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Lack of bone lesions at diagnosis is associated with inferior outcome in multisystem langerhans cell histiocytosis of childhood

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Summary

Skeletal involvement is generally, but not universally, characteristic of Langerhans cell histiocytosis (LCH). We investigated whether the presence of bone lesions at diagnosis is a prognostic factor for survival in LCH. Nine hundred and thirty-eight children with multisystem (MS) LCH, both high (386 RO+) and low (RO−) risk, were evaluated for bone lesions at diagnosis. Risk organ (RO+) involvement was defined as: haematopoietic system (haemoglobin <100 g/l, and/or white blood cell count $<4{\cdot}0 \times 10^9$/l and/or platelet count $<100 \times 10^9$/l), spleen (>2 cm below the costal margin), liver (>3 cm and/or hypoproteinaemia, hypoalbuminaemia, hyperbilirubinaemia, and/or increased aspartate transaminase/alanine transaminase). Given the general view that prognosis in LCH worsens with increasing extent of disease, the surprising finding was that in MS+RO+ LCH the probability of survival with bone involvement 74 ± 3% (n = 230, 56 events) was reduced to 62 ± 4% (n = 156, 55 events) if this was absent (P = 0·007). An even greater difference was seen in the subgroup of patients with both liver and either haematopoiesis or spleen involvement: 61 ± 5% survival (n = 105; 52 events) if patients had bony lesions, *versus* 47 ± 5% (n = 111; 39 events) if they did not (P = 0·014). This difference was retained in multivariate analysis (P = 0·048). Although as yet unexplained, we conclude that bone involvement at diagnosis is a previously unrecognized favourable prognostic factor in MS+RO+ LCH.

Keywords: langerhans cell histiocytosis, multisystem disease, bone lesion, risk organ.

Langerhans cell histiocytosis (LCH) is a rare disease mainly observed in children, although it may occur at any age (Favara *et al*, 1997; Aricò *et al*, 2003). Clinical manifestations range from a solitary, asymptomatic osteolytic lesion to severe disease with multisystem (MS) involvement. When confined to the skeleton, LCH may spontaneously heal or reactivate over years, but it is not life threatening. 'Multisystem disease' (MS+LCH), i.e., involving two or more organs

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or systems, however, has an unpredictable course, especially when there is involvement of risk organs (RO), frequently associated with a poor prognosis (Lahey, 1981; Aricò & Egeler, 1998; Gadner *et al*, 2001, 2008, 2013). Because of this wide disease spectrum, many different treatments have been applied, ranging from conservative approaches to intensive combination chemotherapy (McLelland *et al*, *1990*; Gadner *et al*, 2001, 2008, 2013).

To improve the outcome of patients with disseminated disease, the Histiocyte Society has run a series of randomized international clinical trials (Gadner *et al*, 2001, 2008, 2013). The use of standardized diagnostic criteria (Broadbent *et al*, 1989) allowed accumulation of cases that were also uniformly assessed for disease extent and activity. As a result, a treatment backbone of vinblastine + steroid could be defined as the current standard of care.

Skeletal involvement is a main characteristic of LCH that was included in the original description of '*eosinophilic granuloma*' (Lichtenstein & Jaffe, 1940). In all large series reported since then, about 80% of children show variable patterns of bone involvement (Weitzman & Egeler, 2005; Kim *et al*, 2014). The number and site of osteolytic lesions largely contribute to define the clinical pattern of the disease, especially in patients with multifocal lesions. The prototype of this pattern is the association of multiple osteolytic lesions of the skull, with exophthalmos and diabetes insipidus, also termed '*Hand-Schuller-Christian*' disease. Almost every bone region may be affected by LCH with the relative exception of the bones of hands and feet, which are spared in most cases.

Although the currently available standard of care provides rapid and adequate disease control in most patients, the probability of survival for patients with MS disease and involvement of RO (i.e. liver, spleen and haemopoietic system; MS+RO+) remains <85% (Gadner *et al*, 2001, 2008, 2013). The subset of patients with inferior outcome has been defined in the three clinical trials by the following indicators: insufficient early response by week 6 of initial treatment and RO+ (Gadner *et al*, 2001, 2008, 2013). In their large experience, the Argentine group also supported the adverse prognostic value of similar parameters (hypoalbuminaemia and/or anaemia-thrombocytopenia) (Braier *et al*, 2010).

In her single centre analysis of 70 patients treated between 1961 and 1982, Broadbent (1986) reported that those patients who had bone involvement as part of MS disease had a more favourable outcome than those who did not, and this was most pronounced in the youngest age group. In their univariate analysis of 348 patients, the French LCH Group showed that patients with one or two sites of bone involvement had a borderline significant advantage in survival (The French Langerhans' Cell Histiocytosis Study Group, 1996). A specific prognostic value of bone involvement in patients with MS LCH has never been addressed before.

This study investigated the pattern of bone involvement in patients with MS+LCH enrolled in the trials LCH-I, LCH-II and LCH-III, and explored the outcome of treatment in patients with or without bone involvement at the diagnosis of LCH, to define its possible prognostic value. To address this issue, we also explored the role of bone involvement in the subsets of patients with higher risk of treatment failure.

Methods

Study design and objectives

To address the relevance and the prognostic value of bone involvement in patients with MS LCH, we pooled the data from patients enrolled in the three consecutive trials LCH-I, LCH-II, and LCH-III.

The LCH-I trial was started on April 1, 1991, and closed to accrual on October 1, 1995. A total of 148 patients with a confirmed diagnosis of MS LCH were entered and randomly assigned to treatment arms (Gadner *et al*, 2001).

The LCH-II trial was run between May, 1996 and March, 2001; a total of 279 patients were enrolled in the risk group and 193 were randomized, 93 to Arm A and 100 to Arm B. Risk patients in this trial either had RO involvement (i.e. liver, lung and haematopoietic system or spleen) or had disease onset at younger than 2 years of age (Gadner *et al*, 2008).

The LCH-III study (NCT00276757) was open to accrual from April, 2001, to February, 2008. A total of 554 patients were enrolled (Gadner *et al*, 2013). Risk group in this trial was defined by the involvement of at least one risk organ as defined above, regardless of age at diagnosis.

Eligibility criteria for the trials were age <18 years, definitive diagnosis of LCH (Broadbent *et al*, 1989), MS involvement, no prior systemic treatment for LCH, and informed consent obtained in accordance with the Declaration of Helsinki.

RO and their involvement were initially defined according to modified Lahey criteria (Lahey, 1981) as follows: haematopoietic system—anaemia (haemoglobin <10 g/l, infants <9 g/l) and/or leucopenia (white blood cell count $<4{\cdot}0 \times 10^9$/l) and/or thrombocytopenia (platelet count $<100 \times 10^9$/l); liver—enlargement of more than 3 cm below the costal margin and/or dysfunction (hypoproteinaemia, hypoalbuminaemia, hyperbilirubinaemia and/or increased liver enzymes), spleen (enlargement to more than 2 cm below the costal margin).

In patients with MS LCH but no RO involvement (MS+RO−), the systems involved can include skin, bone, lung, lymph nodes, thymus, hypophysis and/or mucous membranes (oral, gastrointestinal, genital).

According to more recent data obtained by our group in the previous trials (Gadner *et al*, 2001, 2008, 2013) and in keeping with previous experience (Braier *et al*, 2004), beginning with the current LCH-IV trial, lungs are no longer considered to be a risk organ. Thus for the purpose of the present analysis, the definition of RO has been updated accordingly, and lung is no longer considered as such. In an

exploratory analysis we also took into consideration the definition of organ dysfunction for the liver by abnormal laboratory test (hypoproteinaemia, hypoalbuminaemia) and not by hepatomegaly alone. Furthermore, we have recently identified that, even among patients with MS+RO+ disease, involvement of selected organs (i.e. liver and haematopoietic tissue (bone marrow) or spleen) is associated with an unfavourable prognosis, which we thus defined at 'very-high risk' (VHR).

The treatment schedules applied in the three trials have been reported elsewhere (Gadner *et al*, 2001, 2008, 2013). Response to treatment was assessed by the International LCH Study Group criteria (Broadbent *et al*, 1989) and in patients with RO involvement (MS+RO+), reflected RO response.

The objective of this analysis is the evaluation of the prognostic relevance of bone involvement at the diagnosis of LCH in patients with MS disease, enrolled and treated in the LCH-I, LCH-II and LCH-III trials.

Statistical analysis

The primary endpoint was overall survival (OS), which was calculated from date of diagnosis to date of death from any cause; patients with no event were censored at the date of last follow-up. The univariate statistical analysis was performed in several prospectively identified subgroups defined by clinical study, patient age and organ involvement at diagnosis. Based on the considerations that (i) we did not find any evidence for interaction between study and bone involvement ($P = 0{\cdot}472$ in the entire population, adjusted for age, skin and lung), and (ii) prognostic value of bone was evident in the three trials independently even though it was not statistically significant in each trial (LCH-I: 50 ± 10% for RO+ Bone− and 69 ± 7% for RO+ Bone+ with $P = 0{\cdot}035$; LCH-II: 53 ± 7% for RO+ Bone− and 50 ± 6% for RO+ Bone+ with $P = 0{\cdot}382$; LCH-III: 75 ± 5% for RO+ Bone− and 85 ± 4% for RO+ Bone+ with $P = 0{\cdot}076$); we analysed the prognostic value of bone on the basis of the pooled data from the three clinical trials. Kaplan-Meier estimates and Log-Rank test (Kaplan & Meier, 1958) were used to evaluate OS. Further, multivariate Cox regression analysis (Cox, 1972; Altman, 1991) was performed to study the impact of possible confounding factors on the defined outcome. The statistical analysis was done with the SAS System V9.2 (2008, SAS Institute, Cary, NC, USA). All *P*-values below 5% were considered significant.

Results

Of the 938 patients with MS LCH enrolled in the three trials and fully evaluable for this analysis (Table I), 684 (72·9%) had bone involvement (Bone+) at diagnosis while 254 (27·1%) had no bone lesions (Bone−). The distribution of the involved bones is summarized in Table II.

Despite the progress observed over the years in the three sequential trials, a group of patients with MS LCH who are at considerable risk for death is still present. In our study population, the probability of survival of the three risk groups of patients with MS+LCH (i.e. RO−, RO+ without VHR and VHR) was significantly different, with a 97%, 88% and 54% proportion at 5 years, respectively (Fig 1). Thus we decided to perform a subgroup analysis of the impact of bone involvement in these subsets of patients, with special regard to the highest risk of death.

The interaction between RO involvement (risk group) and bone was analysed and is presented in the forest plot analysis (Fig 2B).

In order to define the prognostic value of bone involvement at diagnosis of MS + LCH, we calculated the

Table I. Distribution of bone lesions at the time of diagnosis in 684 patients with multisystem Langerhans cell histiocytosis (LCH).

	Bone sites involved	Study		
		LCH-I	LCH-II	LCH-III
Patients with bone involvement at diagnosis	**684**	**96**	**199**	**389**
Bone sites involved	**1435**	**203**	**388**	**844**
Skull	**769**	**105**	**224**	**440**
Spine	**127**	**12**	**20**	**95**
Cervical vertebra	22	1	2	19
lumbar vertebra	26	3	3	20
thoracic vertebra	47	4	4	39
vertebra, not specified	32	4	11	17
Chest	**120**	**24**	**33**	**63**
clavicle	20	3	9	8
ribs	71	15	17	39
scapula	24	5	7	12
sternum	5	1	0	4
Upper limbs	**78**	**16**	**22**	**40**
humerus	59	14	15	30
ulna	6	0	2	4
radius	8	1	1	6
hand	5	1	4	0
Lower limbs	**153**	**24**	**47**	**82**
femur	105	18	34	53
tibia	39	5	10	24
fibula	6	1	2	3
foot	3	0	1	2
Pelvis	**96**	**19**	**23**	**54**
sacral bone	7	0	2	5
ilium	46	13	7	26
ischiatic bone	7	0	3	4
pubic bone	6	3	1	2
acetabulum	3	0	0	3
pelvic bone, not specified	27	3	10	14
Not specified or missing	**92**	**3**	**19**	**70**
bone (not specified)	15	0	5	10
missing	77	3	14	60

Subtotals are in bold.

probability of survival of patients treated in the three LCH trials, according to the presence or absence of bone lesion(s) and risk organ(s). Based on the considerations that: (i) the prognostic value was confirmed in the three trials independently; (ii) we did not find any evidence for interaction between study and bone involvement; (iii) combining the data across the three trials may help to provide meaningful interpretation, thus we analysed them in a cumulative population of the three studies.

First of all, given that we have reported an improved outcome over the three sequential trials, we explored the impact of bone involvement on outcome in each of these trials. The

Table II. Overview of the study population with respect to age, involvement of bone and risk organs, and original clinical trial.

		MS+RO−		MS+RO+	
	Total	Bone−	Bone+	Bone−	Bone+
Patients number	938	98	454	156	230
LCH-I study	138	14	50	28	46
Age <2 years	95	8	26	25	36
Age ≥2 years	43	6	24	3	10
LCH-II study	281	29	130	53	69
Age <2 years	184	16	64	50	54
Age ≥2 years	97	13	66	3	15
LCH-III study	519	55	274	75	115
Age not specified	1	0	1	0	0
Age <2 years	310	33	122	68	87
Age ≥2 years	208	22	151	7	28

LCH, Langerhans cell histiocytosis; MS+RO−, multisystem LCH without risk organ(s) involvement; MS+RO+, multisystem LCH with risk organ(s) involvement.

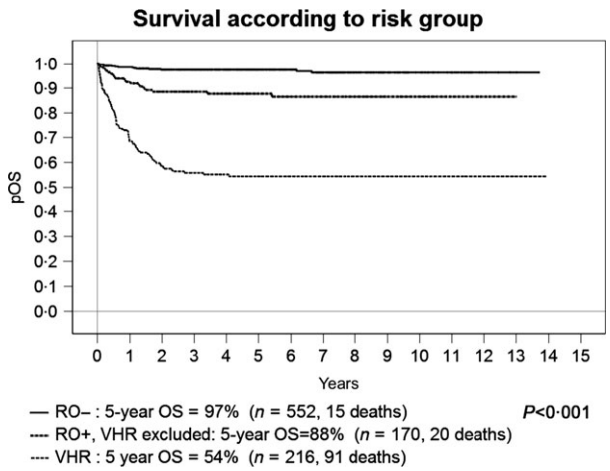

Fig 1. Probability of survival in multisystem Langerhans cell histiocytosis patients with bone involvement according to the risk organs involved (Log-Rank $P < 0{\cdot}001$). Patients with multisystem Langerhans cell histiocytosis had a significantly worse outcome if they had involvement of specific, VHR organ(s) (liver and haematopoietic tissue or spleen) at the time of the diagnosis. RO, risk organ(s); VHR, very high risk; OS, overall survival; pOS, probability of OS.

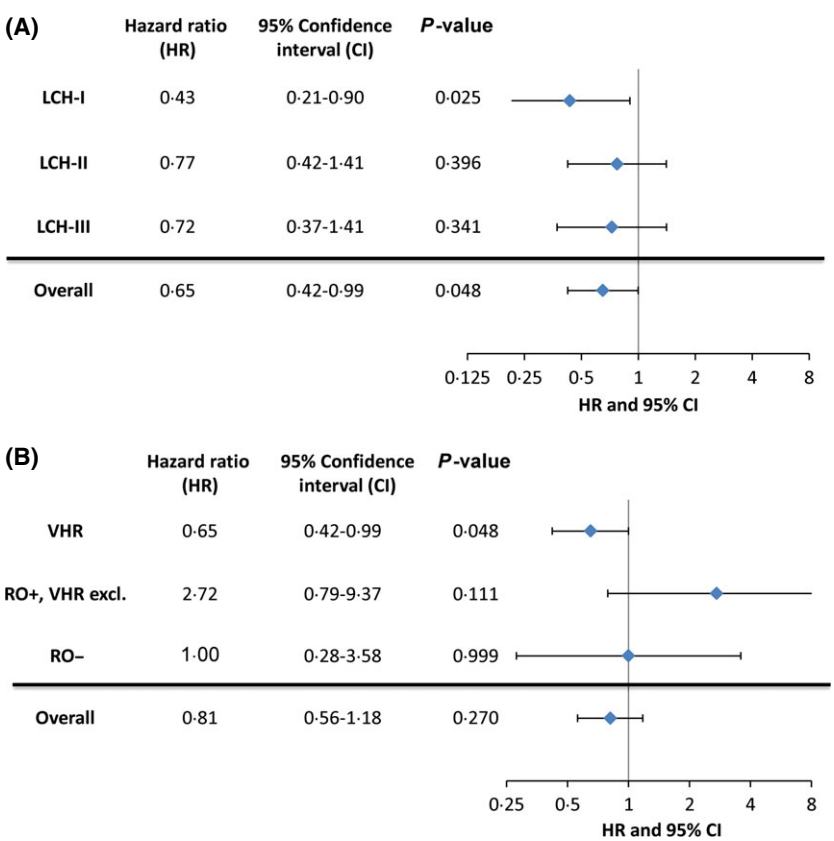

Fig 2. Forest plot analysis of interactions. Interaction between clinical trial and bone involvement. Interaction between risk group and bone-involvement. The Hazard Ratio is indicated by a diamond, and the horizontal line corresponds to the 95% confidence intervals (CI). LCH, Langerhans cell histiocytosis; HR, Hazard Ratio; RO, risk organ(s); VHR, very high risk; excl. excluded.

probability of OS for RO+Bone− patients was 50 ± 10% (28 patients, 14 events) and for RO+Bone+ 69 ± 7% (46 patients, 13 events) in LCH-I (Log-Rank $P = 0{\cdot}035$). In LCH-II OS was 53 ± 7% (53 patients, 24 events) in RO+Bone−, and 60 ± 6% (69 patients, 27 events) in RO+Bone+ (Log-Rank $P = 0{\cdot}382$). In the more recent LCH-III study, OS was 75 ± 5% (75 patients, 17 events) in RO+Bone− and 85 ± 4% (75 patients, 17 events) in RO+Bone+ (Log-Rank $P = 0{\cdot}076$).

Altogether, among the 552 MS+RO− patients (defined in the trial as 'low risk'), the probability of survival was outstanding for both Bone+ (97 ± 1%; 454 patients, 12 events) and Bone− (98 ± 2%; 98 patients, 3 events) (Log rank $P = 0{\cdot}798$). In the group of 386 MS + RO+ patients the probability of survival was 74 ± 3% (230 patients, 56 events) for Bone+ and 62 ± 4% (156 patients, 55 events) for Bone−, respectively. This difference was highly significant ($P = 0{\cdot}007$) (Fig 3). We also analysed this cohort of patients by a more stringent definition of risk organ for the liver (i.e. not by hepatomegaly alone but rather by organ dysfunction as defined in the methods section), and the above results were modified as follows: 73 ± 3% (216 patients, 54 events) for Bone+, and of 61 ± 4% (149 patients, 54 events) for Bone−. This difference was also statistically significant ($P = 0{\cdot}007$).

The impact of bone involvement was evident for those patients with involvement of both liver and haematopoiesis or spleen: the probability of survival was 61 ± 5% (105 patients; 52 events) for Bone+, and 47 ± 5% (111 patients; 39 events) for Bone− ($P = 0{\cdot}014$) (Fig 4).

We also addressed the potential impact of age (cut-off, 2 years) and its relationship with bone lesions. Patients younger than 2 years of age with involvement of at least one risk organ had a probability of survival of 73 ± 4% (177 patients, 44 events) if Bone+ and of 61 ± 4% (143 patients, 52 events) if Bone−. This difference was highly significant ($P = 0{\cdot}018$). For patients older than 2 years the difference was not significant (probability of survival of 75 ± 6%, 53 patients, 12 events if Bone+; 72 ± 14%, 13 patients, 3 events if Bone−) ($P = 0{\cdot}652$).

Subgroup analysis enabled the identification of a subset of 133 patients (with 51 events) with MS+LCH involving at least one RO and the skin but not the bone, who had a probability of survival of 59 ± 4%, which was significantly worse than the survival probability of the respective group with bone involvement: 68 ± 4% (155 patients, 46 events).

Multivariate analysis was performed by the Cox model on 938 patients with MS+LCH enrolled in the three trials. The variables considered were: risk group (RO−, RO+ without VHR, and VHR), clinical trial (studies LCH-I, LCH-II, LCH-III), age group (cut-off 2 years), involvement of bone, the interaction between bone and risk group, as well as involvement of bone, lung and skin. The results showed that risk group ($P < 0{\cdot}001$; RO− *versus* VHR: Hazard ratio [HR] 0·05, 95% confidence interval [CI] 0·02–0·16, $P < 0{\cdot}001$; RO+ without VHR *versus* VHR: HR 0·09, 95% CI: 0·03–0·29, $P < 0{\cdot}001$) and clinical trial ($P < 0{\cdot}001$; LCH-I vs. LCH-III: HR 2·40, 95% CI: 1·49–3·86, $P < 0{\cdot}001$; LCH-II *versus* LCH-III: HR 2·15, 95% CI: 1·42–3·25, $P < 0{\cdot}001$) had an independent, significant association with inferior survival. The interaction between risk group and bone is close to statistical significance ($P = 0{\cdot}089$). For the subgroup of 209 patients with both liver and haematological or spleen involvement (VHR) the presence of bone lesions was associated with lower risk of failure and this result was statistically

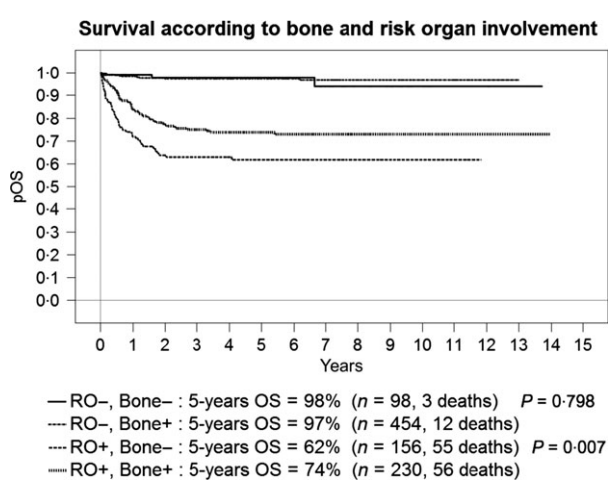

Fig 3. Probability of survival according to bone and risk organ involvement (Log-Rank $P = 0{\cdot}007$). Patients with multisystem Langerhans cell histiocytosis and risk organ(s) involvement have a significantly worse outcome if they did not have associated bone lesion(s) at the time of the diagnosis. RO, risk organ(s); OS, overall survival; pOS, probability of OS.

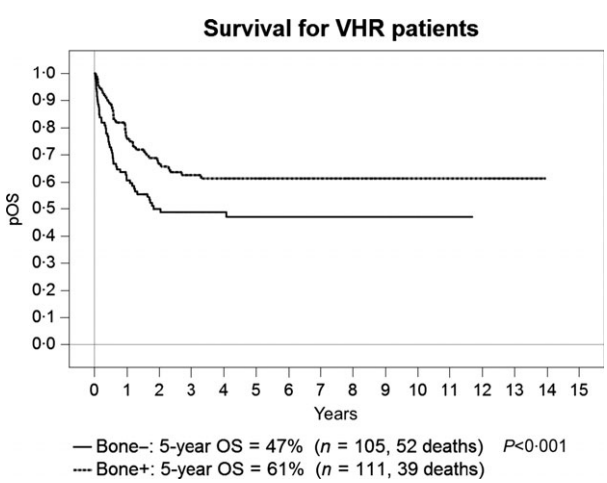

Fig 4. Probability of survival for very high risk patients with multisystem Langerhans cell histiocytosis according to bone involvement (Log-Rank $P < 0{\cdot}001$). Patients with multisystem Langerhans cell histiocytosis and involvement of at least one of the very high risk organs (liver and haematopoietic tissue or spleen) had a worse outcome if they did not have bone involvement at the time of the diagnosis.

significant (HR = 0·65, 95% CI: 0·42–1·00, $P = 0·048$) in the multivariate analysis (Table III).

Discussion

The lack of an animal model has hampered progress in understanding the pathobiology of LCH. Thus, treatment strategy development was based on a purely empiric approach (Minkov, 2012). Similarly to other rare diseases, reliable knowledge on the clinical features, natural course and impact of treatment regimens in paediatric LCH was possible only through cooperative multicentre randomized trials. The three therapeutic trials conducted by the Histiocyte Society have clearly documented that patients with involvement of selected RO and insufficient response to initial therapy represent a subset at risk for mortality; this is now taken into account for patient stratification in the ongoing LCH-IV trial (*EudraCT Number: 2011-001699-20*).

Bone involvement is a hallmark of LCH, observed in around 80% of the cases. Clinical observations of individual patients over the years have raised the hypothesis that patients with MS+LCH who lack bone involvement had a higher risk for treatment failure. Given that, by study design, bone involvement was not a reason for patient allocation, those patients have been exposed to a common treatment; this allowed us to explore its prognostic impact. The availability of a common database of the three consecutive trials LCH-I to LCH-III offered us the unique opportunity to address this issue with a sufficient number of patients prospectively treated and uniformly assessed. In this setting, we analysed the prognostic impact of this variable, and the results clearly define the lack of bone lesion(s) in patients with risk organ involvement as a novel, independent, adverse prognostic factor in childhood MS+LCH.

Table III. Multivariate analysis performed by Cox Regression analysis of the prognostic value on survival in patients with MS+LCH.

Parameter	*P*-value	Hazard Ratio	95% Confidence Interval	
Risk group				
versus VHR	<0·001			
RO−	<0·001	0·05	0·02	0·16
RO+ without VHR	<0·001	0·09	0·03	0·29
Interaction: bone and risk group	0·089			
Bone				
RO−	0·999	1·00	0·28	3·58
RO+ without VHR	0·111	2·72	0·79	9·37
VHR	0·048	0·65	0·42	1·00
Skin +				
versus Skin -	0·148	1·48	0·87	2·53
Lung +				
versus Lung -	0·264	1·24	0·85	1·82
Age >2 years				
versus Age <2 years	0·424	0·81	0·48	1·36
Study*				
versus LCH-III	<0·001			
LCH-I	<0·001	2·40	1·49	3·86
LCH-II	<0·001	2·15	1·42	3·25

*No significant interaction between study and bone.

MS+LCH, multisystem Langerhans cell histiocytosis; VHR, very high risk (involvement of liver and haematopoietic tissue or spleen); RO+, with risk organ(s) involvement; RO−, without risk organ(s) involvement.

In patients with MS+LCH, the absence of bone involvement was associated with a more aggressive disease, as documented by a significantly higher number of RO involved in children who lacked skeletal lesions; in order to understand if the presence or absence of bone involvement was associated with a different outcome, we analysed the subset of patients with the highest risk of death. Previous studies had confirmed that RO involvement was associated with a risk of mortality (Gadner *et al*, 2008, 2013); when we refined this analysis, it was clear that, among the cumulated population enrolled in the three LCH trials, those patients who had involvement of liver and haematopoietic tissue (bone marrow) or spleen had the worst prognosis, due to the highest risk of death. Thus we analysed those 'very high risk' patients and found that among them, the absence of bone involvement was associated with the highest risk of mortality, with a 5-year probability of survival of only 47%. This result is significantly inferior to that achieved with the current standard of therapy in the remaining patients.

The evidence of an adverse prognostic impact may suggest that patients with LCH spreading within the body but sparing the skeleton have an intrinsic reason for developing an insufficient response to standard chemotherapy. A speculative tentative explanation for the negative impact of absence of bone involvement might be the kinetics of the disease; i.e., considering bone involvement as positive and the reason for a better response, vs. lack of bone involvement as 'causing' in some way a negative outcome. That is, lytic bone lesions by their nature must take time to develop; in contrast, it is possible that the primary cellular/cytokine processes of LCH that underlie risk organ involvement may develop more rapidly. Thus bone involvement could reflect more slowly developing disease that is more amenable to successful treatment.

The pathogenesis of LCH has remained elusive for many years and recent findings may probably contribute to its clarification (Badalian-Very *et al*, 2013; Delprat & Aricò, 2014). A very recent paper showed that patients with active, high-risk LCH carry the *BRAF* V600E mutation in both circulating (CD11c+ and CD14+) dendritic cell precursors and bone marrow (CD34+) myeloid progenitors, whereas the mutation is restricted to lesional CD207+ dendritic cells in low-risk LCH patients (Berres *et al*, 2014). This observation is consistent with the findings in an animal model (Berres *et al*, 2014) and suggests that patients with disseminated disease but without bone lesions may represent a subset due to somatic *BRAF* V600E mutation in a myeloid progenitor. Yet,

why this may happen in individual patients remains elusive. The issue of a viral triggering agent in LCH has been raised again, some years after others have found no evidence of many other viruses, by Murakami *et al* (2014), who recently reported elevated amounts of Merkel cell polyomavirus DNA in the peripheral blood cells in 2 of 3 LCH patients with high-risk organ involvement and lower levels in an additional 12 LCH tissues. This finding is interesting but deserves confirmation in other series. The possible correlation of alterations described in LCH, such as the novel cell fusion pathway in an *ex vivo* model (Coury *et al*, 2008), the excess of spontaneous chromosomal breaks (Scappaticci *et al*, 2000) recalling the genomic instability induced by respiratory syncytial virus, hepatitis C virus or Epstein-Barr virus (Scappaticci *et al*, 2000; Kamranvar *et al*, 2007), the *JAG2*-mediated NOTCH activation (Hutter *et al*, 2012 and the spontaneous expression of the pro-survival member of the BCL2 family, BCL2A1 (Olsson Åkefeldt *et al*, 2013), deserves specific investigation. Evaluation of the proportion of cases showing *BRAF* V600E mutation (Badalian-Very *et al*, 2010) among patients with or without bone involvement appears warranted at the present stage, as well as revisiting the gene-expression profile of these two subgroups of patients.

Based on our findings, MS+LCH patients with involvement of liver, spleen or haematopoiesis, but who lack bone involvement, might represent a subgroup of patients suitable for early treatment intensification or alternative therapeutic approaches (Haroche *et al*, 2013). The observation that, in the most recent LCH-III trial, the probability of survival of this patient subgroup, although persistently inferior to the remaining patients, has markedly improved may be reassuring. This is probably due to improved rescue therapy based on acute myeloid leukaemia-directed regimens (Bernard *et al*, 2005) and supportive care. The available data suggest that their risk of fatality remains confined to the initial phase, reaching a plateau within 2 years from diagnosis. Thus, efforts must attempt to bring these patients into disease remission, even with intensified therapy, which in most cases will open the way to survival and cure.

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Authorship contributions

M.A. and M.M. wrote the paper; U.P. performed the statistical analysis; M.M., N.G., and E.T. contributed to study coordination, data collection, and analysis; M.A., J.B., J.D., I.A., J.-I.H., G.J., K.M., S.W., and K.W. made clinical contributions and provided critical manuscript review. All authors were involved in study design and final approval of the paper.

Disclosure of conflict of interest

No conflict of interest to disclose by any of the authors.

Ethics statement

Written informed consent for the data collection was obtained by the attending physician for all patients as well as from all healthy donors. The data were collected as part of the clinical trials LCH-I, LCH-II, LCH-III, all of which had been approved by the Institutional Review Boards of the participating institutions. All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki.

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