## Discussion

More than 40 patients with refractory LCH have been reported to have received SCT since 1987.[15] The OS of the 29 patients who underwent myeloablative SCT was 48% (14/29 patients), and the transplant-related mortality was 45%. However, in a recent study reported from Europe,[10] seven (78%) of nine patients survived with no signs of disease activity after RIC-SCT. The conditioning regimen consisted of fludarabine, melphalan, low-dose total lymph node irradiation (2 Gy), and either of antithymocyte globulin or MabCampath (anti-CD52 antibody). In Japan, four patients with LCH underwent SCT between 1994 and 1997, and two of them (50%) survived.[16] In this study, 11 of the 15 patients have survived with no evidence of disease; 8/10 (80%) with myeloablative conditioning and 3/5 (60%) with RIC-SCT, and the 10-year OS rate was superior (73.3 ± 11.4%). In particular, two patients with RIC-SCT had organ dysfunction before SCT, suggesting that a less toxic conditioning regimen is also effective for patients with organ dysfunction.

In the recent report of 22 patients who underwent SCT, the donor source was a sibling in 17 (77%) and a matched unrelated donor in five (23%).[11] The stem cell source was BM in 12 (55%), PB in six (27%), and CB in four (18%).[11] In this study, 10 of 15 patients received UCB, and eight of these have survived with no evidence of disease, including three with RIC-SCT. Therefore, our results support the use of UCB as an alternative donor source when neither a sibling donor nor a matched unrelated donor is available.

The appropriate timing of SCT for LCH is unclear. In this study, nine of 15 patients underwent SCT within 12 months after initial diagnosis, including seven with risk organ involvement at diagnosis. This group had a life-threatening clinical course, and they required prompt SCT as the only potentially curative treatment. On the other hand, three patients without risk organ involvement at initial diagnosis underwent SCT 7 years or later after diagnosis, resulting in no evidence of disease. A total of six patients in the low-risk group underwent SCT because of PD or disease refractory to conventional chemotherapy. Although it is not known which LCH patients really require SCT, our findings suggest that SCT should also be considered in patients in the low-risk group at diagnosis who develop active or PD even after long-term chemotherapy. A large-scale prospective study could provide useful information to select subsets of LCH that definitely need SCT.

It has recently been shown that various cytokines have an important role in the pathogenesis of LCH, suggesting that eradication of the pathologic cells associated with cytokine production could be effective for refractory LCH. In the previous report, two patients with refractory LCH who underwent RIC-SCT showed resolution of disease after SCT.[10] Kinugawa *et al.*[16] reported one patient who failed engraftment, followed by complete autologous recovery and resolution of disease activity.[16] In this study, one patient who underwent allo-BMT (patient 9) had a similar clinical course. Two patients who relapsed after auto-PBSCT showed resolution of disease following conventional chemotherapy (patients 10 and 12). Although the disease state of the two patients at 12 or 14 weeks after chemotherapy was GR, multiple recurrences had occurred, and their disease state at SCT was PD. These two patients were rescued by a myeloablative conditioning regimen with infusion of donor T lymphocytes, which prevented deterioration of the LCH. Steiner *et al.*[17] also reported one patient who achieved complete remission after RIC-SCT, despite post transplant mixed chimerism, in which only a T-cell subset proved to be of donor origin. He emphasized that a strong immunomodulating influence, mainly exerted by allogeneic T-cells, rather than eradication of the LCH cell clone, may be potentially curative in LCH. Correction of inappropriate immunological crosstalk by the replacement of allogeneic donor cells may be pivotal.

Bernard *et al.*[9] reported that 7 of 10 patients with refractory LCH had achieved sustained complete remission after treatment with 2-CdA and Ara-C. In this study, two patients (patients 6 and 8), who failed to respond to the combination of 2-CdA and Ara-C, underwent SCT, and one has been alive with no disease after RIC-SCT. Prior utilization of 2-CdA may help tailor the indications for SCT.

In conclusion, the improved outcomes of SCT for refractory LCH show that it is a promising new salvage approach. RIC-SCT is desirable for young children, especially with non-malignant disease. Further investigations are required to establish the SCT strategy for refractory LCH.

## Conflict of interest

The authors declare no conflict of interest.

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# LETTER TO THE EDITOR
# VCR/AraC Chemotherapy and ND-CNS-LCH

To the Editor: We read with great interest the recent article by Allen et al. [1] on the use of vincristine (VCR) and cytosine arabinoside (AraC) combination for neurodegenerative central nervous system Langerhans cell histiocytosis (ND-CNS-LCH). The authors chose to employ this treatment regimen for two reasons: first, AraC can cross the blood–brain barrier, although no significant pharmacokinetic studies were done at the dose used; second, this combination was originally described by Egeler et al. [2] to be effective for patients with LCH at onset or relapse of disease.

Starting in 1996, we have been testing whether induction therapies that consist of VCR/AraC/prednisolone (PSL), called the JLSG-96 and JLSG-02 protocols, are effective for newly diagnosed patients with LCH in Japan [3,4]. By the end of 2009, more than a total of 340 multifocal LCH cases have been treated with these two protocols. Prior to 1996 there was no clinical trial. Even after 1996 the patients not participating on the study were treated with individual non-VCR/AraC regimens. We compared the cases of ND-CNS as defined by the Vienna group [5,6], in the VCR/AraC-treated group and the group who received other treatments for newly diagnosed LCH. Of the 16 cases in total 13 cases developed ND-CNS after systemic treatment for LCH; 8 had MRI abnormalities only and 5 had neurological symptoms. One case already had ND-CNS at the time LCH was diagnosed and the remaining two cases already had ataxia before LCH was diagnosed (Table I).

Of the 13 cases who developed ND-CNS after treatment, 5 had been treated with JLSG-96/02 protocols. The remaining eight had received other regimens (mostly a combination of vinblastine and PSL), of which four were the cases treated before 1996. The ND-CNS incidence for the JLSG-96/02 group was 1.5% but it could not be calculated for the other group because the total number of patients with LCH treated with other regimens was unknown. The incidence of ND-CNS LCH in the JLSG-96/02 protocol looks low compared with other international treatment protocols [7], but we must wait for longer and more comparable follow-up. When we used the EDSS [8] to score the neurological symptoms as minimal (<2.5), moderate (2.5–5), or severe (>5.0), we found that, with the follow-up of a median 6.2 (range; 0.5–18) years, 9 of the 16 patients have moderate to severe ND-CNS disease. One case with ND-CNS at onset has been stable with JLSG-02 treatment as there was no progression of neurological symptoms. The remaining two cases who had presented with ataxia before the diagnosis of LCH and received JLSG-02 protocol, have the worst prognosis as their neurological symptoms are now severe. We are also testing whether high-dose intravenous immunoglobulin (IVIG) regimen can prevent further progression of neurological symptoms particularly in cases with minimal to moderate EDSS scores [9].

It remains unknown whether the initial systemic chemotherapy for multifocal LCH patients can limit the later occurrence of ND-CNS. However, our data suggest that ND-CNS can also develop in LCH patients who were initially treated with the VCR/AraC combination. Thus, while this drug combination can be effective on active non-CNS LCH lesions, it is at present unclear whether it also acts on neurodegenerative lesions with the same mechanism.

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**TABLE I. Clinical Characteristics of the 16 Cases of ND-CNS-LCH That Are Registered in JLSG**

| Onset of ND-CNS | n | Initial treatment for LCH* | Time to ND-CNS from onset (years) | IVIG for ND-CNS (n/total) | Current status of ND-CNS** |
|---|---|---|---|---|---|
| After LCH treatment | 5 | JLSG-96/02 | 3.5 (2.0–6.9) | 4/5 | Minimal (n = 3)<br>Moderate (n = 1)<br>Severe (n = 1) |
| | 8*** | Other regimens | 4.5 (1.5–13.5) | 4/8 | Minimal (n = 3)<br>Moderate (n = 4)<br>Severe (n = 1) |
| At diagnosis of LCH | 1 | JLSG-02 | 0 | 1/1 | Minimal (n = 1) |
| Ataxia preceded the diagnosis of LCH | 2 | JLSG-02 | −0.5, −0.8 | 2/2 | Severe (n = 2) |

* VCR/AraC was employed in the JLSG-96/02 protocol but not in the other regimens. ** EDSS scores: minimal (<2.5), moderate (2.5–5.0), and severe (>5.0), *** Of which, four had the onset of LCH before 1996.

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ORIGINAL ARTICLE

# Tyrosine phosphatase SHP-1 is expressed higher in multisystem than in single-system Langerhans cell histiocytosis by immunohistochemistry

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**Abstract** Langerhans cell histiocytosis (LCH) is a proliferative disorder of Langerhans cell (LC)-like CD1a-positive cell (LCH cell) with unknown causes. LCH consists of two subtypes: single-system LCH (LCH-SS) with favorable prognosis and multisystem LCH (LCH-MS) with poor prognosis. LCH has been indicated as a neoplastic disorder from monoclonal characteristics of LCH cells. This study aimed to investigate an expression of tyrosine phosphatase SHP-1 in LCH, since its expression levels were variously reported in many tumors, overexpression in ovarian cancers (a candidate oncoprotein), and downregulation by methylation in gastric cancers, prostate cancers, malignant lymphomas, and leukemias (a putative tumor suppressor). By immunohistochemistry (IHC), the SHP-1 expression in LCs and LCH cells was compared in LCH (two subtypes: LCH-SS=21, LCH-MS=12), dermatopathic lymphadenopathy (DLA) ($n$=9) and normal epidermal LCs ($n$=3) near LCH lesion. IHC results were analyzed semiquantitatively using a Photoshop software. The mean intensity score (IS) of DLA, LCH-SS, LCH-MS, and LCs were 47, 100, 139, and 167 (in arbitrary unit), respectively. The IS had significant differences among LCH-SS, LCH-MS, and DLA ($p<0.01$). SHP-1 is expressed significantly higher in LCH-MS than in LCH-SS. SHP-1 can be a progression marker of LCH. SHP-1 is also useful for differential diagnosis between LCH in lymph nodes and DLA.

**Keywords** Langerhans cell histiocytosis · Tyrosine–protein phosphatase nonreceptor type 6 (PTPN6, SHP-1) · Immunohistochemistry · Photoshop-assisted image analysis · Staining intensity

**Abbreviations**

| | |
|---|---|
| B-ALL | B lymphoblastic leukemia |
| CMYK | Cyan/magenta/yellow/key |
| IF | Immunofluorescence |
| IHC | Immunohistochemistry |
| IS | Intensity score |
| ISs | Intensity scores |
| LC | Langerhans cell |
| LCH | Langerhans cell histiocytosis |
| LCH cell | Langerhans cell-like CD1a-positive cell |

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| | |
|---|---|
| LCH-MS | Multisystem LCH |
| LCH-SS | Single-system LCH |
| LCS | LC sarcoma |
| meV | Viable motheaten mice |
| MHC | Major histocompatibility complex |
| PTPN6 | Tyrosine–protein phosphatase nonreceptor type 6 |
| SHP-1 | Src homology region 2 domain-containing phosphatase 1 |
| SHPS-1 | Substrate of SHP-1 |
| T-ALL | T lymphoblastic leukemia |

## Introduction

Langerhans cells (LCs) are specialized immature dendritic cells (DCs) in the skin. When LCs encounter exogenous antigens, they capture and process them to generate major histocompatibility complex (MHC)/peptide complexes on their surface. LCs migrate from epidermis to draining lymphoid tissues in order to initiate and present the MHC/peptide complexes to the T cells. During this migration, LCs mature, and the expressions of their cell-surface molecules are changed [1, 2]. Langerhans cell histiocytosis (LCH) is a proliferative disorder of Langerhans cell (LC)-like CD1a-positive cell (LCH cell) with unknown causes. Patients with LCH develop as two clinical subtypes: single-system LCH (LCH-SS) and multisystem LCH (LCH-MS).

LCH tissues are known to have monoclonal characteristics of LCH cells by the *human androgen receptor* gene assay [3, 4]. LCH cells of two LCH patients suffering from T lymphoblastic leukemia (T-ALL) have T cell receptor rearrangement of the same cell origin as T-ALL cells [5]. In addition, LC sarcoma (LCS) cells in a patient who suffered from B lymphoblastic leukemia (B-ALL) were found to have identical immunoglobulin heavy chain rearrangements in both LCS cells and B-ALL cells [6]. Signal transduction pathways, such as the IL-17A-dependent pathway, have been reported to have abnormalities in LCH cells [7, 8]. More recently, *BRAF* mutation (V600E) was reported in 57% of LCH cases [9].

These accumulated data indicate that LCH is a neoplastic disease with abnormalities of signal transduction pathways. Tyrosine–protein phosphatase nonreceptor type 6 (PTPN6, src homology region 2 domain-containing phosphatase 1 (SHP-1), Hep, HepH, HPTP1C, SH-PTP1, HCP) is known to be one of nonmembranous type phosphatases and plays an important role in many signal transduction pathways especially in hematopoietic neoplasms. Downregulation of SHP-1 was widely reported in lymphomas and leukemias at a high rate by our group and other groups [10, 11], while SHP-1 was overexpressed in epithelial tumors such as ovarian cancers [12, 13]. In cases of downregulation, epigenetic mechanisms such as methylation have been proposed, and loss of SHP-1 function in cytokine signaling has been shown to have a relationship with neoplastic mechanisms. In contrast, affected signaling events of overexpressed SHP-1 were not known in ovarian cancers [13], although some epithelial cell lines such as 293 cells and Hela cells, SHP-1 acts positively for stimulation such as epidermal growth factor [14, 15].

LCH cells in LCH-MS are known to have an immature character of LC compared with LCH-SS [16], and one of the substrates of SHP-1, termed as SHPS-1, was reported to regulate the induction of murine LCs into maturation [17]. However, to date, little is known about the role of SHP-1 in both LCs and LCH cells in humans. Such paucity of data has driven us to analyze SHP-1 in human LCs and LCH tissues. We hypothesized that downregulation of SHP-1 might have a role in the pathogenesis of LCH because LC is one myeloid-derived dendritic cell. The objective of the present study was to clarify the relationship of the expression levels of SHP-1 between tissues from patients with LCH-SS and LCH-MS and other control tissues, the dermatopathic lymphadenopathy (DLA) as a control of mature LC, and the epidermal LC as a control of immature LC, using the immunohistochemical method.

## Materials and methods

### Patients, samples, and clinical data

The study was approved by the Institutional Review Board of Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan. Tissues of LCH were obtained from patients registered with the Japan LCH Study Group (JLSG) between 2002 and 2009. Tissues of DLA and normal skin tissues contained in the dermal tumors (such as dermatofibroma) were from patients who visited Okayama University Hospital between 2002 and 2009. Histological sections and immunohistochemistry (IHC) such as the CD1a of all the specimens were reviewed by pathologists to confirm the diagnosis. Information on patients including laboratory data at onset of disease was obtained from the JLSG registration center and the medical records of patients at Okayama University Hospital. The clinical data are as follows: hemoglobin, platelet, white blood cell, lymphocyte, total protein, albumin, total bilirubin, lactate dehydrogenase, glutamic–oxaloacetic transaminase (aspartate aminotransferase), glutamic pyruvic transaminase (alanine transaminase), C-reactive protein, erythrocyte sedimentation rate, and soluble interleukin-2 receptor.

### Antibodies for immunofluorescence and immunohistochemistry

Rabbit polyclonal antibody directed against SHP-1 (SH-PTP1 (C-19): sc-287, Santa Cruz Biotechnology, Inc., CA, USA) was used at 1:1,600 dilution. Anti-CD1a antibody (monoclonal mouse anti-human CD1a antibody (IgG1, kappa), O10, Dako Japan, Kyoto, Japan) was used at 1:100 dilution.

### Immunofluorescence

Tissue samples were fixed in 10% neutral-buffered formalin and embedded in paraffin. Sections were deparaffinized in xylene and rehydrated in a graded ethanol series. Antigen retrieval was performed by heating slides in 0.01 M citrate-buffered solution, pH 6.0, in a pressure cooker at 98°C for 15 min. The slides were incubated for 1 h in 5% skim milk, then primary antibodies were applied against both SHP-1 and CD1a, and the slides were maintained for 3 h at 37°C. After washing first antibodies, second antibodies, Alexa Fluor® 555 goat anti-mouse IgG (H+L) and Alexa Fluor® 488 goat anti-rabbit IgG (H+L) (Molecular Probes, Inc., Eugene, OR, USA) were applied for 1 h at room temperature. Images were captured and analyzed using Image Gauge version 3.3 (Fujifilm Corporation, Tokyo, Japan).

### Immunohistochemistry

For IHC, treatment of tissue samples is the same as described in immunofluorescence (IF). For blocking endogenous peroxidase activity, hydrogen peroxide was used. Antigen retrieval and incubation were the same as described. Next, 1 h of incubation of secondary antibody at room temperature and staining were performed using the DAKO EnVision+system (Dako Japan, Kyoto, Japan) and 3,3′-diaminobenzidine as a chromogen. The slides were counterstained with hematoxylin. As appropriate, positive controls and negative controls, normal lymph nodes, and skin samples, respectively, were used throughout. An isotype control was not performed.

### Digital image capture

Digital images for photomicroscopy of the best-stained area of the sections as representative of the overall immunostaining were acquired with the Olympus DP71 microscope digital camera (Olympus, Tokyo, Japan). All images were obtained using a ×40 objective of the Olympus BX51 microscope. The software used was the Olympus DP2-BSW. For the entire study, the camera was manipulated with manual controls with its auto mode turned off and used to adjust the image intensity, which was kept at an identical level during the entire study. Conditions were as follows: manual exposure, 720 μs; ISO 1600; tagged image format, 4,140×3,096 pixel shift. Files were saved in uncompressed TIFF format so that their sizes were fixed to 36.7 MB.

### Quantification of immunohistochemical chromogen

The determination of immunostaining intensity was assisted by the use of Adobe Photoshop, version 5.0 J (Adobe Systems Incorporated, Japan office, Shinagawa, Tokyo, Japan) as described by Lehr et al. [18]. Using the magic wand tool in the select menu of Photoshop, the cursor was placed on SHP-1-positive cytoplasm. The tolerance level of the magic wand tool was adjusted so that the entire positive cytoplasm was selected automatically. This selection was confirmed by checking both the selected figure and its inverted figure, which was made using the inverse tool. A yellow optical density plot of the selected area was generated using the histogram tool in the image menu as described by Pham et al. [19]. The mean staining intensity was calculated as follows: intensity score (IS)=255−mean of brightness of yellow channel score on a cyan/magenta/yellow/key color model (in arbitrary unit, AU). Immunohistochemical data as IS (AU) of LC, DLA, LCH-SS, and LCH-MS were plotted using the SPSS software (version 15.0 J, SPSS Japan Inc., Tokyo, Japan).

### Necrosis and mitotic index in LCH specimens

In LCH specimens, we also determined necrosis (+ or −) and mitotic index (mitotic number/10 high-power field using an Olympus BX50 microscope with SWH10X-H/26.5 eyepieces), compared between LCH-SS and LCH-MS subtypes.

### Statistical analysis

Analyses were performed using the SPSS software or Microsoft Office Excel 2003 (Microsoft Corporation, Tokyo, Japan). Comparisons of immunohistochemical data of SHP-1 between LCH and DLA, between LCH-MS and LCH-SS, were performed using Wilcoxon's signed-rank test. Immunohistochemical data of SHP-1 and histological data such as necrosis were compared using Wilcoxon's signed-rank test. The correlation of SHP-1 data, mitotic index, and clinical data were determined using regression analyses. Comparisons of the LCH subtypes and histological data such as necrosis or mitotic index were performed using Fisher's exact test or Wilcoxon's signed-rank test. Differences between values were considered statistically significant at $p<0.01$ or $p<0.05$.

## Results

### Characteristics of patients

The characteristics of patients are summarized in Table 1. The numbers of patients were 21 patients with LCH-SS, 12 patients with LCH-MS, and nine patients with DLA. The median ages of the LCH-SS patients were 6 years 6 months old (range, 0–71 years old). The median ages of the LCH-MS patients were 1 year 6 months old (range, 0–62 years old). The median age of DLA patients was 78 years old (range, 72–87 years old). The male to female ratio of LCH-SS was 16:5. The male to female ratio of LCH-MS was 6:6. The male to female ratio of DLA was 6:3. Epidermal LCs were analyzed from three dermal LCH.

### IF: identification of SHP-1-and CD1a-double positive cells

We first demonstrated that CD1a-positive LCs [20] in normal epidermis were proven to express SHP-1 by double staining. Spinous cells showed weak positivity and granular cells showed strong positivity (Fig. 1a).

### Quantification of IHC of SHP-1 in epidermal LC, DLA, and LCH

LCs in the epidermis expressed SHP-1, and spinous cells showed weak positivity and granular cells showed just a little strong positivity (Fig. 1b). LCs in DLA and LCH cells in LCH showed negative to high expression of SHP-1 as shown in Fig. 1c–f. Immunohistochemical data as ISs (AU)

**Table 1** Clinical characteristics of LCH patients and SHP-1 intensity score

| Patient number | Age | Sex | Subtype | Biopsy site | Necrosis | MI | IS |
|---|---|---|---|---|---|---|---|
| 1000114 | 2 years 3 months | M | SS | Bone | + | 0 | 68 |
| 1000115 | 1 year 1 month | M | SS | Bone | − | 0 | 63 |
| 1000179 | 58 years | F | SS | LN | − | 15 | 96 |
| 1000202 | 71 years | M | SS | Skin | − | 12 | 78 |
| 1000219 | 2 years 11 months | M | SS | Bone | + | 4 | 114 |
| 1000222 | 5 years 1 month | F | SS | Bone | − | 1 | 118 |
| 1000227 | 1 years 5 months | M | SS | Bone | − | 0 | 103 |
| 1000267 | 4 years 2 months | M | SS | Bone | − | 0 | 97 |
| 1000275 | 6 years 6 months | M | SS | Bone | − | 2 | 140 |
| 13001 | 11 years | F | SS | Bone | + | 0 | 126 |
| 130011 | 65 years | M | SS | Orbit | + | 2 | 112 |
| 130012 | 29 years | M | SS | Lung | − | 0 | 100 |
| 130015 | 32 years | M | SS | LN | − | 9 | 62 |
| 130016 | 61 years | M | SS | Lung | + | 2 | 105 |
| 130017 | 2 years 5 months | M | SS | Bone | − | 23 | 60 |
| 13002 | 39 years | M | SS | Lung | − | 0 | 58 |
| 13003 | 7 years | M | SS | Bone | + | 3 | 93 |
| 13005 | 0 month | M | SS | Bone | + | 1 | 111 |
| 13006 | 4 years | F | SS | Bone | + | 4 | 158 |
| 13007 | 4 years | F | SS | Bone | + | 0 | 131 |
| 13008 | 7 years | M | SS | Bone | + | 0 | 97 |
| 1000159 | 2 years | F | MS | Bone | − | 0 | 108 |
| 1000178 | 1 month | F | MS | Liver | − | 1 | 97 |
| 1000185 | 10 months | M | MS | LN | + | 6 | 85 |
| 1000201 | 5 months | M | MS | Bone | + | 1 | 128 |
| 1000216 | 1 year 1 month | F | MS | LN | + | 20 | 179 |
| 1000230 | 4 months | F | MS | Skin | + | 20 | 179 |
| 1000234 | 4 year 2 months | F | MS | Bone | − | 0 | 161 |
| 1000249 | 62 years | M | MS | Skin | − | 17 | 176 |
| 1000273 | 7 years 8 months | M | MS | Bone | − | 1 | 94 |
| 1000277 | 0 month | M | MS | Skin | − | 4 | 180 |
| 130014 | 7 years | M | MS | Skin, LN | + | 1 | 128 |
| 13009 | 8 years | F | MS | Bone | + | 4 | 150 |

*MI* mitotic index, *M* male, *F* female, *LN* lymph node

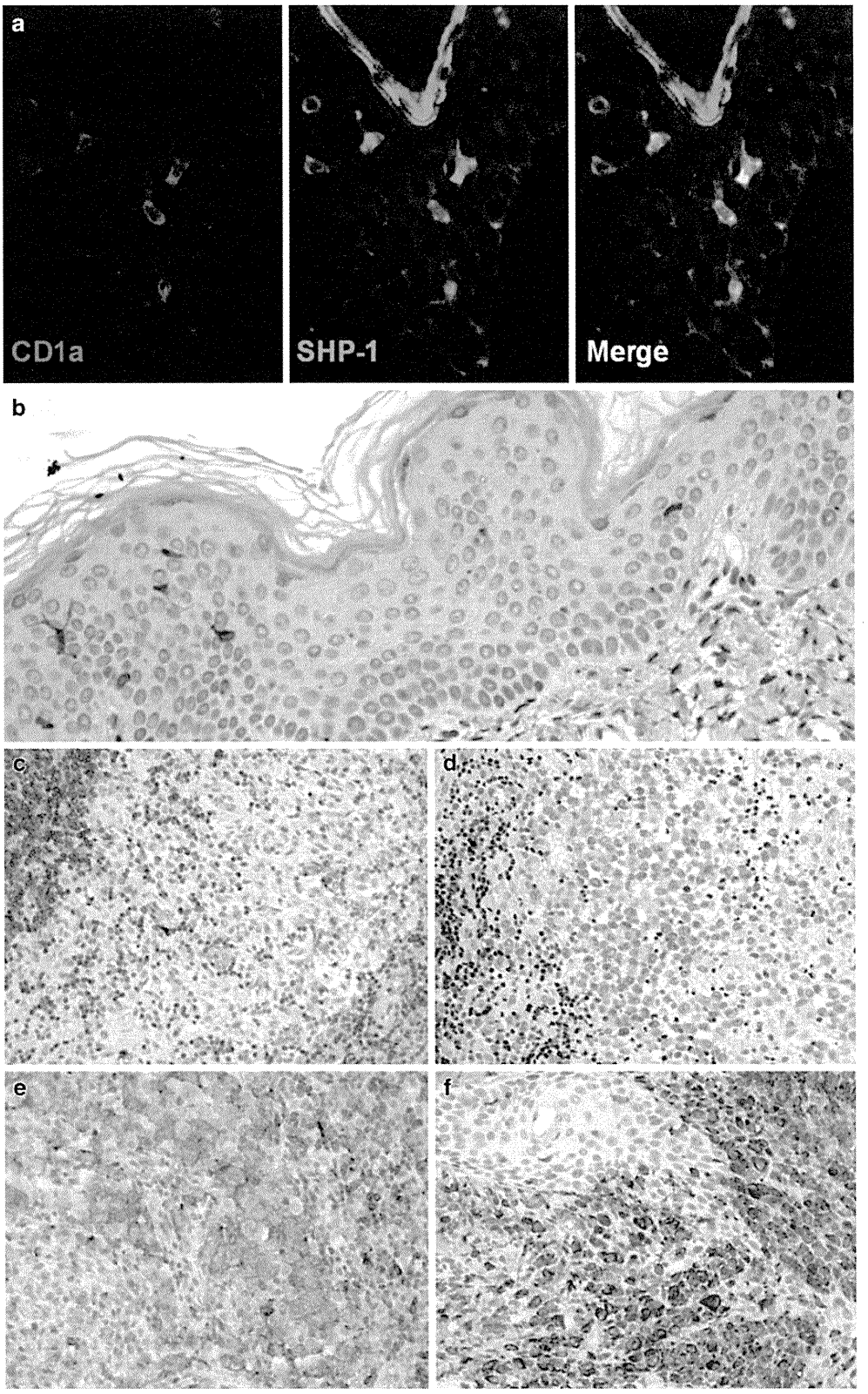


**Fig. 1** **a** Detection of CD1a and SHP-1 in skin (epidermis) by IF is shown from a 55-year-old female abdominal skin. Left, CD1a positive LCs. Middle, IF of SHP-1 revealed the strong immunoreactivity in LCs and granular cells, and weak positivity in spinous cells. Right, merge of left and middle images. **b** Detection of SHP-1 protein expression by IHC in normal skin of baby with LCH-MS (1000277). LCs (IS=164 AU) and granular cells in epidermis showed positivity for SHP-1. Spinous cells in epidermis showed vague positivity. **c–f** Detection of SHP-1 protein in the tissues from DLA and LCH by IHC. **c** LCs (IS=45 AU) in DLA of 87-year-old male (150034678). Lymphocytes showed positivity. **d** Bone lesion (IS=93 AU) of LCH-SS (13003). **e** Lung lesion (IS=105 AU) of LCH-SS (130016). **f** Skin lesion (IS=179 AU) of LCH-MS (1000230)

of epidermal LC, DLA, LCH-SS, and LCH-MS were plotted respectively, as shown in Fig. 2, using the SPSS software. As shown, ISs of SHP-1 ranged from 42 (negative) to 180 (strong staining) in the cytoplasm of LCs/LCH cells of DLA and LCH. On the other hand, ISs of SHP-1 expressed in the cytoplasm of LCs in normal epidermis of dermal LCH cases were 164 (patient 1000277), 165 (patient 1000249), and 173 (patient

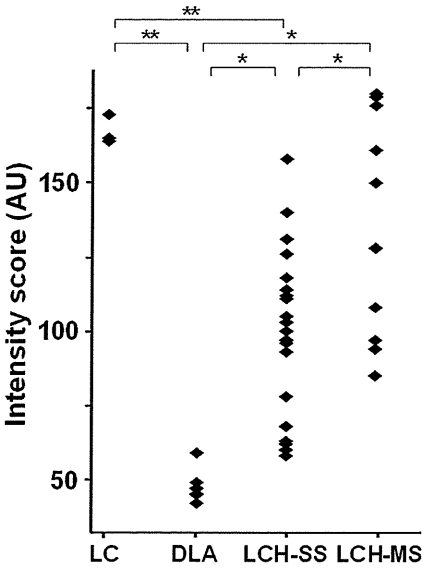


**Fig. 2** Dot plots of IS of LCs in the normal epidermis of LCH patients, proliferating cells in DLA, LCH-SS, and LCH-MS. The *x*-axis shows LC, DLA, LCH-SS, and LCH-MS. An *asterisk* means $p<0.01$. A *double asterisk* means $p<0.05$

1000230). The mean IS of DLA, LCH-SS, LCH-MS, and epidermal LC were 47, 100, 139, and 167 AU. A significant difference of SHP-1 reactivity was observed between DLA and LCH-SS ($p<0.01$), DLA and LCH-MS ($p<0.01$), and LCH-SS and LCH-MS ($p<0.01$). Also, a significant difference of SHP-1 reactivity was observed between epidermal LC and DLA ($p<0.05$) and epidermal LC and LCH-SS ($p<0.05$). Between epidermal LC and LCH-MS, there was no significant difference. In skin samples, the dermis as negative control did not give background staining.

Correlation of LCH subtypes with necrosis/mitotic index

LCH subtypes did not have a relationship with the rates of necrosis (Fisher's exact test) or mitotic index (Wilcoxon's signed-rank test) in LCH tissues.

Correlation of SHP-1 expression with necrosis/mitotic index and various clinical variables in LCH

The expression levels of SHP-1 did not correlate with the rates of necrosis (Wilcoxon's signed-rank test) or mitotic index in LCH tissues (regression analyses), nor with any clinical variables (regression analyses).

## Discussion

LCs are specialized immature DCs in the skin. Ramachandran et al. showed that SHP-1 was an important inhibitor of mice DC signaling, targeting multiple activation pathways [21]. Accumulated data showed that SHP-1 exerted either putative oncogenic or tumor suppressive functions, depending on the cellular context. Considering the fact that no data of SHP-1 in human LCs were available in the past, our data could be the first in human LCs

The aim of our study was to determine whether SHP-1 expression was related to LCH. Our hypothesis was that SHP-1 was negative or showed low expression in LCH, as in other hematopoietic cell neoplasms such as malignant lymphoma or leukemia. The results of SHP-1 expression analysis showed the opposite of this hypothesis. Higher SHP-1 expression was correlated with worse LCH subtype and thus with worse prognosis (LCH-MS).

Our study had five novel findings: (1) LCs in the epidermis were highlighted compared with spinous cells in the epidermis, except for granular cells, using highly diluted anti-SHP-1 polyclonal antibody (1,600:1). At a dilution of 1,600:1 of anti-SHP-1 antibody, LCs in epidermis (not LCH cells that were in the dermis) of patients 1000249, 1000277, and 1000230 were strongly positive and the epidermis showed faint positivity in IHC and IF, as shown in Fig. 1a, b. Differences of sensitivity between IF and IHC were thought to cause faint positivity of granular cells as well as spinous cells in IHC of Fig. 1b compared with IF of Fig. 1a. (2) Semiquantitative analyses could be performed among LCH cells using highly diluted anti-SHP-1 antibody (1,600:1). (3) LCH cells in LCH-MS did not show a significant difference in SHP-1 intensity compared with LCs in the epidermis. (4) SHP-1 intensity of LCH cells in LCH-SS was weaker in IHC compared with that of LCH-MS (Fig. 1d–f). (5) Proliferating nonneoplastic LC of DLA showed negative or very weak reaction of SHP-1 in IHC (Fig. 1c).

Based on the formerly described data about cell volume of LCs [22], LCH cell diameters [23], and LCH cell nucleus diameters [24], LCH cell cytoplasm volumes are thought to reach 8.7 times those of epidermal LCs. The cell size differences between LCH cells and epidermal LCs and calculation of SHP-1 staining intensity in AU indicate difficulty to compare total volumes of SHP-1 between LCH cells and epidermal LCs.

From the pathological point of view, LCH-SS was described to have more necrotic tissues compared with LCH-MS by Bank et al. [25]; however, our data of almost all Japanese patients were different from those of Bank et al., and we could not confirm this. SHP-1 expression correlates with neither mitotic index in LCH tissue nor any other clinical data at onset of disease.

Low or negative expression of SHP-1 in LCs in DLA is compatible with the theory that SHP-1 negatively regulates LCs migration to lymph nodes from epidermis [17]. Thus, our SHP-1 data could be compatible with data by Geissman

et al. that LCH cells of self-healing or isolated cutaneous LCH (LCH-SS) have a more mature phenotype (with low expression of SHP-1) than those in disseminated disease (LCH-MS) [16]. SHP-1 is also useful for the differential diagnosis between LCH in lymph nodes and DLA. Organs which LCH involves are various. A lymph node may be a presenting and only site of LCH. LCH involves only skin and lymph nodes [23]. Those cases without characteristic features such as increased eosinophil levels give a diagnostic problem at the point of differential diagnosis with DLA containing proliferating LCs in lymph nodes [26].

Motheaten mice or viable motheaten mice (meV) lacks the SHP-1 expression [27]. meV shows normal LC numbers in the epidermis at birth, but the numbers decrease along with mouse maturation [28, 29]. SHP-1 might be necessary to maintain the LC number in the epidermis by maintaining immaturity of the mouse skin. Data on such mice LCs and human LCs in DLA may suggest that SHP-1 in the skin (keratinocytes) and/or SHP-1 in LCs themselves play a role in maintaining the immaturity of LCs.

Very recently, *BRAF* mutation was described in LCH including LCH-SS cases involving the lungs [9]. *BRAF* mutation is also related with many cancers [30]. Such data point out the neoplastic possibility of LCH as well as a clonal association between LCH and a lymphoblastic leukemia [5, 6]. It is an important question whether LCH-SS involving the lungs is neoplastic or nonneoplastic. Our data about SHP-1 expression of two cases LCH-SS involving the lungs looks same as in other LCH-SS cases, but two cases are not enough to talk about the role of SHP-1 expression in this differential diagnosis statistically. SHP-1 exists in some signaling pathways relating to BRAF [31, 32]. Thus, it is an important question whether SHP-1 relates or not with BRAF or mitogen-activated protein/extracellular signal-regulated kinase pathways in LCH. We are currently analyzing roles or functions of SHP-1 using transfectants derived from a cell line with LC character.

In conclusion, SHP-1 expressions were negative, low and high among DLA (nine patients), LCH-SS (21 patients), and LCH-MS (12 patients), respectively, with a significant difference. This difference can be used to provide insight into LCH and differential diagnosis among DLA, LCH-SS, and LCH-MS, especially between LCH in LNs and DLA.

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**Conflict of interest** We declare that we have no conflict of interest.

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CASE REPORT

# Treatment of patients with hypothalamic-pituitary lesions as adult-onset Langerhans cell histiocytosis

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**Abstract** We report four cases of adult-onset Langerhans cell histiocytosis (LCH) with central nervous system (CNS) lesions in the hypothalamic-pituitary region. The first clinical symptoms were diabetes insipidus (two patients), hypothyroidism (one patient), and decreased libido/erectile dysfunction (one patient). Diagnosis was delayed as the CNS lesion was not initially suspected to be secondary to LCH, with a median time from symptom onset to treatment of 3.0 (range <1–5.3) years. In three patients, the tumor mass was effectively reduced by chemotherapy; however, all patients continue to exhibit hypopituitarism. Early diagnosis and initiation of treatment are required to improve the outcome of CNS-LCH in adult patients.

**Keywords** Langerhans cell histiocytosis · Medical oncology · Brain tumors · Hypothalamic-pituitary mass · Hypopituitarism

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## 1 Introduction

Langerhans cell histiocytosis (LCH) is a rare proliferative disorder of cells with the phenotype of activated Langerhans cells [1, 2]. About two-thirds of LCH cases develop in childhood while the remaining one-third develops in adulthood. LCH is a single or multi-system disease of the skin, bones, soft tissues, hematopoietic system and central nervous system (CNS) [2]. With regard to LCH with CNS involvement, diabetes insipidus (DI) is the most frequent symptom. It is diagnosed by magnetic resonance imaging (MRI) that reveals the absence of the posterior pituitary bright signal with or without a thickened pituitary stalk and/or space-occupying mass lesions at the hypothalamic-pituitary region (HPR) [3, 4]. However, even when such lesions are detected, LCH diagnosis is often delayed, particularly when the CNS lesion is the only LCH site. This is because such lesions can also be caused by other diseases [5–7]. Patients with LCH-induced lesions at the HPR and DI can eventually develop hypopituitarism [8], also known as anterior pituitary dysfunction. This has been observed in both children and adults [4, 9, 10]. However, reports of

adult cases are very limited, which make the treatment of adult patients further difficult.

LCH-induced DI has been considered not reversible in the majority of patients with this complication. It was shown recently that 2-chlorodeoxyadenosine (2-CDA) effectively induces a complete radiographic resolution of enhancing mass lesions in the CNS [11]. 2-CDA successfully reversed DI in a pediatric patient when it was given early [12]. While LCH-induced endocrine dysfunctions other than DI appear to be permanent and not reversible by any known treatment [9, 10], it remains to be determined whether the early introduction of 2-CDA or any specific therapy can reverse hypopituitarism and/or DI. Since the information on adult LCH is still fragmentary, we report here the cases of four adult-onset LCH patients with lesions at the HPR hoping that these case series may unravel critical issues to improve the outcome of the adult LCH in future.

## 2 Case report

### 2.1 Case 1

A 20-year-old girl first complained of polyuria/polydipsia and amenorrhea. Eight months later, she was diagnosed with hypopituitarism in association with a gadolinium (Gd)-enhanced mass at the HPR that was detected by a brain MRI (Fig. 1a). An immediate open biopsy of the mass confirmed the diagnosis of LCH. Localized irradiation (21 Gy) markedly reduced the mass. Two years later, the patient was referred to us with re-growth of hypothalamic mass, continued amenorrhea, poorly controlled DI and generalized cutaneous LCH, which was confirmed by biopsy. On admission, she was very obese (159 cm, 89.5 kg) with significantly low serum hormone levels (Table 1). Multiagents' chemotherapy was started, consisting of vinblastine (VBL; once a month), methotrexate (once a month), oral prednisolone (PSL; 5 days a month) and daily oral 6-mercaptopurine (6-MP), followed by a combination of vincristine/cytarabine/methotrexate/PSL with daily oral 6-MP for an additional 5 months. DDAVP and hormonal replacement therapy (estrogen/progesterone, thyroxin) were continued. The size of hypothalamic mass was reduced significantly (>50%) along with complete resolution of skin lesions (Table 1). Five years after LCH onset, her serum prolactin and estradiol levels returned to normal. Thereafter, she has become free from DDAVP, but without restoring of menstrual cycles.

### 2.2 Case 2

A 36-year-old female developed DI in association with amenorrhea and was placed on DDAVP. The thickened pituitary stalk detected by MRI remained under observation but was not treated because of a presumed diagnosis of lymphocytic hypophysitis. However, the thickened pituitary stalk progressed fairly rapidly into a Gd-enhanced mass at the HPR (Fig. 1b). One and a half years later after onset, a tumor biopsy revealed typical LCH histology. When referred to us, she was obese (156 cm, 87.0 kg) and had amenorrhea, fatty liver, reduced glucose tolerance and reduced hormone levels (Table 1). Five courses of 2-CDA (5 mg/day × 5 days) and PSL (20 mg/day) over 4.5 months, followed by daily oral 6-MP, significantly reduced the mass size (>50%) and pituitary stalk thickening. LCH lesions outside the CNS were not found. The patient remains markedly obese and diabetic.

### 2.3 Case 3

A 38-year-old male was first diagnosed with primary hypothyroidism. Nineteen months later, he developed the symptoms of thirst, fatigue and disturbed consciousness along with disorientation and abnormal behaviors. A brain MRI revealed a Gd-enhanced mass at the HPR (Fig. 1c).

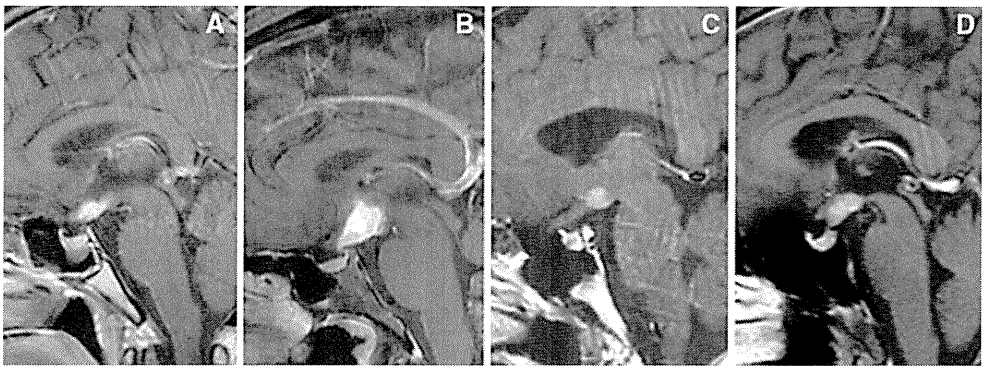


**Fig. 1** Sagittal magnetic resonance imaging view of the gadolinium-enhanced mass at the hypothalamic-pituitary region in four adult patients at disease onset. **a–d** Cases 1–4, respectively

**Table 1** Patient profiles

| Cases | Age (years)/ sex | LCH type | Symptoms | Hormonal dysfunction[a] | Time (years) to biopsy of CNS mass from onset of symptoms | Time (years) to treatment from onset of symptoms | Treatment | Reduction of mass size[b] (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | 20/F | MS[1] | DI, amenorrhea, obesity, skin lesions | Reduced (LH, FSH, freeT4, ACTH, GH) Elevated (PRL) | 0.7 | <1.0 | Irradiation (21 Gy), multiagents' chemo[c] | 75.5 |
| 2 | 36/F | CNS alone | DI, amenorrhea, obesity | Reduced (GH, LH, FSH, freeT4) Elevated (PRL) | 1.6 | 1.8 | 2-CDA/PSL, 6-MP | 87 |
| 3 | 38/M | MS[2] | DI, polyendo-crinopathy, bone lesions | Reduced (GH, LH, FSH, ACTH) Elevated (PRL) | 1.8 | 4.1 | 2-CDA/PSL, 6-MP | 20 |
| 4 | 46/M | MS[3] | DI, decreased libido, erectile dysfunction, bone lesions | Reduced (LH, FSH, freeT4, ACTH) Elevated (PRL) | 4.0 | 5.3 | VBL/PSL 2-CDA/PSL, 6-MP | 58 |

*CNS* central nervous system, *MS* multisystem type ([1]CNS + skin lesions, [2]CNS + multiple bone lesions, [3]CNS + spinal bones), *DI* diabetes insipidus, *VBL* vinblastine, *6-MP* 6-mercaptopurine, *PSL* prednisolone, *2-CDA* 2-chlorodeoxyadenosine, *GH* growth hormone, *LH* luteinizing hormone, *FSH* follicle-stimulating hormone, *ACTH* adrenocorticotropic hormone, *PRL* prolactin

[a] Plasma antidiuretic hormone levels were within normal ranges at onset but later dropped markedly in all cases

[b] Mass size change was calculated based on the 3-dimensional (axial, sagittal and coronal) MRI findings, for the recurred CNS mass in Case 1 with use of VBL-containing multiagents' chemotherapy, and for the primary CNS mass in Cases 2–4 with use of 2-CDA/PSL

[c] See text

He also showed complicated polyendocrinopathy consisting of DI, hypogonadism, hyperthyroidism, adrenal crisis and severe orthostatic hypotension (Table 1). The mass was presumptively diagnosed as pilocytic astrocytoma on the basis of an endoscopic biopsy. DDAVP was started together with corticosteroid, thiamazole and testosterone propionate. Four years later, osteolytic bone lesions appeared on the right femur and left clavicle, which was diagnosed as LCH by a biopsy. Eventually, the histology of the CNS mass was confirmed to be a LCH lesion. The patient received five courses of 2-CDA/PSL chemotherapy, followed by daily oral 6-MP. However, these treatments only minimally (<50%) reduced the mass size.

### 2.4 Case 4

A 46-year-old male first presented with decreased libido and erectile dysfunction 7 years after total gastrectomy for gastric adenocarcinoma. Four years later, a Gd-enhanced mass at the HPR was detected (Fig. 1d). Until then, he had ignored his polydipsia/polyuria symptoms. The CNS mass biopsy led to a diagnosis of LCH. A systemic MRI survey also revealed multiple spinal involvements. Based on the patient's hormone deficiency (Table 1), hormonal replacement therapy with human chorionic gonadotropin, follitropin alpha and human growth hormone was started. The patient also had loss of concentration and short-term memory deficits that were suggestive of mild neurodegenerative disease. Eventually, chemotherapy (VBL/PSL) was given but stopped after three courses because liver dysfunction grade 2 was observed. However, a year later, the patient decided to receive five courses of 2-CDA/PSL followed by daily oral 6-MP. This markedly reduced the CNS mass size (>50%).

## 3 Discussion

LCH-induced hypopituitarism has been described in both adults and children [4, 9, 10, 13]. In most cases, the initial symptoms are the polyuria/polydipsia signs of DI. As LCH-induced hypopituitarism, while children generally show growth hormone deficiency [14], adults are often associated with additional deficiencies of sex hormone or hypogonadism and hyperprolactinemia besides growth hormone deficiency [10, 13]. Indeed, all four adult patients described here had LH–FSH deficiency and hyperprolactinemia. The initial symptoms of Cases 1 and 2 were DI and amenorrhea, Case 3 presented with hypothyroidism, and Case 4 had decreased libido and erectile dysfunction (for some reason, the diagnosis of DI in the two male patients was delayed). Although not common, primary hypothyroidism associated with LCH-induced DI has been described previously [15, 16]. The female patients had problematic morbid obesity.

When a CNS lesion at the HPR is found as an apparent sole disease, it is critical to have histological confirmation of LCH by biopsy because several possibilities besides LCH should be considered, including germ cell tumors, lymphocytic hypophysitis [7], Erdheim–Chester disease, and juvenile xanthogranulomatosis [4–6]. However, a biopsy of CNS mass needs to be decided under careful consideration. In cases of histiocytic disorders like LCH, extra-CNS lesions may exist at the onset of disease, thus a systemic survey should always be performed before CNS mass biopsy. Also, it is possible that extra-CNS LCH lesions develop later. Of our cases, the CNS mass of Case 1 was immediately biopsied, although the patient also had eczematous scalp lesions which were highly suspicious of LCH. Case 2 had no extra-CNS lesions, thus a CNS biopsy was performed after observation until mass grew. Cases 3 and 4 developed bone LCH after CNS mass biopsy. Notably, the diagnosis of LCH was significantly delayed in the latter three cases, probably because LCH was thought uncommon in adults, thus other causes were searched.

In three of these 4 adult patients, we observed >50% CNS mass reduction with use of chemotherapy including VBL (Case 1) and 2-CDA/PSL (Cases 2 and 4); however, in none of the patients hormonal deficiencies reversed. These results made us to conclude that we were able to control mass lesions in the HPR with chemotherapies such as VBL or 2-CDA but late mass reduction is not sufficient enough to reverse endocrine dysfunction. In other words, benefits of such treatment are questionable for adult LCH patients with already existing endocrine deficits. Thus, the most critical question is, are LCH-associated endocrine disorders reversible if they are treated early and appropriately? Although rare and controversial, some reports suggest that DI can be reversed [12, 17, 18]. Particularly, a previous report indicated that hypothalamic-pituitary radiation therapy can effectively reverse DI if it is provided early, namely <14 days after DI diagnosis [18].With regard to anterior pituitary dysfunction, while most reports suggest that it cannot be reversed [6, 9, 10], Makras et al. [19] have described the case of a 35-year-old female who resumed normal menstruation following steroid administration. Case 1 became free from DDAVP several years after treatments with immediate hypothalamic-pituitary radiation therapy for the primary mass and rapid introduction of multiagents' chemotherapy for the recurred CNS mass, but not resumed menstruation. With regard to 2-CDA treatment, the HPR lesions were reported to respond well to it [6, 11]. Of our three cases treated with 2-CDA/PSL, two responded quite well (the tumor size was reduced by >50%), the remaining patient responded minimally. In these cases, no hormonal recovery has yet been attained.

As typically seen in this report, for CNS lesions in LCH, reducing a LCH-related HPR mass lesion has been one of the therapeutic goals, which is achievable with different options including radiation therapy and/or systemic chemotherapy. However, in the majority of the patients, such trials were too late to reverse endocrine dysfunction which already exists. Thus, to ward off such undesirable situation, introducing early treatment in association with rapid diagnosis and with the CNS mass reduction as early as possible is essential [20]. However, a major obstacle associated with adult patients with LCH is that they are reluctant to take a long leave of absence from their jobs for examination/treatment. This often prevents the disease from being evaluated fully or being treated sufficiently with chemotherapy. Indeed, three of our patients only took one week off during their 4-week 2-CDA treatment. Given the adverse effects of 2-CDA, this limited treatment schedule forced us to give smaller 2-CDA doses (5 mg/day × 5 days a month). This may have been partly responsible for the limited responsiveness to 2-CDA that we observed. Nevertheless, the treatment still caused grade 2 adverse hepatic effects (two cases) and neutropenia and thrombocytopenia (one case). A rapid diagnosis and novel measures are needed to improve the outcome of adult-onset LCH-induced DI and hypopituitarism. For that purpose, future studies must clarify the preventive role of systemic treatment with respect to reversal of endocrine deficits with controlled prospective trials, although the numbers are too small that international collaboration is required on adult patients with LCH lesions at the HPR.

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ORIGINAL ARTICLE

# Analysis of 43 cases of Langerhans cell histiocytosis (LCH)-induced central diabetes insipidus registered in the JLSG-96 and JLSG-02 studies in Japan

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**Abstract** To determine the ability of recent systemic chemotherapy protocols to reduce the incidence of central diabetes insipidus (CDI) in Langerhans cell histiocytosis (LCH), 43 CDI cases that belonged to a cohort of 348 pediatric patients with multi-focal LCH who were treated with the JLSG-96/-02 protocols were analyzed. The overall incidence of CDI was 12.4%, but in 24 cases CDI was already present at the time LCH was diagnosed. Thus, CDI developed during or after systemic chemotherapy over a follow-up period of 5.0 (0.2–14.7) years in only 19 patients (5.9%), with 7.4% at 5-year cumulative risk by Kaplan–Meier analysis. In two cases, complete resolution of CDI was noted. Anterior pituitary hormone deficiency was detected in 13 cases, while CDI-associated neurodegenerative disease was observed in six cases. The JLSG-96/-02 protocol appears to effectively reduce the occurrence of CDI. However, novel therapeutic measures are required to reverse pre-existing CDI and to prevent CDI-associated neurological complications.

**Keywords** Central diabetes insipidus · Langerhans cell histiocytosis · Anterior pituitary hormone dysfunction · Neurodegenerative disease

## 1 Introduction

Langerhans cell histiocytosis (LCH) is a rare proliferative disorder of cells with the phenotype of activated Langerhans cells. It is thought to be the major cause of central diabetes insipidus (CDI) in children. Although LCH develops most often at extra-cranial sites such as the skin, bones, and lymph nodes as a multi-focal disease, LCH lesions can also arise in the hypothalamic–pituitary region (HPR), which can induce arginine vasopressin (AVP) deficiency and trigger CDI [1, 2]. In early studies of LCH, CDI was reported in 15–50% of patients [3]. However, since the advent of modern systemic chemotherapy, CDI has been observed in 7–20% of patients [4]. Notably, CDI can develop before, concurrently with, or subsequent to the

For Japan LCH Study Group.

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diagnosis of LCH on the basis of various extra-cranial lesions; it can also arise during chemotherapy for systemic LCH or after therapy has concluded. Thus, it should be kept in mind that the aforementioned incidences of CDI represent all occurrences of CDI. To clarify whether a particular treatment regimen can prevent the development, or produce a resolution of CDI, it is necessary to perform an appropriately designed randomized prospective trial; however, due to small numbers of LCH patients, no such data are available to date. As an alternate way, a comparison could be made for the incidence of CDI that develops after chemotherapy or off therapy with those in previous reports.

In terms of LCH-induced CNS complications, it has been reported that an anterior pituitary hormone deficiency (APHD) may develop following CDI during life-long follow-up [3, 5, 6]. In addition, it has been reported that neurodegenerative (ND) disease can occur [7, 8].

The present study investigated whether the JLSG-96/-02 protocols for pediatric patients with multi-focal LCH in Japan can significantly reduce the incidence of LCH-induced CDI or related neurological complications by analyzing data obtained by these protocols that were performed in the last 14 years [9, 10]. To this end, the details of patients participating in these studies who developed LCH-induced CDI were analyzed retrospectively.

## 2 Patients and methods

The JLSG study was approved by the institutional review board of the Kyoto Prefectural University of Medicine, where the registration office is located. The study was performed in accordance with institutional ethical standards and the tenets of the Helsinki Declaration. In total, 348 multi-focal LCH cases including 222 multi-system (MS) disease and 126 cases of single system multi-focal (SS-m; 116 bone and 10 skin or lymph node) disease were studied. These included 91 and 257 cases that were registered in the 1996–2001 JLSG-96 study and the 2002–2009 JLSG-02 study, respectively. These treatment protocols involved essentially the same drug combinations (vincristine, cytosine arabinoside, prednisolone, methotrexate and 6-mercaptopurine), but the latter protocol differed from the earlier protocol in terms of a mild modification and by involving a longer treatment period (7.5 months as opposed to 12 months) [9, 10]. Continuing prospective data collection of the therapeutic results of the registered patients revealed that by March, 2010, CDI was found in a total of 43 patients, of whom 12 and 31 patients had been registered in the JLSG-96 and JLSG-02 studies, respectively, where diagnosis of CDI was made either by clinical features alone or by water deprivation test.

The median age of these 43 CDI patients at the time LCH was diagnosed was 2.6 (range 0.4–17.0) years, and the male/female ratio was 0.8. All of these patients had multi-focal LCH, of which 72% had cranio-facial bone lesions. The median follow-up period for all 348 LCH cases in the two studies was 5.0 (range 0.2–14.0) years, with the shortest follow-up of surviving patients being 0.8 years from the initiation of treatment.

The prospectively collected data sheets of the 43 CDI cases that were recorded during the follow-ups (6 weeks, 6 months and 1 year, and thereafter every year) were analyzed for the cumulative risk of CDI by the Kaplan–Meier method [11]. In addition, to clarify clinical features of CDI cases, the data from newly prepared CDI-oriented questionnaires that were sent to each physician-in-chief who was taking care of the LCH patients with CDI were analyzed in details including CDI-related neurological consequences. The questionnaires asked are summarized in Supplementary Table.

Complete and partial CDI were defined with modified criteria of Sands et al. [12]. The response of CDI to any type of chemotherapy was evaluated as complete remission (CR), namely no need for 1-deamino-8-D-arginine vasopressin (DDAVP) or normalization of MRI findings including recovery of the posterior bright spot; partial remission (PR), namely >50% reduction in DDAVP dosage or improvement in MRI findings with resolution of the stalk thickening; or no response (NR), namely persistent symptoms of polydipsia/polyuria with dependence on DDAVP. The diagnosis of ND disease was based on the criteria described by Wnorowski et al. [7]. Statistical analysis was performed with the Chi-square test. $p$ values less than 0.05 were considered to be significant.

## 3 Results

### 3.1 Incidence of CDI

In all, CDI occurred in 12.4% (43/348), which consisted of 15.8% (35/222) in MS and 6.3% (8/126) in SS-m diseases ($p = 0.01$). Between JLSG-96 and JLSG-02 studies, the incidence of total CDI did not differ significantly (12/91 vs. 31/257; $p = 0.78$). Thus both studies were combined and analyzed. In 55.8% (24/43) of CDI cases, CDI was already detectable at the time LCH was diagnosed. The remaining 44.2% (19/43) developed after the initiation of treatment, with the overall incidence of late-onset CDI (namely, during chemotherapy or off therapy) was only 5.9% (19/348−24 = 324). The cumulative risk of CDI after treatment was estimated as 7.4% at 5 years and 12.8% at 10 years, respectively, with use of Kaplan–Meier method

**Table 1** Incidence of CDI in LCH; Comparison of our data with historical data

| Author (reference) | Cohorts | Incidence of CDI | | |
|---|---|---|---|---|
| | | Simple calculation/KM | | |
| | | Total (%) | At LCH Dx (%) | After treatment (%) |
| Dunger et al. ([1]) | $N = 52$ (SS-m 20, MS 32) | 28.8 (15/52) | 13.3 (2/15) | 26 (13/50)<br>42 (KM 4 years) |
| Grois et al. ([3])[a] | $N = 199$ (SS-s 93, SS-m + MS 166) | 9.5 (19/199) | 42.1 (8/19) | 5.8 (11/191)<br>11 (KM 5.3 years) |
| Grois et al. ([4])[b] | $N = 1741$, Of which 1183 (SS-s 509, SS-m 154, MS 520) were studied for CDI after treatment | 12 (212/1741) | 48.1 (102/212) | 9.3 (110/1183)<br>16 (KM 5 years<br>20 (KM 10 years) |
| Current study | $N = 348$ (SS-m 126, MS 222) | 12.4 (43/348) | 55.8 (24/43) | 5.9 (19/324)<br>7.4 (KM 5 years)<br>12.8 (KM 10 years) |

*SS-s* single system single site, *SS-m* single system multi-sites, *MS* multi-system, *Dx* diagnosis, *KM* Kaplan–Meier analysis

[a] DAL-HX83 study (1983–1993)

[b] DAL-HX83/90 + LCH-I + LCH-II studies

(Table 1; Fig. 1). Clinical features of the 43 CDI cases are summarized in Table 2.

### 3.2 Diagnosis of LCH/CDI

In all but three cases, LCH was diagnosed on the basis of biopsies of extra-cranial lesions. In remaining three cases in which CDI developed early as the only sign of LCH, the lesion at the HPR was biopsied. One of these cases was first diagnosed as low grade glioma, but a second biopsy performed 2 years later revealed it to be LCH. CDI was diagnosed in two-thirds of the cases by water deprivation/hyperosmolar salt loading tests. The plasma AVP and plasma osmolarity (pOSM) levels at onset were determined in 14 cases: the median AVP value was 0.45 (range <0.15–1.5) pg/ml and the median pOSM value was 285 (range 273–311) mOsm/kg $H_2O$.

### 3.3 Characteristics of CDI and treatment response

In terms of the MRI abnormalities that were noted at the onset of CDI, a thickened stalk was observed in 72.1% (31/43), while loss of a hot signal (T1-weighted MRI) in the pituitary posterior lobe was observed in at least 95.0% (41/43) and a hypothalamic mass was noted in 18.6% (8/43) of the cases, either on its own or in combination with the loss of the hot signal. All CDI patients except one received nasal DDAVP, most often at a dose of 5–10 μg. Although the JLSG-96/02 chemotherapy was largely effective for LCH at extra-cranial sites [9, 10], it was

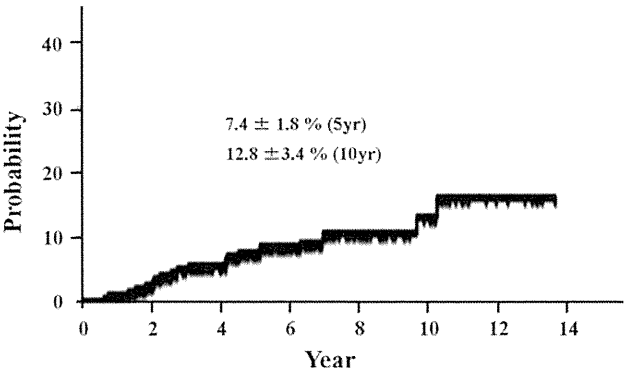


**Fig. 1** Cumulative incidence and risk of CDI after treatment in 323 patients with multi-focal LCH disease, analyzed by Kaplan–Meier method

unable in the majority of cases to reverse CDI that was present before or at the time of the diagnosis of LCH: of these 25 cases, only two attained a CR (Table 3). It is worth commenting that both patients had partial CDI at the time LCH was diagnosed. One of these patients exhibited clinical improvement of CDI symptoms within 2 weeks of commencing the JLSG-96 protocol, prior to the introduction of DDAVP; this was associated with the normalization of the pituitary stalk thickening within 1.5 months of JLSG-96 treatment (figure not shown). The other case was first treated with JLSG-02 and then with 2-chlorodeoxyadenosine/cytosine arabinoside over a period of 2.8 years, which resulted in a CR along with the recovery of the hot signal in the posterior lobe after 3 years (Fig. 2). With regard to the late-onset CDI cases, where CDI arose during