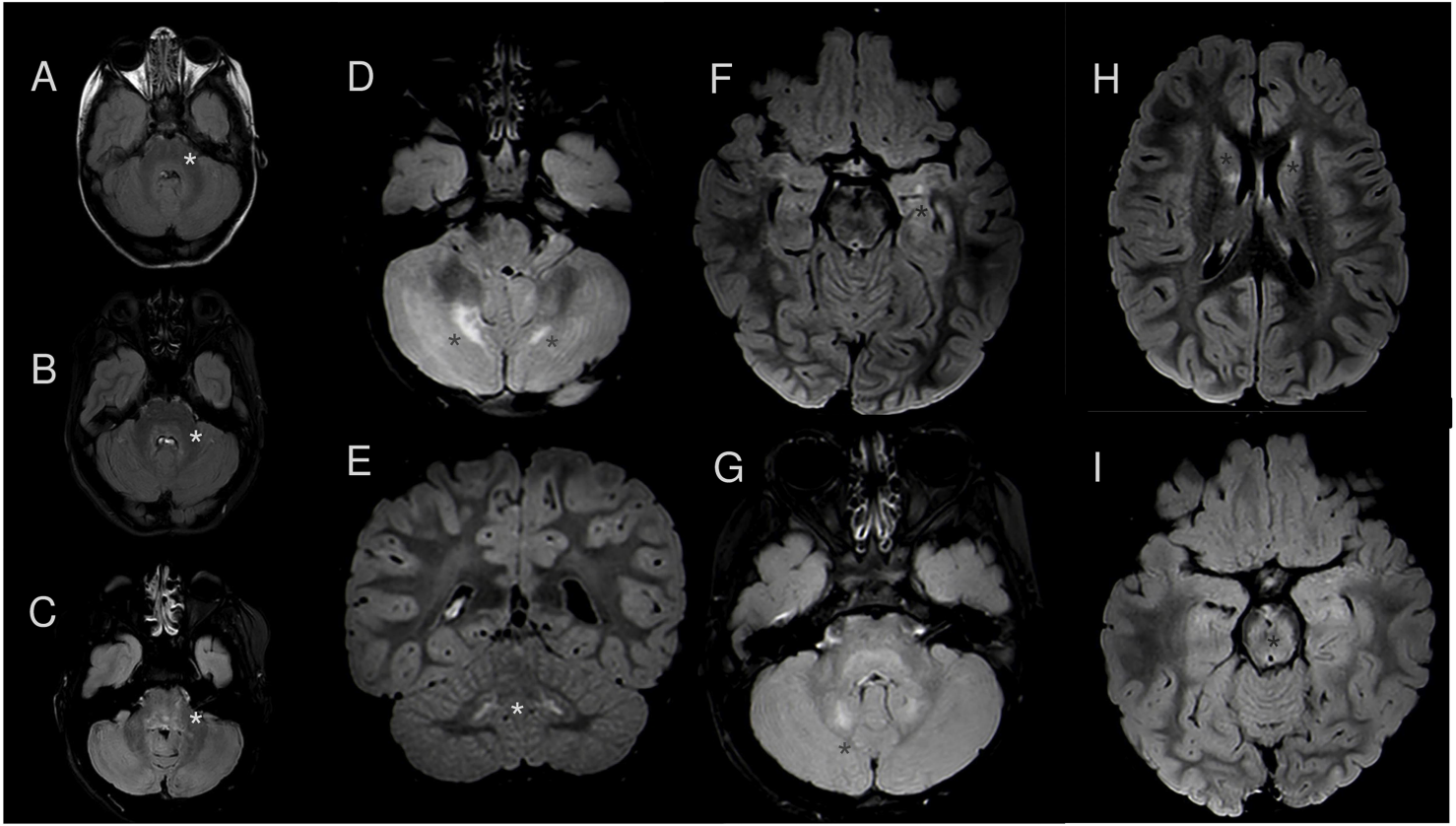

Asymptomatic n=38
Mild MRI, n=26
Severe MRI, n=12
Mild n=15
Mild MRI, n=12
Severe MRI, n=3
SARA 1-3, n=10
Abnormal EP, n=15
Overt clinical symptoms n=10
Mild MRI, n=3
Severe MRI, n=7
Cerebellar, n=6
Cognitive, n=4
Brainstem, n=2
Behavioural, n=3
Asymtomatic n=27
MRI worsening (3→4), n=1
BRAFi, n=1
CT, n=1
Mild n=6
Mild MRI, n=4
Severe MRI, n=2
SARA 1-3, n=4
Abnormal PE, n=6
Untreated, n=2
IVIG, n=4
Overt symptoms n=5
Severe MRI, n=5
Cerebellar, n=5
Cognitive, n=2
Brainstem, n=1
IVIG, n=1
BRAFi, n=1
CT, n=1
Untreated, n=2
Worsened, n=2
IVIG, n=1
BRAFi, n=1
Mild n=13
Untreated, n=3
IVIG, n=10
Overt symptoms n=1
Severe MRI, n=1
Cerebellar, n=1
Overt symptoms n=2
Mild MRI, n=1
Severe MRI, n=1
Cerebellar, n=2
Behavioural, n=1
IVIG, n=2
IVIG, n=1
IVIG+BRAFi, n=1
BRAFi, n=1
Untreated, n=7
Worsened, n=4
IVIG, n=4

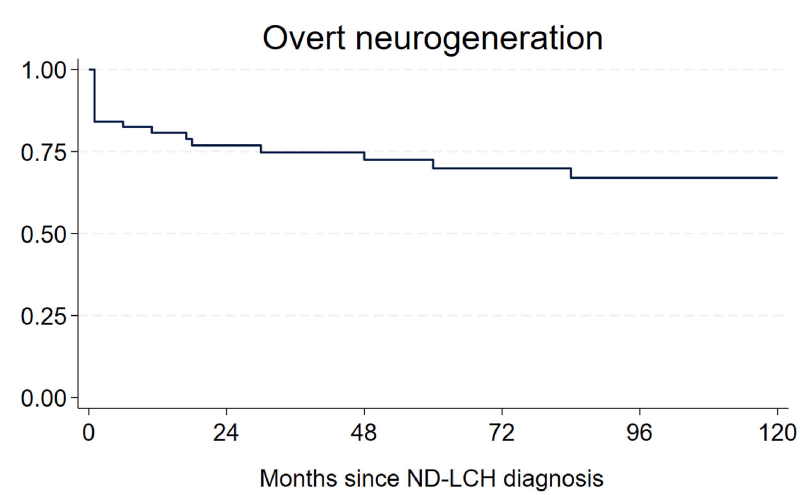
A - Treated

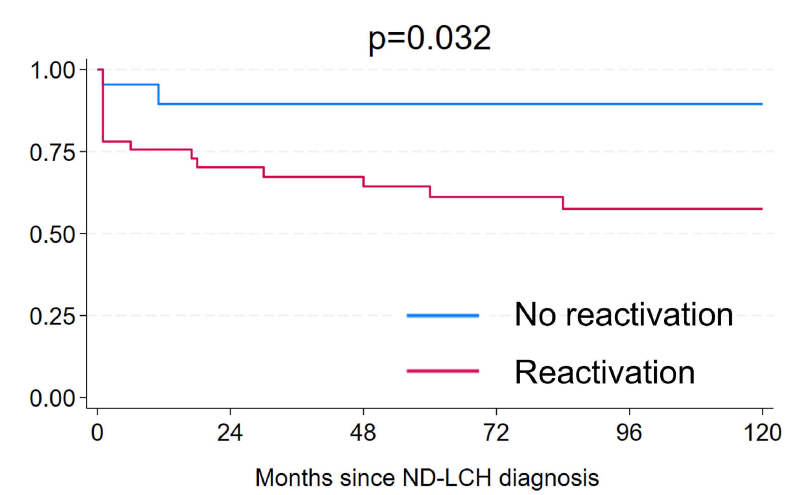


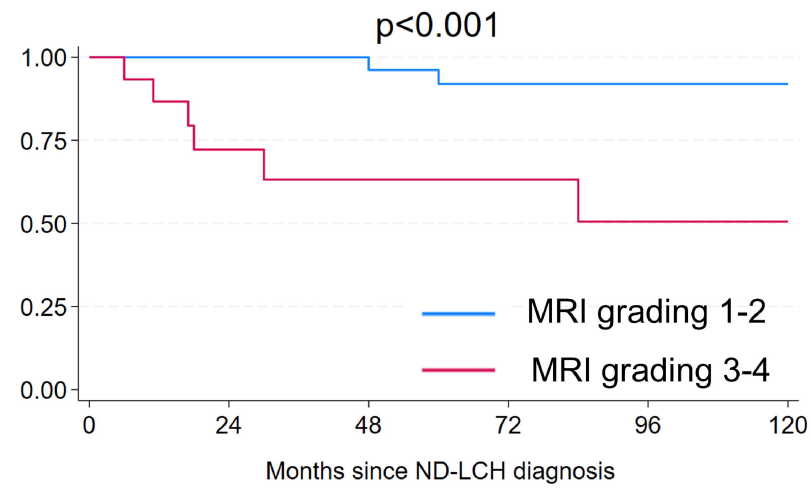
B - Untreated

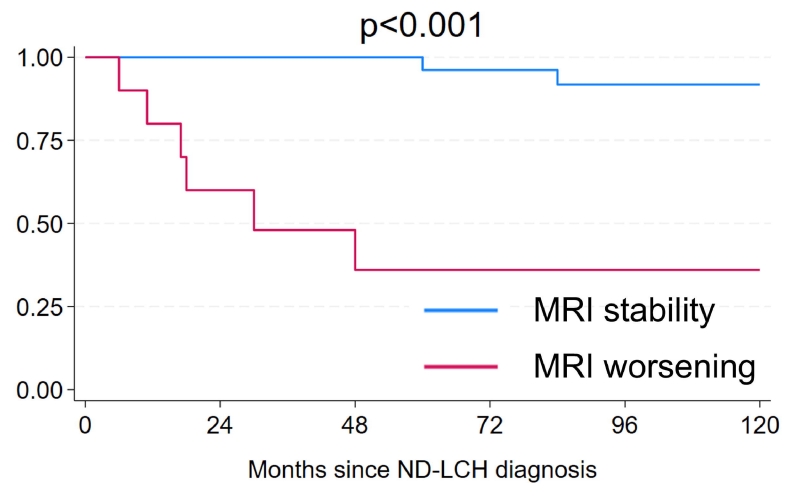



A
B
C
D
E
F
G
H
I


Overt neurogeneration
1.00
0.75
0.50
0.25
0.00
0
24
48
72
96
120
Months since ND-LCH diagnosis


p=0.032
1.00
0.75
0.50
0.25
0.00
No reactivation
Reactivation
0
24
48
72
96
120
Months since ND-LCH diagnosis


p<0.001
1.00
0.75
0.50
0.25
0.00
MRI grading 1-2
MRI grading 3-4
0
24
48
72
96
120
Months since ND-LCH diagnosis


p<0.001
1.00
0.75
0.50
0.25
0.00
MRI stability
MRI worsening
0
24
48
72
96
120
Months since ND-LCH diagnosis

Supplementary methods

Data collection

Data on demographics, LCH presentation (organ involvement at diagnosis, lesional *BRAF* status, treatment, and reactivation), and ND-LCH characterization and follow-up (MRI, neurological, neurophysiological assessment treatment) were retrieved from the Registry or collected from clinical charts by local physicians. The *BRAF* mutational status was assessed on diagnostic fresh biopsies or Formalin-Fixed Paraffin-Embedded (FFPE) tissue biopsies by digital droplet PCR.

Treatments were administered according to the center expertise and included immunoglobulin (IVIG), BRAF inhibitors (BRAFi) such as vemurafenib or dabrafenib, MEK inhibitors (MEKi) such as cobimetinib and trametinib, and chemotherapy according to the LCH-IV protocol (NCT02205762) alone or in combination with other agents.

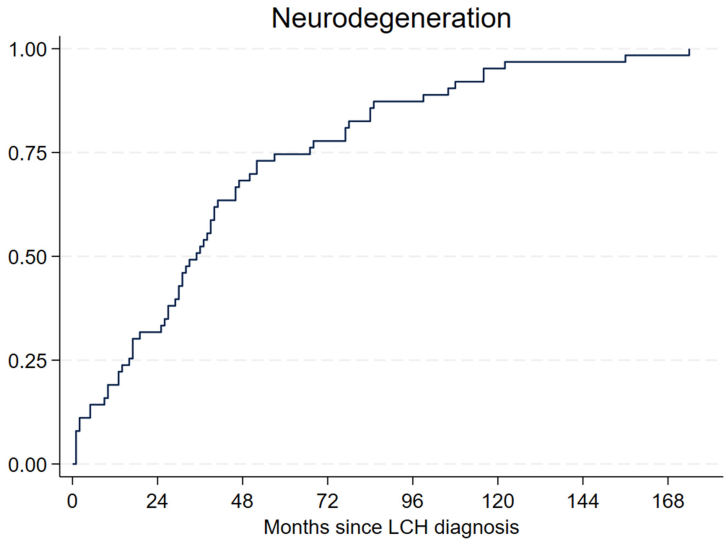
Statistical analysis

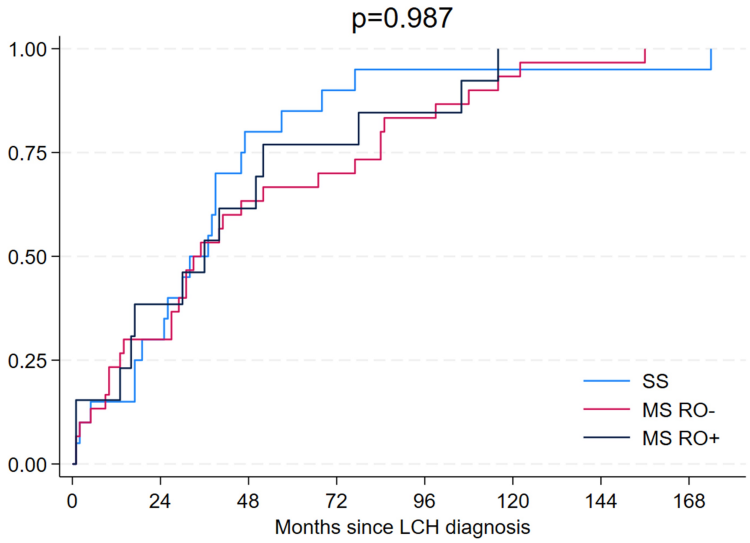
Continuous variables were presented as medians and interquartile range (IQR) while categorical variables as absolute numbers and percentages. Comparisons between groups were made using the chi-square or Fisher's exact tests for categorical variables, and the Mann-Whitney test for continuous variables. Predictors of overt clinical ND-LCH were investigated through an univariable logistic regression model. Odds ratios (ORs) were expressed by exp(B) values and reported with their respective 95% confidence intervals (95%CIs). In the multivariable Cox regression model, variables were entered using a forward selection method, after checking for multicollinearity. For time-to-event analysis we designed Kaplan-Meier curves with the log-rank test. Two-sided p-value<0.05 was considered statistically significant. Statistical analyses were performed using Stata, version 17.

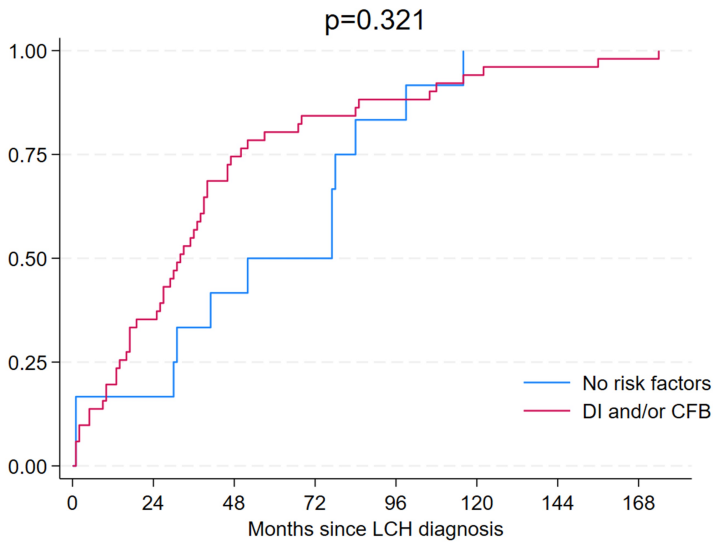
Supplementary figures

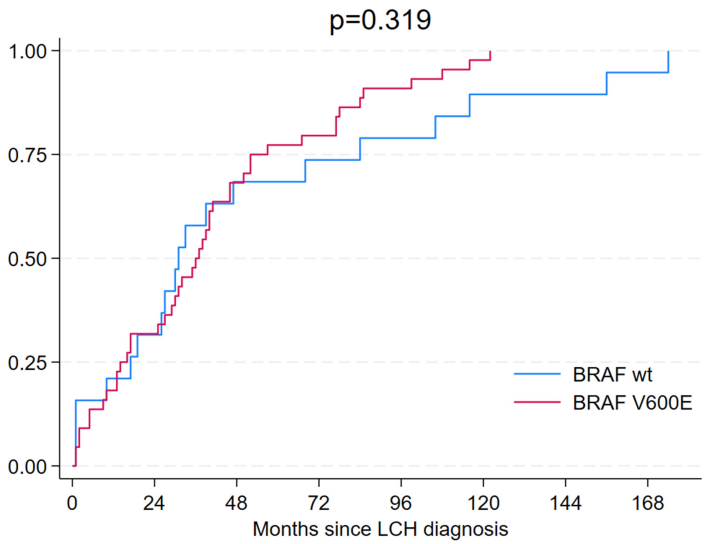
Figure S1 – Kaplan-Meier time to ND-LCH diagnosis: LCH: Langerhans Cell Histiocytosis; ND-LCH: neurodegenerative Langerhans Cell Histiocytosis; DI: diabetes insipidus; CBL: craniofacial bone lesion; SS: single-system; MS: multi-system; RO: risk organ.

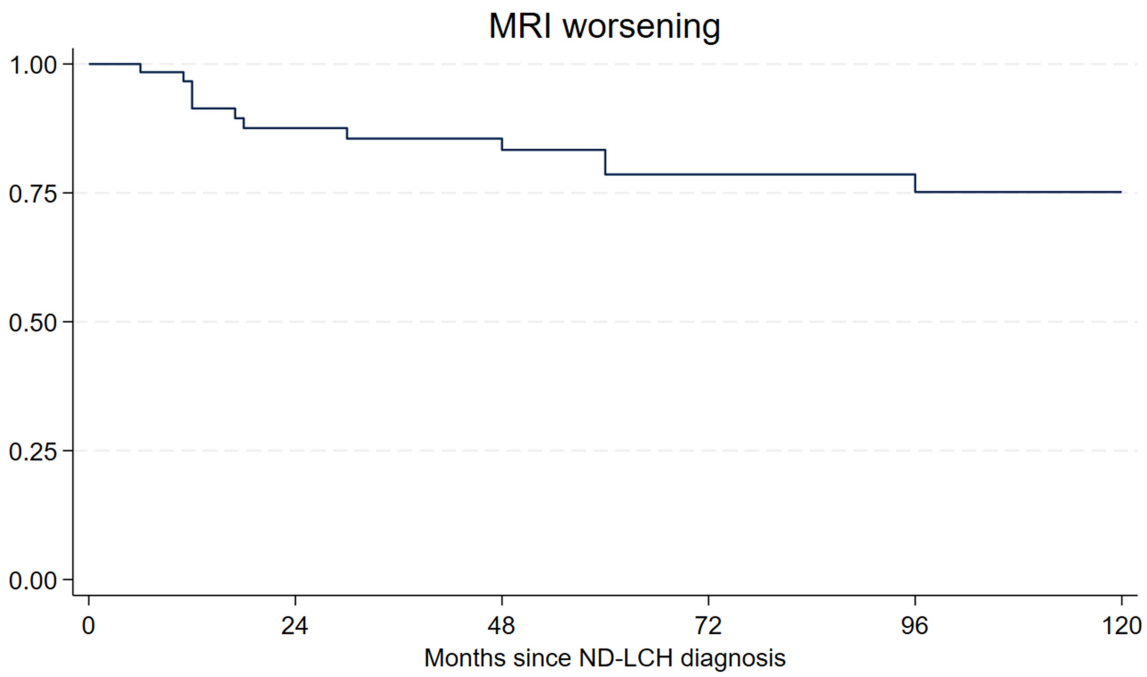
Figure S2 - Kaplan-Meier time to brain MRI abnormalities worsening: LCH: Langerhans Cell Histiocytosis; ND-LCH: neurodegenerative Langerhans Cell Histiocytosis; MRI: magnetic resonance imaging.


Neurodegeneration
1.00
0.75
0.50
0.25
0.00
0
24
48
72
96
120
144
168
Months since LCH diagnosis


p=0.987
1.00
0.75
0.50
0.25
0.00
0
24
48
72
96
120
144
168
Months since LCH diagnosis
SS
MS RO-
MS RO+


p=0.321
1.00
0.75
0.50
0.25
0.00
0
24
48
72
96
120
144
168
Months since LCH diagnosis
No risk factors
DI and/or CFB


p=0.319
1.00
0.75
0.50
0.25
0.00
0
24
48
72
96
120
144
168
Months since LCH diagnosis
BRAF wt
BRAF V600E


MRI worsening
1.00
0.75
0.50
0.25
0.00
0
24
48
72
96
120
Months since ND-LCH diagnosis