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Frequency and spectrum of central nervous system involvement in 193 children with haemophagocytic lymphohistiocytosis

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Summary

Haemophagocytic lymphohistiocytosis (HLH) may cause meningoencephalitis and significant neurological sequelae. We examined the relationship between neurological symptoms and cerebrospinal fluid (CSF) at diagnosis, and long-term outcome, in all children enroled in the HLH-94-study prior to July 1, 2003, for whom information on CSF at diagnosis was available (n = 193). Patients were divided into four groups: (i) normal CSF (cells/protein) and no neurological symptoms (n = 71); (ii) normal CSF but neurological symptoms (n = 21); (iii) abnormal CSF but no symptoms (n = 50); and (iv) abnormal CSF with neurological symptoms (n = 51). At diagnosis, neurological symptoms were reported in 72/193 (37%) (seizures = 23); abnormal CSF in 101/193 (52%), and either or both in 122/193 (63%). Altogether 16/107 (15%) survivors had neurological sequelae at follow-up (median 5·3 years). Multivariate hazard ratios (HR) for mortality were 0·98 [95% confidence interval (CI) = 0·42–2·31], 1·52 (0·82–2·82) and 2·05 (1·13–3·72) for groups 2–4, compared with group 1. Moreover, sequelae were more frequent in group 4 (7/21, 33%) compared to groups 1–3 (9/86, 10%) (P = 0·015). Patients with abnormal CSF at diagnosis had significantly increased mortality [HR = 1·78 (95% CI = 1·08–2·92), P = 0·023]. Thus, a substantial proportion of HLH survivors suffer neurological sequelae, and children with abnormal CSF have increased risk of mortality and neurological sequelae. Prompt treatment of HLH at onset or relapse may reduce these complications.

Keywords: central nervous system, encephalitis, haemophagocytic lymphohistiocytosis, meningitis, sequelae.

The term haemophagocytic lymphohistiocytosis (HLH) encompasses the inherited form familial haemophagocytic lymphohistiocytosis (FHL), an autosomal recessive disease mostly affecting young children, and secondary HLH, predominantly associated with infections or malignancies (Janka, 1983; Henter *et al*, 1998). Common characteristics of HLH include fever, cytopenia, hypertriglyceridaemia, hypofibrinogenaemia and hepatosplenomegaly (Janka, 1983; Henter *et al*, 1991a,b, 1998; Arico *et al*, 1996). Haemophagocytosis is a classical finding but not always demonstrated. Most patients display a pronounced inflammatory response with hypercytokinaemia and hyperferritinaemia, in association with a deficient lymphocyte cytotoxic activity (Perez *et al*, 1984; Esumi *et al*, 1989; Komp *et al*, 1989; Henter *et al*, 1991c; Schneider *et al*, 2002; Janka & Schneider, 2004; Horne *et al*, 2005a). Importantly, many patients also develop a meningoencephalitis that may be severe and cause permanent neurological sequelae (Henter & Elinder, 1992; Haddad *et al*, 1997; Henter & Nennesmo, 1997).

Pathophysiological studies have shown that FHL is associated with deficient apoptosis triggering (Henter *et al*, 1996; Fadeel *et al*, 1999). Subsequent genetic investigations revealed

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mutations in the perforin gene (*PRF1*) to be the underlying cause of the disease in 20–40% of FHL patients (Stepp *et al*, 1999; Ericson *et al*, 2001). More recently, mutations in the genes encoding the proteins hMunc13-4 and syntaxin 11 have also been reported (Feldmann *et al*, 2003; zur Stadt *et al*, 2005; Rudd *et al*, 2006). The impairment in natural killer and cytotoxic T-lymphocyte cellular cytotoxicity is associated with an accumulation of non-malignant macrophages (histiocytes) and T lymphocytes in lymph nodes, spleen, liver and other organs, such as the central nervous system (CNS). Patients with HLH may develop meningoencephalitis, in which the characteristic neuropathological findings include a lymphohistiocytic infiltration in the leptomeninges and perivascular spaces (Haddad *et al*, 1997).

The multinational treatment protocol HLH-94, introduced in 1994, included immunomodulating and cytotoxic treatment with the aim of inducing disease control followed by an allogeneic haematopoietic stem cell transplant (SCT) to achieve a permanent cure in patients with familial, persistent and recurrent disease (Henter *et al*, 1997, 2002). The survival of children with HLH has improved markedly and the overall 3-year survival observed in the HLH-94 trial is now around 55% (Henter *et al*, 2002). It has also become apparent that long-term sequelae in some of the survivors may be severe, with neurological impairment being the most troublesome (Haddad *et al*, 1997; Henter *et al*, 2002; Horne *et al*, 2005b). Data on the frequencies and characteristics of CNS symptoms in large patient cohorts are limited, as is information regarding to what extent CNS involvement at diagnosis can predict long-term outcome, including survival and persistent neurological deficits. This report analysed these questions in a large cohort of children ($n = 193$).

Patients and methods

Patients

Patient data were collected from the HLH-94 database in Stockholm, to which patient information was submitted by treating physicians and national co-ordinators on follow-up forms. The inclusion criteria for this study were: age 15 years or less at diagnosis; no other disease and no previous cytotoxic or ciclosporin A (CSA) therapy; all diagnostic criteria of HLH fulfilled at diagnosis or familial disease; information available on CSF cell count and/or protein level at diagnosis; and treatment or intention to treat according to the HLH-94 protocol prior to July 1, 2003. All patients with HLH were included, primary as well as secondary, which is also the clinical situation at onset in most patients. As the recruitment period was between 1994 and 2003, the vast majority of patients were not studied with regard to gene mutations. Altogether, 193 patients were eligible for the current analysis. When comparing the 193 children studied and the 44 patients otherwise eligible but not included in the study due to missing information on CSF at diagnosis, there was no statistically significant differences in distribution regarding sex, age, consanguinity, familial history of disease, frequencies of neurological symptoms at diagnosis or neurosequelae at last follow-up, or estimated 3-year probability of survival between the groups. Patient data and outcome were analysed as of April 22, 2005.

Evaluation of CSF and neurological findings

The CSF was considered abnormal in the event of elevated leucocyte cell counts and/or protein levels ('yes' or 'no' answers were reported, with values being provided in certain cases). Where the referring institution had provided a value but not confirmed abnormal or normal, we carried out this assessment using age-adjusted reference values (Nicholson & Pesce, 2000). Pathological neurological findings were assessed by the treating physician at each referral centre (answering 'yes' or 'no') and if neurological symptoms were confirmed, the clinician was required to specify these in free-text.

To evaluate if neurological symptoms and/or abnormal CSF had any association with the long-term outcome, we divided the patients into four CNS disease groups: normal CSF and no neurological symptoms (CNS group 1); normal CSF but neurological symptoms (CNS group 2); abnormal CSF but no neurologic symptoms (CNS group 3); and abnormal CSF with neurological symptoms (CNS group 4).

Neuroradiological studies

Computed tomography (CT) scan and magnetic resonance imaging (MRI) findings were evaluated by a radiologist at the treating hospital ('yes' or 'no' answers were required, with a free-text option).

HLH-94 therapy

The HLH-94 protocol consisted of 8 weeks initial therapy with etoposide (VP-16) and dexamethasone (Henter *et al*, 1997, 2002). In addition, intrathecal methotrexate was recommended to patients with progressive neurological symptoms after 2 weeks of therapy, or if an abnormal CSF had not improved. Continuation therapy consisted of CSA daily plus alternating weekly pulses of VP-16 or dexamethasone (Henter *et al*, 1997, 2002). Of the 193 patients, 188 (97%) were initially treated according to HLH-94; the remaining five were enroled on the HLH-94 study with intention to treat. Nine patients changed treatment after initial therapy with HLH-94 protocol. At the time of analysis, 102 patients (53%) had undergone an SCT.

With regard to their HLH therapy status, the patients were classified as being 'off-therapy' if they had been off therapy without disease re-activation for at least 1 year after stopping therapy, and as 'not off-therapy' if an SCT had been performed or HLH therapy had been administered during the last follow-up year.

Table I. Characteristics of the 193 patients with respect to CNS group.

	CNS group 1 (No neurological symptoms and normal CSF)	CNS group 2 (Neurological symptoms but normal CSF)	CNS group 3 (No neurological symptoms but abnormal CSF)	CNS group 4 (Neurological symptoms and abnormal CSF)	Total
No. evaluated patients	71	21	50	51	193
Male	41 (58)	10 (48)	26 (52)	30 (59)	107 (55)
Familial history	15 (21)	3 (14)	12 (24)	13/50 (26)	43/192 (22)
Consanguinity	9/69 (13)	5 (24)	6/47 (13)	17/49 (35)	37/186 (20)
Age <12 months at onset	26 (37)	10 (48)	38 (76)	32 (63)	106 (55)
Pathological neuroradiology at onset	5/37 (14)	2/10 (20)	6/26 (23)	22/42 (52)	35/115 (30)

Values in parenthesis are in percentage.

Statistics

Differences in distribution were compared by using the chi-squared test or, where frequencies were small, two-tailed Fisher's exact test. Survival rates were analysed using the Kaplan–Meier life table method, and univariate comparison of survival using the log rank test. Multivariate analysis using Cox proportional hazards regression was performed, with time to death as the endpoint and using the maximum follow-up time available. The covariates used were: sex; age at start of treatment; SCT and CNS disease group. All analyses were carried out using the Statistical Package for the Social Sciences (SPSS), version 11.5 (Chicago, IL, USA). The study was approved by the Scientific Committee of the Histiocyte Society and the Ethics Commitee of the Karolinska Institute. The fundings sources had no involvement with any part of the study design, results or publication.

Results

Patients

The 193 patients studied originated from 25 countries. The characteristics of these patients and their CNS disease groupings are detailed in Table I. The vast majority fulfilled all the diagnostic criteria (181, 94%), and the remaining 12 (6%) had a familial disease (affected sibling). In total, 43/192 (22%) (no data = 1) had familial disease and there was known consanguinity in 37/186 (20%) cases (no data = 7). The median age at disease diagnosis was 9 months (range: 12 days–14 years); 106 (55%) were less than 1 year of age at diagnosis. With regard to infections, 11 of the patients studied were reported to have specified viral infections at diagnosis: Epstein–Barr virus (EBV) ($n = 5$), cytomegalovirus (CMV) ($n = 4$), EBV and CMV ($n = 1$) and hepatitis virus A ($n = 1$).

Neurological symptoms at diagnosis

Clinical neurological abnormalities at diagnosis were reported in 72 patients (37%). In 70 of these 72 cases (97%) the nature of the neurological symptoms was specified, with a wide variety of neurological symptoms being described, ranging from quite mild to severe. Figure 1 presents these symptoms and their association with abnormal CSF. Seizures of both general and focal nature were reported, affecting a total of 23 patients (33%), and irritability was the most common finding, reported in 24 patients (34%). Based on clinical findings only, 17 patients (24%) were assessed to have meningitis, with findings such as neck stiffness, opisthotonus, a bulging fontanel and papilledema. Eight children (11%) were described as having disturbances in the levels of consciousness. A developmental delay was present at diagnosis in six patients (two of whom were born prematurely). Other findings reported were cranial nerve palsy ($n = 6$), ataxia ($n = 4$), spasticity ($n = 1$) and hemiparesis ($n = 1$).

CSF findings at diagnosis

In the CSF analysis performed at time of diagnosis, 101 patients (52%) were reported as having abnormal CSF with elevated cell and/or protein content. Elevated CSF protein was reported in 76/188 (40%) and elevated cell counts in 79/189 (42%). Among the 184 patients with CSF data for both cell counts and protein level, 20 had pleocytosis with normal protein levels whereas 17 had normal cell counts but elevated protein levels. Notably, the pleocytosis was often only mild or moderate and 26% of the patients with pleocytosis had a cell count of only $6–10 \times 10^6$/l and 51% had a cell count $<20 \times 10^6$/l. Only 22% of the children had a cell count $>50 \times 10^6$/l.

CSF findings related to neurological symptoms at onset and at follow-up. With regard to the correlation between CSF findings and neurological symptoms at onset, the three most common symptoms (seizures, meningismus and irritability) were all associated with abnormal CSF in 70–80% of the children (Fig 1). Altogether 109 patients were alive 1 year after diagnosis, data on late sequelae were available for 107. Of these, 11/46 (24%) patients with abnormal CSF at onset had sequelae compared to only 5/61 (8%) patients with normal

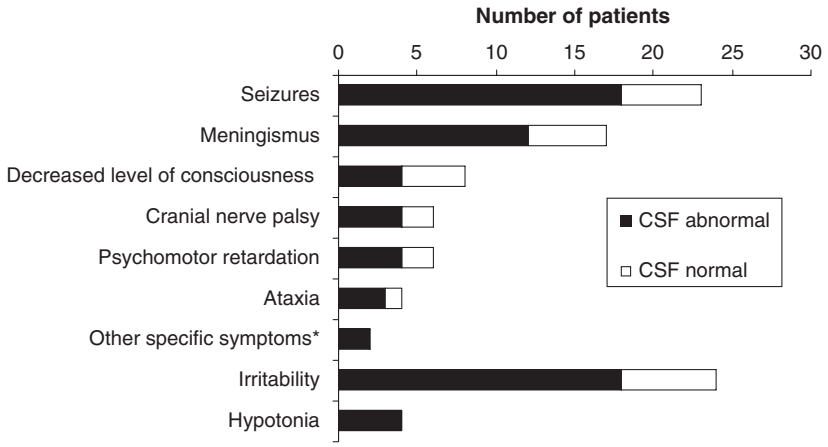

Fig 1. Neurological symptoms at diagnosis and their relation to abnormal CSF in 193 children with HLH. The bars refer to the actual number of patients reported as affected by each symptom; black sections refer to patients that also had abnormal cerebrospinal protein and/or cell count (for definitions see text). *includes spasticity ($n = 1$) and hemiparesis ($n = 1$).

CSF at onset ($P = 0{\cdot}024$, chi-squared test). It was not possible to perform multivariate analysis because of too few patients in each group (11 and five respectively).

Neuroradiology

A neuroradiological study was performed in the early phase of the treatment in 115 patients (60%) of which the studies in 35 patients (30%) were reported to be abnormal, including 25 of 75 MRI studies (33%) and 17 of 70 CT investigations (24%) (some patients had both CT and MRI performed). The neuroradiological findings were specified in 27 of these 35 patients. The single most common radiological finding was generalized cerebral atrophy ($n = 16$) followed by white matter lesions and demyelination ($n = 5$). The high number of patients with cerebral atrophy has to be considered with caution, since the neuroradiological investigations were performed after onset of steroid therapy in some patients. Other findings were non-specific inflammatory changes ($n = 4$), intracerebral bleeding ($n = 2$) and brain oedema ($n = 1$). Cerebellar involvement was specifically reported in five children. The highest proportion of pathological findings was found in CNS group 4 (Table I).

Mortality and CNS involvement

Overall, the 3-year probability of survival (with 95% confidence interval, CI) was 56% (±7%). At the time of analysis, 109 patients (56%) were alive, with a median follow-up period of 5·3 years (range 0·2–10·0 years). Of the 109 survivors, 67 (61%) had been transplanted, 37 (34%) were classified as being 'off-therapy', and five non-transplanted patients had received HLH therapy during the previous year (Table II).

Forty-nine patients died before SCT and the reported cause of death for 18 of these (37%) was CNS involvement (no data on the cause of death = 6). Of the 35 deaths after SCT, six were reported as relapse of HLH; four of these had CNS involvement.

Neurological symptoms at follow-up

Non-transplanted patients. At time of analysis, a total of 42 patients were alive without an SCT. Of these, 37 (88%) were 'off-therapy' without neurological sequelae; the majority of these ($n = 25$) were in CNS group 1 (Table II). Two of the 42 were lost to adequate follow-up (neurological follow-up was <1 year). In the remaining three patients with ongoing treatment, one was described as being neurologically intact whereas two had severe developmental delay.

Transplanted patients. Altogether, 102 patients had undergone SCT, of whom 67 (66%) were alive at time of analysis. The median follow-up time after SCT with regard to neurological status was 5·3 years (range 1·4–9·9 years). Of these 67, 14 (21%) were reported to have neurological sequelae at their last follow-up. The most common sequelae were neurodevelopmental retardation ($n = 7$) and epilepsy ($n = 4$). In addition, attention deficit/hyperactivity disorder (ADHD) ($n = 2$), hearing loss ($n = 2$), minimal cerebral palsy ($n = 1$) and hemiplegia ($n = 1$) were also reported. The ability to play in these 14 patients with neurological sequelae was normal in seven, mildly moderately reduced in five and moderately severely reduced in one (no data = 1).

CNS disease groups

When differences in distribution of characteristics between the four CNS groups were compared (Table I), there were significantly more patients (38/50, 76%) aged less than 12 months at time of diagnosis in CNS group 3 compared with all other patients (68/143, 48%) ($P = 0{\cdot}001$, chi-squared test). None of the patients in CNS group 4 reached 'off-

Table II. Outcome with respect to CNS group in 193 patients.

	CNS group 1 (No neurological symptoms and normal CSF)	CNS group 2 (Neurological symptoms but normal CSF)	CNS group 3 (No neurological symptoms but abnormal CSF)	CNS group 4 (Neurological symptoms and abnormal CSF)	Total
No. evaluated patients	71	21	50	51	193
Clinical status 2 months after start of therapy					
Alive without neurological symptoms	63 (89)	14 (67)	41 (82)	26 (51)	144 (75)
Alive with neurological symptoms	2 (3)	3 (14)	2 (4)	20 (39)*	27 (14)*
Dead	6 (8)	4 (19)	7 (14)	5 (10)	22 (11)
Stem cell transplant					
SCT performed	32 (45)	8 (38)	31 (62)	31 (61)	102 (53)
Neurological symptoms at SCT	8/29 (28)	2/8 (25)	3/30 (10)	11/30 (37)	24/97(25)
Treatment status at last follow-up					
Alive after SCT	21 (30)	8 (38)	19 (38)	19 (37)	67 (35)
Alive on therapy without SCT	2 (3)	0	0	3 (6)	5 (3)
Alive off therapy†	25 (35)	6 (29)	6 (12)	0	37 (19)
Dead	23 (32)	7 (33)	25 (50)	29 (57)	84 (44)
Clinical status at last follow-up					
Alive without neurological sequelae	43 (61)	13 (62)	21 (42)	14 (27)	91 (47)
Alive with neurological sequelae	4 (6)	1 (5)	4 (8)	7 (14)	16 (8)
Alive but follow up <1 year	1 (1)	0	0	1 (2)	2 (2)
Dead	23 (32)	7 (33)	25 (50)	29 (57)	84 (44)

Values in parenthesis are in percentage.

CNS, central nervous system; CSF, cerebrospinal fluid; SCT, stem cell transplant; HLH, haemophagocytic lymphohistiocytosis.

*One patient had undergone a SCT.

†No HLH-therapy for ≥1 year without previous SCT; presumably secondary HLH.

therapy' status whereas 37/142 (26%) of the other patients did ($P < 0{\cdot}001$, chi-squared test) (Table II). The incidence of neurological sequelae in long-term survivors was higher in CNS group 4 (7/21, 33%) compared to CNS groups 1–3 (9/86, 10%) ($P = 0{\cdot}015$, Fisher's exact test) (Table II).

Survival

Univariate analyses of survival for the four CNS groups are shown in Fig 2. The probability of survival 3 years after start of treatment was 67% (±11%) for CNS group 1, 67% (±20%) for group 2, 50% (±14%) for group 3 and 44% (±14%) for group 4. Multivariate analyses with death as the endpoint showed no significant difference in survival between groups 1 and 2. Univariate hazard ratio (HR) indicated a significantly reduced survival for both groups 3 and 4 when compared with group 1 (Table III). However, after adjusting for potential confounding factors, the multivariate HR for group 3 compared with group 1 was 1·52 (0·82–2·82), whereas the HR for group 4 compared with group 1 was 2·05 (1·13–3·72) and therefore remained statistically significant, i.e. there was an increased risk of mortality for patients with neurological symptoms and abnormal CSF findings when compared with patients with no neurological symptoms and normal CSF. To further strengthen the analyses, the model was also run with a time-dependent covariate that evaluated the risk of dying before or after SCT in relation to the time between start of treatment and time for SCT, which did not alter any results (data not shown).

Of the 121 patients without neurological symptoms at diagnosis, 73 (60%) were alive at last follow-up compared to 36/72 patients (50%) with neurological symptoms ($P = 0{\cdot}162$; chi-squared test). With regard to CSF at diagnosis, 62/92 (67%) of children with normal cell and protein content were alive as compared to 47/101 (47%) with abnormal values ($P = 0{\cdot}004$, chi-squared test). Multivariate analysis, adjusted for age, sex and SCT, also showed a significantly increased risk of mortality in patients with abnormal CSF at diagnosis compared to those with normal CSF [HR 1·78 (CI = 1·08–2·92), $P = 0{\cdot}023$].

Discussion

This study suggests that a very high proportion (63%) of patients with HLH have neurological symptoms and/or abnormal CSF findings at the time of diagnosis, with seizures, meningismus and irritability being the three most common symptoms. In long-term survivors, neurological sequelae were reported in approximately 15%, the majority of these being neurodevelopmental delay and epilepsy. Children with both abnormal CSF and neurological symptoms (CNS group 4) fared worse with both a higher incidence of neurological sequelae in long-term survivors and an increased risk of mortality.

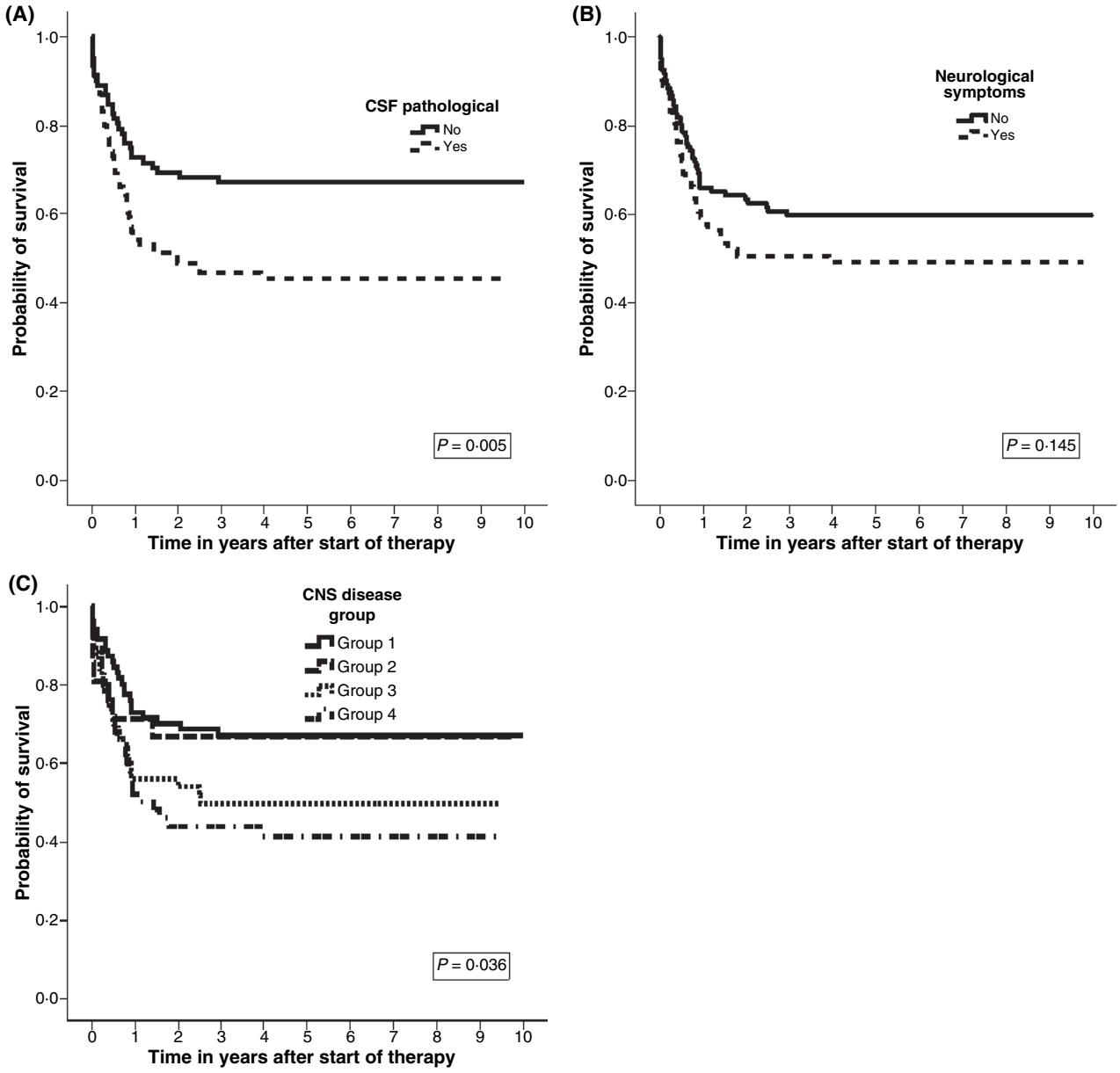

Fig 2. Survival in HLH with regard to the presence of neurological symptoms and cerebrospinal fluid (CSF) abnormalities at diagnosis. (A) Survival in patients with normal and abnormal CSF at diagnosis. (B) Survival in patients with and without neurological symptoms at diagnosis. (C) Survival with regard to both neurological symptoms and CSF abnormalities at diagnosis. CNS group 1: No neurological symptoms and normal CSF; group 2: Neurological symptoms with normal CSF; group 3: No neurological symptoms but abnormal CSF; group 4: Neurological symptoms with abnormal CSF. The survival rates were analysed using the Kaplan–Meier life table method, and univariate comparison of survival using the log rank test.

Importantly, late sequelae were significantly more frequent in patients with abnormal CSF at onset (24%) compared to patients with normal CSF (8%). Moreover, abnormal CSF also appeared to have a greater impact on mortality than evidence of neurological symptoms as adjusted multivariate analysis showed a significantly increased risk of mortality in patients with abnormal CSF at diagnosis but not in patients with neurological symptoms reported at diagnosis. Notably, it may be difficult to evaluate neurological symptoms in infants (<12 months at diagnosis). In our study, the proportion of infants was higher in CNS group 3 (no neurological symptoms but abnormal CSF) and it is possible that some infants that appeared neurologically intact at diagnosis actually may have had neurological symptoms.

The frequency of neurological symptoms at diagnosis was 37%, of CSF abnormalities at diagnosis 52%, and either/both of these findings 63%. Overall, these results are in line with earlier smaller studies reporting CSF abnormalities at diagnosis in the range of 52–73% (Janka, 1983; Henter *et al*, 1991b; Arico *et al*, 1996; Haddad *et al*, 1997). Many of the wide

Table III. Results of Cox proportional hazards regression analysis of survival on 193 patients with HLH.

	Univariate HR			Multivariate HR		
Covariate	HR of death	95% CI	*P*-value	HR of death	95% CI	*P*-value
Sex (Male)	1·11	0·72–1·71	0·644	1·19	0·76–1·86	0·438
Age <12 months at start*	1·93	1·22–3·05	0·005	2·31	1·36–4·05	0·002
CNS group 1 (No neurological symptoms and normal CSF)	1			1		
CNS group 2 (Neurological symptoms but normal CSF)	1·11	0·47–2·58	0·816	0·98	0·42–2·31	0·969
CNS group 3 (No neurological symptoms but abnormal CSF)	1·78	1·00–3·13	0·046	1·52	0·82–2·82	0·187
CNS group 4 (Neurological symptoms and abnormal CSF)	2·17	1·26–3·76	0·006	2·05	1·13–3·72	0·018
SCT performed	0·47	0·30–0·73	0·001	0·30	0·19–0·48	<0·001

CNS, central nervous system; CSF, cerebrospinal fluid; SCT, stem cell transplant; HLH, haemophagocytic lymphohistiocytosis; HR, hazard ratio.
*at start = at start of therapy.

variety of symptoms reported in our study were severe and included seizures, consciousness disturbances, cranial nerve palsies, ataxia, spasticity and hemiparesis. Physicians should be aware that HLH is now being reported also in adolescents and young adults (Clementi *et al*, 2002), and that cerebromeningeal involvement can be the first manifestation of HLH (Henter & Elinder, 1992; Kieslich *et al*, 2001; Feldmann *et al*, 2005) and, as such, can mimic a wide range of disorders including various infectious, inflammatory, ocular and neoplastic disorders and may even masquerade as child abuse (Appen *et al*, 1976; Kollias *et al*, 1994; Rooms *et al*, 2003). Swelling of the cerebellum with downward tonsillar herniation may also occur (Astigarraga *et al*, 2004).

Data on the incidence of neuroradiological abnormalities in HLH is limited, in particular with regard to findings at time for diagnosis (Kollias *et al*, 1994; Haddad *et al*, 1997; Imashuku *et al*, 2002; Fitzgerald & McClain, 2003). In this study, CT/MRI of the brain at onset was performed in an early phase of the disease in 60% of cases, of which 30% were reported as having pathological findings. Interestingly, abnormal neuroradiology was also reported in five of 37 patients with normal CSF findings and without neurological symptoms (CNS group 1), and it is possible that neuroradiological findings may precede apparent clinical neurological manifestations. However, as many as 52% (22/42) of the patients with both abnormal CSF and neurological symptoms also had pathological neuroradiological findings (Table I). This is in line with a recent study of 48 patients with primary HLH, which identified a trend towards a poorer prognosis for patients with clinical neurological manifestations and abnormal neuroimagery features before SCT (Ouachée-Chardin *et al*, 2006). To better define the predictive value of neurological evaluation and CNS imaging, their systematic use have both been incorporated in the current HLH-2004 trial (Henter *et al*, 2007).

Three major strengths of our study are: (i) its size: it is by far the largest study on HLH and CNS involvement; (ii) it is the first documented study to classify CNS groups, which enables a closer evaluation of the association of symptoms, pathological CSF and disease outcome; and (iii) it is, to the best of our knowledge, the first study to test such associations by multivariate analysis. An inevitable drawback of large multicentre studies is that multiple treatment centres can mean a lack of consistency in neurological evaluation and supportive care. The evaluation of neurological symptoms was also challenging because the majority of the children were very young at diagnosis made. All patients for whom the treating physician reported a neurological symptom are included in Fig 1. In a study such as this, it may be difficult to estimate the association between CNS group and survival because some patients have had an SCT and others have not. In our multivariate analysis, we therefore adjusted for SCT. To further strengthen the analyses, we also ran the model with a time-dependent covariate which evaluated the risk of dying before or after SCT in relation to the time between start of treatment and time for SCT, which did not alter any results.

To conclude, in light of the increasing probability of cure in HLH patients, long-term sequelae have become even more important to evaluate thoroughly. The most important of these are CNS sequelae, which may be severe. The present study, representing the largest report on CNS involvement in HLH, documents that abnormal CSF bears a significant, independent adverse prognostic value with regard to late sequelae as well as mortality. Moreover, in children with both abnormal CSF findings and neurological symptoms at diagnosis, only 27% were alive without neurological sequelae at follow-up. Our findings stress the importance of carrying out rigorous CNS examinations, by means of clinical neurological examination, CSF investigation and neuroradiology (preferably MRI). Prompt treatment of active HLH at onset or relapse may reduce neurological sequelae, and increasing awareness of the seriousness of CNS disease in HLH is an essential prerequisite for achieving this goal.

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Stockholm County Council (ALF), and the Histiocytosis Association of America.

Conflict of interest statement

We declare that we have no conflicts of interest.

Contributions

JIH initiated the HLH-94 study which was designed by JIH, GJ, MA, ME, AF, HG, SI, SL, and DW with JIH as PI, and these individuals all also served as subcentre co-ordinators. ACH and HT performed the data analysis for this paper and drafted the manuscript, which was finalized by JIH. All authors reviewed the paper and approved the final version.

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