DOI: 10.1002/pbc.26770

WILEY Pediatric Blood & Cancer
SIOP INTERNATIONAL SOCIETY OF PAEDIATRIC ONCOLOGY
aspho The American Society of Pediatric Hematology/Oncology

Abstracts from the 33rd Annual Meeting of the Histiocyte Society Singapore, October 3-4, 2017

Treatment of LCH of Bone Using Indomethacin

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Purpose: Indomethacin is an anti-inflammatory drug which inhibits prostaglandin synthesis by cyclo-oxygenase. Prostaglandins are synthesized in Langerhans Cell Histiocytosis (LCH) cells which has led to investigation of indomethacin for treatment of LCH. We describe the treatment of four patients with bone involvement of LCH (mandible, iliac bone) with dramatic results.

Methods: Four case reports.

Results: Patient 1 presented at age 3 years with 3 weeks of right lower extremity pain and right iliac crest fullness. MRI showed an aggressive tumor, arising from the right iliac wing with cortical expansion and destruction. Biopsy revealed LCH. Evaluation for other sites of disease was negative. Treatment with indomethacin 1mg/kg BID was instituted. By 3 months all symptoms resolved. MRI showed Active Disease (AD) Better. Patient 2 presented at 4 years of age with 2 months of pain in the left upper gluteal region with fullness on examination. MRI showed a localized bony defect in the left iliac bone with intrapelvic and extrinsic soft tissue masses. Biopsy showed LCH. Investigations were negative for other sites of disease. Indomethacin 1mg/kg BID was started. After 6 weeks all symptoms resolved and MRI showed AD Better. Patients 3 and 4 presented at 17 and 24 months of age respectively,with several weeks of progressive jaw swelling and pain. CT showed a single lytic expansile mandibular lesion and associated soft tissue mass in each case. Biopsies showed LCH to be present. No other sites of disease were found. Treatment ensued with indomethacin 1mg/kg BID. On evaluation both patients showed response by 8 weeks with AD Better.

Conclusion: LCH presenting with a bone lesion and large soft tissue mass may have imaging characteristics of an aggressive malignant bone tumor. Despite the large soft tissue component a biopsy followed by indomethacin therapy can be effective treatment in patients with localized disease.

Early Onset of HLH and Inherited UNC13D and JAK3 Mutations in a Patient. A Difficult Diagnostic and Therapeutic Challenge

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Purpose: Several HLH-associated genes are required for cytotoxic lymphocyte exocytosis. HLH is the first manifestation of other primary immunodeficiencies, including severe-combined-immunodefiency (SCID). The type and combination of mutations correlate with age at onset, clinical presentation and severity of cytotoxic impairment. We present a case diagnosed with early-onset HLH and monoallelic UNC13D and JAK3 mutations.

Methods: We reviewed clinical, laboratory, immunological and pathological data. Familial genetic study was performed by whole-exome-sequencing (WES) and confirmed by Sanger sequencing.

Results: A girl was diagnosed with CMV infection, severe HLH and bowel involvement at 2 months and responded well to HLH-2004 therapy. Initial genetic studies detected a heterozygous UNC13D c.1021C>G p.Gln341Glu mutation inherited from the father, but immunological functional assays didn't impaired lymphocyte exocytosis. The following years the patient had failure to thrive and mild respiratory infections, but SCID was ruled out. Later she had intermittent episodes of fever, megalies and cytopenia with complete resolution, followed by progression to persistent hepatosplenomegaly, abdominal adenopathies and hepatitis. WES revealed two rare JAK3 variants c.1142+3G>T and c.878G>A p.Cys293Tyr, which were confirmed by Sanger sequencing in patient and the mother. In sequential immunological studies, NK cell and cytotoxic T cell functional assays were not impaired drastically. At the age of 5, after mild pneumonia and rhinovirus infection, she developed severe HLH

with autoimmune hepatitis. She responded partially to HLH-therapy, but died (sepsis and fungal infection). Autopsy confirmed HLH-immunopathology.

Conclusion: Genetic screening by high-throughput sequencing, immunological phenotyping and functional assays are important in order to establish correct diagnosis in HLH patients. Monoallelic and polygenic inherited defects in the genes UNC13D (from father) and JAK3 (from mother) may have colluded for development of fatal HLH. It is not clear how HLH and SCID-associated gene mutations might combine for a digenic inheritance, but combined mutations represent a diagnostic and therapeutic challenge.

Rosai Dorfman Disease In Children – A Rare Disease with Diverse Clinical Course

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Purpose: Rosai Dorfman (RD) disease is a monocyte/macrophage related histiocytic disorder usually presents with lymphadenopathy causing diagnostic confusion with malignancy and infection.

Methods: This is a retrospective review of three children in two institutions treated for RD with different strategies.

Results: Case 1- A 14 year old girl presented with isolated right upper cervical lymphadenopathy of 1 month duration without any systemic symptoms. Diagnosis of RD disease was confirmed on lymph node excision biopsy. She remains in complete remission 4yrs after initial diagnosis without any treatment. Case 2-A 10 year old boy with right upper cervical lymphadenopathy and intermittent fever for 3 months. A large conglomerated mass of lymph nodes causing vascular compression on internal jugular vein was seen with no other disease on clinical or radiological examination. Autoimmune Lymphoproliferative Syndrome (ALPS) screening was negative. He was treated with prednisolone 2 mg/kg initially tapered slowly over 5 months. He remains in complete remission 4 years off treatment. Case 3-A 3 year old boy presenting with bilateral cervical lymphadenopathy and diarrhoea with low albumin. Lymph node biopsy confirmed RD disease and improved with 6 weeks of steroids. He had two subsequent recurrences after 12 and 19 months with lymphadenopathy, hepatosplenomegaly, direct antiglobulin test (DAT) positive haemolytic anaemia treated with prolonged steroids at first relapse and then steroid with Rituximab 100 mg once a week for 4 weeks at second relapse. He had elevated double negative T cells suggesting diagnosis of ALPS. Unfortunately he had subsequent relapse with lymphadenopathy and had a sudden unexplained death in remission 3 years from initial diagnosis.

Conclusion: Three very different clinical course of the same disease as illustrated makes the diagnosis and management of RD disease quite challenging. Treatment has to be tailored according to disease characteristics, immunosuppression necessary only for vital organ compression or autoimmune haematological problems.

Vaccine Associated Soft Tissue Infiltrate Can Predispose Multi-System Langerhans Cell Histiocytosis

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Ethiology and ways of dissemination of Langerhans cell histiocytosis (LCH) still remains obscure. Some cases demonstrate both immunologic and neoplastic theory.

Purpose: The study of multy-system LCH (MS-LCH) developed after unsuccessful treatment of purified vaccine associated infiltrate.

Methods: 2 cases were evaluated. Repeat imaging including magnet resonance tomography and computer tomography was performed. Biopsy with immunohystochemistry tests and direct DNA sequencing with real time PCR were used.

Results: We observed 2 cases of MS-LCH treated in our clinic initially as vaccine associated soft tissue infiltrates. Both kids were male of their second year of age. Both had several months long history of surgical treatment of soft tissue infiltrate with purification in hip, which occurred in place of vaccination. Performed biopsy revealed LCH. BRAF mutations were found in both cases. Extensive search for another lesions more typical for LCH was performed but nothing was found. Infiltrates were removed and after course of antibiotics healed up completely. No steroides or chemotherapy treatment was performed. After 6 months of follow up we observed multy-focal bone disease in 1 case. Solitary pituitary stalk thickening with central diabetes insipidus occurred after 9 months of follow up. Both kids were examined. No active lesion in the place of primary infiltrate was found. Systemic chemotherapy with vinblastine and prednisone was successful in both cases.

Conclusions: These cases demonstrate the possibility of development BRAF positive MS-LCH after long time treatment of solitary soft tissue lesion associated primary with vaccination.

Langerhans Cell Histiocytosis and NK/T Lymphoma After T-Cell Acute Lymphoblastic Lekemia

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To report the disease evolution and treatment of an 5-year-old boy achieved complete remission of T-cell ALL for 18 months and skin rashes pathology ascertained LCH and T-cell lymphoma. An 5-year-old man due to fever, hepatomegaly and splenomegaly was diagnosed with T-cell acute lymphoblastic leukemia (T-ALL). He was treated with induction chemotherapy using the China CCLG group protocol and achieved complete remission. Eighteen months after the diagnosis of T-ALL, he developed a severe multiorgan histiocytosis that is clinically suggestive of LCH.We prescribed the secondline treatment for LCH (cytarabine and 2'-chlorodeoxyadenosine), he achieved partial remission. The rashes disappeared for 2 weeks and recurred. T lymphoma was found by review of skin pathology, and NK / T cells were done by bone marrow of flow cytometry.2 courses of chemotherapy of CHOP-E were used, but the disease was progressively exacerbated with more rashes, hepatomegaly, splenomegaly and pancytopenia. LCH with T lymphoma was considered by third skin biopsy and immunohistochemistry. Rashes, peripheral blood, bone marrow and cerebrospinal fluid flow cytometry were all provided evidence for NK-T lymphoma. The secondline treatment for LCH (cytarabine and 2'-chlorodeoxyadenosine) was applied again, the condition was transitional improved for 2months with rashes disappeared, liver and spleen shrinked, and normal blood. Hepatomegaly, splenomegaly and pancytopenia were recurred and hematopoietic stem cell transplantation was put on the agenda. Only few literatures of T-ALL with LCH were reported, and so was LCH with lymphoma. Maybe the case of T-ALL with LCH and NK / T lymphoma was firstly reported. The disease evolution and effect of conventional chemotherapy application were described, and bone marrow hematopoietic stem cell transplantation had been conducted. The further follow-up for final prognosis would be done.

Lenalidomide-Dexamethasone: A Promising Therapy For Langerhans Cell Histiocytosis Without Risk Organ Involvement

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Background: Treatment of refractory/ relapse Langerhans Cell Histiocytosis (LCH) is still unsatisfactory. Currently recommended cladribine-cytarabine protocol is quite toxic. Hence lenalidomide-dexamethasone combination, if effective, may be a cheap and well tolerable option for the treatment.

Case History: Two and half years-old-girl presented with scaly papular skin lesions over scalp, polyuria/polydipsia for 3 months. Investigations revealed diagnosis of multisystem LCH (skin and multifocal bone) with no-risk organ involvement with diabetes insipidus. LCH-3 protocol was administered to her. At 6 weeks assessment skin lesions resolved. At the end of treatment, bone scan was normal. She had mal-occlusion of teeth. She was kept on follow up. Two years later, malocclusion of teeth was still persistent. PET CT revealed metabolically active (SUV 4.18) lytic lesion in the left mandibular ramus with soft tissue component resulting in floating teeth. The right maxillary sinus was expanded by the soft tissue, resulting in thinning and erosions of the sinus walls. The high parietal lytic lesion was not taking uptake. She received 12 cycles of lenalidomide (2.5 mg for <15kg and 5 mg for >15 kg; for 3 weeks every 4 weekly cycles) and dexamethasone (0.8 mg/kg every weekly). After completion of 6 cycles, PET-CT scan revealed reduction in soft tissue and SUV (3.05). After 12 cycles, PET-CT showed reduction in size of metabolically active soft tissue in the right maxillary sinus region with increase in sclerosis in the right maxilla. Ill-defined lytic area in body of mandible on left side appeared less prominent. In view of unknown long term safety of lenalidomide in children, we decided to continue with pulse prednisolone 40 mg/m2 × 5 days every 3 weeks till complete remission.

Conclusion: Dexamethasone lenalidomide combination is a cheap, well tolerated and effective regimen at least for non-risk organ disease.

The Role of Soluble Interleukin-2 Receptor (SIL-2R) in Diagnosis of Adult Hemophagocytic Lymphohistiocytosis (HLH): A Single Center Retrospective Study

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Purpose: Serum soluble interleukin-2 receptor (sIL-2r) is considered an important disease marker in hemophagocytic lymphohistiocytosis (HLH). The HLH-2004 diagnostic criteria report sensitivity of 93% and specificity of 100% for sIL-2r >2,400U/ml for pediatric HLH. No studies have reported the performance characteristics of this test in adult HLH patients. We conducted a retrospective study to evaluate the clinical utility of sIL-2r in adult HLH patients, including sensitivity, specificity, and prognostic significance.

Methods: Retrospective data was collected on adult patients with at least one sIL-2r level at Vancouver General Hospital in Vancouver, Canada between March 2012 and April 2017. Patients were subdivided into HLH and non-HLH groups. Sensitivity, specificity, prognosis associated with

sIL-2r >10,000U/ml, utility as a marker of disease activity and mean sIL-2r between subgroups of HLH were evaluated.

Results: 79 patients were included, 41 with HLH and 38 with an alternate diagnosis (non-HLH). The sensitivity of sIL-2r >2,400 U/ml was 93% (95% CI 0.79 : 0.98) and specificity 66% (95% CI 0.49 : 0.79). Specificity improved to 92% (95% CI 0.76 : 0.98) with a threshold of sIL-2r >10,000U/ml. Similar to ferritin, sIL-2r levels correlated with disease activity. Within the HLH group, sIL-2r >10,000U/ml was not associated with worse prognosis. Higher sIL-2r levels were seen in malignancy associated HLH (MAHS) as compared to infection associated HLH (IAHS) and macrophage activation syndrome (MAS).

Conclusion: sIL-2r >2,400U/ml is a sensitive test for diagnosis of adult HPS/HLH and is useful in monitoring disease activity. At higher levels (sIL-2r >10,000U/ml), this biomarker loses sensitivity but gains specificity in diagnosing HPS/HLH. Higher sIL-2r levels may indicate MAHS when the underlying etiology is unclear. Further prospective studies are needed to further confirm the utility of sIL-2r in diagnosing adult HLH.

Sclerosing Cholangitis In Identical Twins with Langerhans Cell Histiocytosis

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Introduction: Survival of children with Langerhans cell histiocytosis (LCH) has improved in the past decades. Disease involvement of liver carries a worse prognosis. Sclerosing cholangitis is an uncommon complication of LCH with high mortality. Little is known to its aetiology, disease course or the best treatment. We report a pair of twin brothers who presented with sclerosing cholangitis. The clinical course after treatment with chemotherapy was studied.

Methods: The clinical courses, radiological and histological features were studied. Twin 1 presented at 26 month of age with jaundice, rash and ear discharge, with marked hepatosplenomegaly. Blood test revealed hyperbilirubinemia and markedly elevated ductal enzymes. Magnetic resonance cholangiogram showed features of sclerosing cholangitis. LCH was diagnosed by liver biopsy and skin biopsy. Twin II was asymptomatic other than mild rash. He had no jaundice and liver was only mildly enlarged. Screening blood tests showed markedly elevated alkaline phosphatase but normal bilirubin. Imaging revealed similar findings as Twin I but with less severity. Skin biopsy confirmed LCH.

Results: Twin I received chemotherapy according to LCH-III protocol, with vinblastine and prednisone, followed by maintenance of 6-mercaptopurine for total of 3 years. Liver function improved but radiological features remained static, and the patient developed portal hypertension. Chemotherapy has been stopped for 2 years and the condition remained static. Twin II was also treated with LCH-III chemotherapy. He showed good response with normalisation of liver function. Radiologically there was mild decrease in periportal inflammation.

Discussion: The prognosis of sclerosing cholangitis in LCH is usually poor. Our patients demonstrated the effect of chemotherapy in controlling the disease, Twin II actually had normalisation of liver function. This suggests early diagnosis and timely treatment may lead to better outcome. The occurrence of LCH with SC in monozygotic twin also raised the role of genetic factor in the disease pathogenesis.

Phase 2 Trial of Single-Agent Cobimetinib for Adults With Histiocytic Disorders: Interim Results

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Background: The identification of recurrent BRAFV600E mutations in Erdheim-Chester disease (ECD) and Langerhans cell histiocytosis (LCH) led to a breakthrough in treatment of severe forms of disease with BRAF inhibition. The finding that nearly all BRAF-wildtype ECD/LCH lesions harbor mitogen activated protein kinase (MAPK) pathway alterations raised the possibility of treatment of BRAF-wildtype ECD with MEK inhibition.

Methods: This is a phase 2 trial of Cobimetinib 60mg daily for patients with (1) BRAF-wildtype histiocytosis or (2) BRAFV600-mutated histiocytosis intolerant or without access to BRAF inhibitor therapy. The primary outcome is metabolic response by 18F-FDG PET scan.

Results: 11 patients have enrolled: 8 ECD, 1 Rosai-Dorfman disease (RDD), 1 mixed ECD/RDD, and 1 LCH. Three patients have BRAFV600E mutated disease. Ten patients (4 ECD, 1 RDD,1 RDD/ECD, 1 LCH) have had response assessments. One patient died (Grade 5 respiratory failure, related to infection) before the first response assessment and one patient was removed from study due to toxicity (Grade 3 retinal vein occlusion) related to drug. Two patients withdrew consent from the study to pursue off-trial therapy. Grade 3/4

toxicities have been hyponatremia (27%), lymphopenia (27%), hyperlipidemia (18%), and hyperglycemia (18%). The most common Grade 1–2 toxicities have been hypoalbuminemia (91%), fatigue (73%), increased alkaline phosphatase (73%) and anemia (63%). Five patients have required dose reduction to 40mg. All patients but one have had a metabolic response in target lesions; 30% (3 patients) a complete metabolic response, 40% (4 patients) a partial metabolic response, 10% (one patient) has stable metabolic disease. All patients have had symptomatic benefit as measured by symptom and QOL scales.

Conclusions: Interim results from this trial demonstrate robust efficacy of single-agent Cobimetinib in histiocytic disorders, regardless of BRAF mutational status. Toxicities have been manageable and similar to those observed in previous trials of Cobimetinib.

Hemophagocytic Lymphohistiocytosis Associated With Visceral Leishmaniasis And Epstein Barr Virus-Reactivation in A Scandinavian Male Without Recent Travel History: A Potential Adverse Event To Anti-Tumor Necrosis Factor-Alpha Therapy

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Purpose: To describe the occurrence of hemophagocytic lymphohistiocytosis (HLH) associated with visceral leishmaniasis (VL) in a Scandinavian patient with no recent travel history, following treatment with the Tumor Necrosis Factor-alpha inhibitor Infliximab. And to review data on the occurrence of VL in a non-endemic country.

Methods: Case report and review of existing data.

Results: A 68 year-old Danish male suffering from sarcoidosis received infliximab due to disease aggravation. At the time of treatment he had near-normal hematological parameters. Twenty-six days after infliximab initiation he was hospitalized due to fever. On admission he was pancytopenic and had elevated triglyceride and ferritin levels (137,000 μg/l). HLH was suspected and treatment with dexamethasone, immunoglobulin, and antibiotics was initiated. The patient improved on treatment, however, 4 days later he suffered a retroperitoneal bleed and eventually succumbed to this complication. A bone marrow examination verified ongoing hemophagocytosis. Furthermore, parasites resembling Leishmania amastigotes were visualized. VL was confirmed by PCR (Leishmania donovani complex). Leishmania serology by immunofluorescence antibody testing was borderline positive. Additionally, the patient had active Epstein Barr viremia (initially 7,000 and max 127,000 copies/ml), but was found IgM negative with serology. The patient had never presented symptoms of VL and VL is not endemic in Scandinavia. He had no travel history from highly VL endemic regions but had visited Italy, Spain, and Greece, 6, 10, and 14 years prior to admission.

Conclusion: HLH may be induced iatrogenously by immunomodulatory drugs. In the presented case, Infliximab is believed to have activated a dormant subclinical VL infection in a patient with no recent travel history. As VL-associated HLH often responds favourably to Ambisome treatment, screening for VL, preferably by PCR, should be carefully considered in all HLH patients. Testing for VL may be relevant in spite of identification of alternative HLH-triggers as Epstein Barr Virus infection.

Adult Patients with Mixed Histiocytoses

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Purpose: The histiocytoses are a heterogeneous group of rare, potentially fatal neoplasms characterized by inflammation and infiltration of dendritic cells or macrophages. Histiocytic infiltration can affect multiple organ systems such as bones, central nervous system and retroperitoneum, resulting in tissue damage and organ failure. Histiocytoses are classified as Langerhans cell histiocytosis (LCH) or non-LCHs based on clinical, immunohistochemical and radiographic features. The presence of two histiocytic diseases in a single individual is termed "mixed histiocytosis". Here we illustrate the distinctive clinical and molecular features of patients with mixed LCH and Erdheim-Chester disease (ECD) evaluated at the National Institutes of Health (NIH).

Methods: Biopsy samples from seventy-five patients enrolled in an approved NIH protocol were reviewed to confirm the presence of a histiocytic disorder, and to test for the BRAF V600E mutation.

Results: Four cases (5.3%) of mixed LCH and ECD were found. Of the mixed cases, three tested positive for the BRAF V600E mutation. All patients had bone disease, two patients were diagnosed with diabetes insipidus, commonly seen in LCH and ECD. One patient showed LCH and ECD infiltrates in the lung. One patient presented with mixed

infiltration in the skin, colon and mandible, showing the multisystem involvement of these diseases.

Conclusion: Our findings illustrate the unique clinical and molecular presentations of four mixed LCH and ECD cases, which adds to other reports, suggesting that such cases may not be rare. The discovery of the BRAF V600E mutation in >50% of LCH and ECD patients highlights the overlap of mitogen-activated protein kinase (MAPK) pathway mutations in the pathogenesis of histiocytoses, and in fact, three of our cases had BRAF V600E mutation in LCH and ECD affected tissue. The increasing number of reported mixed histiocytoses expands our understanding of the diversity of these conditions and mutation testing offers improved treatments.

Adrenal Insufficiency and Other Endocrinopathies In Erdheim-Chester Disease

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Purpose: Erdheim-Chester disease (ECD) is a non-Langerhans cell histiocytic neoplasm resulting in chronic inflammation and fibrosis. The majority of patients harbor the BRAF V600E mutation in affected tissue. ECD involves multiple organ-systems and causes endocrinopathies. Abnormalities of the Hypothalamic Pituitary Adrenal (HPA) axis and other endocrine pathways have not been extensively investigated in ECD.

Methods: Sixty consecutive patients with ECD participated in a National Institutes of Health (NIH) approved study.

Results: Forty-seven percent of patients had diabetes insipidus treated with vasopressin. Adrenal gland and pituitary stalk infiltration was present in 21/60 (35%) and 14/55 (25.45%). Both were observed in 5/55 (9.09%). Twenty-five patients (25/55, 45.45%) had no infiltration in the HPA axis. Twenty patients (20/60) had a prior diagnosis of AI. No patient presented with adrenal crisis as the initial manifestation of ECD. All patients with AI reported lack of education toward sick day rules. Glucocorticoid replacement therapy was not required in 11/21 patients with adrenal gland infiltration or in 4/14 with pituitary/stalk infiltration. Mineralocorticoid replacement therapy was not required in all patients. Thirty patients (30/56, 53.5%) harbored the BRAF V600E mutation, and were more likely to have adrenal gland infiltration with comparable rates for pituitary/stalk infiltration. High-sensitivity C-reactive protein was significantly higher in patients with adrenal gland infiltration and positive BRAF V600E mutation. Other endocrine abnormalities included hypogonadism in 60%. Insulin-like growth factor levels were abnormal in one-third of cases, 22% had hypothyroidism. Occasional abnormalities in parathyroid, and prolactin hormones were seen.

Conclusions: Infiltrative processes of the HPA axis in patients with ECD tend to favor the adrenal glands in BRAF-positive patients, without influencing the rates of AI, although there is a poor biochemical-radiological concordance in ECD. Patients with ECD should be educated on the risk for and the management of endocrine abnormalities and followed closely by an endocrinologist.

Computed Tomography (CT) Findings of Pulmonary and Mediastinal Involvement in Erdheim Chester Disease (ECD)

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Purpose: To prospectively evaluate pulmonary and mediastinal findings on computed tomography (CT) and demonstrate their correlation with BRAFV600E mutation in Erdheim Chester Disease (ECD).

Methods: We designed a prospective study of 61 ECD patients (46 males) who gave written informed consent. All patients underwent chest CT and the images were reviewed in consensus by two experienced radiologists. Correlation with BRAFV600E mutation was performed on 58 cases by using Fischer exact test. P-value <0.05 was considered significant.

Results: 16 patients had respiratory manifestations (11 had shortness of breath, 3 had chronic dry cough and 2 had recurrent sinusitis) and 45 patients were symptom free. Pulmonary involvement was seen in 55 patients (90%); among which interstitial lung disease was the most common (44 patients), including interlobular septal thickening in 42 and bronchial wall thickening in 8 patients. Nodular opacities were classified by the pattern of distribution: in 22 cases, nodules were located in subpleural regions, including lung fissures; in 8 of them nodules were diffusely distributed and in 8 cases, nodules were not found in subpleural regions. 9 patients (15%) had pleural involvement and 38 (62%) had mediastinal involvement on CT imaging. Right coronary artery was the most frequent vessel sheathed with histiocytic infiltration (21 patients) followed by thoracic aorta (18 patients). 31 patients tested positive for BRAFV600E mutation; BRAFV600E

mutation positive is significantly associated with higher frequency of both non-subpleural nodules (P-value: 0.04) and sheathing of the coronary arteries (P-value: 0.01).

Conclusion: Even though pulmonary and cardiovascular involvements are common in ECD, patients are usually asymptomatic. This study presents pulmonary and mediastinal involvement of ECD in detail, and evaluates the correlation between BRAFV600E mutation and distribution of findings. Poor prognosis of ECD is increased with presence of cardiovascular involvement; therefore, special attention should be paid to understanding of these findings.

Obstructive Uropathy and Nephropathy in Erdheim-Chester Disease

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Purpose: Erdheim-Chester Disease (ECD) is a rare, life-threatening neoplasm associated with retroperitoneal fibrosis (RPF), which can cause an obstructive uro-nephropathy and irreversible renal dysfunction. Treatment for these complications includes systemic therapy for ECD, ureteral stenting, and rarely, nephrostomy tubes, ureterolysis, or renal transplantation. This study highlights the prevalence and management of obstructive uro-nephropathy (and the association with BRAF mutation status) in a cohort of ECD patients.

Methods: Sixty-one ECD patients gave informed consent for an approved protocol at the National Institutes of Health (NIH). ECD and BRAFV600E status was confirmed by histopathological and molecular analysis of biopsy samples. Radiographic imaging studies were evaluated at NIH.

Results: Twenty-one (34%) patients with RPF had either bilateral or unilateral hydronephrosis/ureter (18 men and 3 women; mean age 59 years), and one had concomitant cystomegaly. This includes two (10%) with symptomatic renal artery stenosis (RAS). Of the twenty-one patients, analysis of renal function showed: mean glomerular filtration rate = 66 mL/min, mean creatinine = 1.3 mg/dL), mean Cystatin-C = 1.38 mg/dL), and mean 24-hr urine protein = 421 mg/dL). Seven of the twenty-one patients (33%) with hydronephrosis/ureter had either bilateral or unilateral ureteral stents, and one of the seven required a nephrostomy tube after stenting. Two patients (10%) had renal artery stents for stenosis. One patient (5%) underwent ureterolysis for severe bilateral obstruction, but was eventually transplanted. Correlation between BRAFV600E status and presence of obstructive uro-nephropathy in the cohort was statistically significant using Fisher exact test (value = 0.007; p-value < 0.05) The stented patients had all been treated for ECD with various agents, but hydronephrosis persisted and all required long-term ureteral and/or renal artery stenting to prevent further renal damage.

Conclusion: ECD-associated RPF can cause an obstructive uro-nephropathy, leading to irreversible renal dysfunction. Despite therapy, some patients require stenting to maintain renal function.

Single-Center Experience in Targeted Therapy of Both Braf V600E Positive And Braf Wt Multisystem Refractory Langerhans-Cell Histiocytosis (LCH) with Risk Organs Involvement in Children: A Report of 11 Cases

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Purpose: To evaluate retrospectively the efficacy and tolerability of the targeted therapy in a cohort of children with multi-system LCH, refractory to or intolerant to standard chemotherapy.

Methods: Among 11 cases of multi-system LCH with risk organ involvement 9 were BRAF V600E positive and 2 were BRAF wild type. Median age of manifestation was 3.4 months (1-12 months), median age at diagnosis was 12 months (3-22 months). Preceding chemotherapy was according to the LCH-IV protocol. All patients received initial therapy with VBL + PRED, 7 received Ara-C + 2-CdA as second line therapy. At the start of vemurafenib therapy all patients had active disease with median DAS 13.4 points (4 : 22). Nine patients were were treated with vemurafenib (median dose was 44.6 mg/kg/day (37-50), median time of follow-up was 201 day (76-407) and 2 patients were treated cobimetinib (median dose 20 mg/day, median time of evaluation - 120 days) with or without concomitant chemotherapy (1 receieved mono vemu, 1 received mono cobimetinib, 1 received cobimetinib + 2-CdA, 5 received vemu + VBL + PRED + MTX + 6-MP, 3 received vemu + low-dose Ara-C + 2-CdA).

Results: All 11 patients had partial or complete response to therapy. At day 28 median DAS was 5.4 points (2 : 11). Main toxicities were skin toxicity (91%) and QTs elongation (45%). 1 patient couldn't tolerate vemurafenib due to sever vomit and weight loss and died later due to disease progression. 1 patient died during vemurafenib intake due to severe liver lesion of unknown origin.

Conclusion: Targeted therapy with either BRAF or MEK inhibitors induces marked clinical and laboratory responses in patients with multi-system LCH, refractory to standard chemotherapy. The optimal schedule, potential toxicities, rational therapeutic combinations and treatment regimens should be studied prospectively.

Needs Assessment of Histiocytosis Physicians in Asia And The Middle East: Results of an "AME-Histio Network" Questionnaire

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Background: Histiocytic disorders can present with a wide range of clinical manifestations and severity. Data on the needs of physicians looking after such patients in Asia and the Middle East (AME) is lacking. A group of experts from these regions convened during the 2016 Histiocyte Society meeting, and created the "AME Histio Network".

Objectives: To assess the challenges that physicians from AME encounter when treating patients with histiocytic disorders, with regards to diagnosis and treatment, availability of molecular/genetic testing, and to assess the existence of national consensus guidelines.

Methods: A 15-item questionnaire was distributed. Questions included: the presence of national registries and patient support groups, availability of specialized diagnostic testing, and existence of treatment guidelines.

Results: Thirty-five participants from ten countries responded. Most did not have a national histiocytosis association, a parent support group, or a histiocytosis registry. Specialized imaging studies were available in almost all centers, while sophisticated genetic/molecular testing such as BRAF-V600E and HLH genetic testing were lacking in many institutions. Most centers have adopted the International LCH and HLH treatment protocols, and most did not have difficulty finding the most common histiocytosis chemotherapy drugs. Novel drugs such as cladribine, clofarabine and BRAF-inhibitors were not readily available in many centers. There was a great interest in participating in national registries, clinical trials, genetic/molecular studies and in exchanging information and resources. Due to the heterogeneity of participating countries, priorities and expectations varied depending on the country and its available resources.

Conclusions: The lack of national histiocytosis associations, parent organizations, and treatment guidelines were noted by most participants. The diagnostic and treatment challenges varied among different countries due to economic, rather than geographic, reasons. Establishing international training scholarships to support physicians from countries with limited resources, and partnerships between centers could be the first steps in facing these challenges.

Secondary Hemophagocytic Lymphohistiocytosis in Patients with Sepsis

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Purpose: Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening condition characterized by uncontrolled inflammation and has common clinical and laboratory features with sepsis. The study is conducted to know the clinical and laboratory features of Secondary HLH in patients with sepsis.

Method: This is a prospective observational study where patients presenting with Sepsis and Bicytopenia are included. The patients underwent relevant investigations for diagnosis of HLH according to HLH 2004 diagnostic criteria. Patients fulfilling the criteria were further analysed regarding the clinical features, laboratory parameters and microbiology investigations for etiology of secondary HLH. The treatment received and the final outcome of the patient was also studied.

Results: There were 16 patients who were screened, out of which 9 fulfilled the diagnostic criteria. There were 4 men and 5 women with secondary HLH. The etiology were Dengue

(1),Pulmonary Aspergillosis (2), HIV (1), Typhoid (1), MDR Tuberculosis (1), Visceral Leishmania (1), Staph aureus (1), Unknown(1). Fever, organomegaly,hyperferritenemia and bicytopenia was seen in all patients. Lymphadenopathy was seen in 2 patients, Neuropsychiatric symptoms in 2 patients. Bone marrow was done in 6 patients as the others did not give consent. Hemophagocytes was seen in 50% of them. Highest ferritin levels was 21,723 seen in the patient with Staph aureus associated Sepsis. Steroids with etoposide according to HLH 1994 treatment protocol was given to 3 patients but unfortunately 2 of them died. 5 patients received only Dexamethasone in tapering doses for 8 weeks with supportive care and all of them survived. 1 patient could not receive any treatment for HLH as she died before the results of investigations were available.

Conclusion: HLH should be suspected in sepsis patients with bicytopenia. Infection associated HLH is treated with steroids. Infection associated HLH in not uncommon and and early recognition and treatment can improve outcome.

Classification of Oral (Bone and/or Mucosae) Lesions in Pediatric Patients with Langerhans Cell Histiocytosis

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Purpose: Langerhans cell histiocytosis (LCH) could affect different organs and tissues. Oral cavity could be implicated and also could even be the first lesion of the disease and in many cases the only clinical involvement. In our knowledge there are not information related to the classification of oral lesions (OL) of LCH. Therefore, we conducted a study to classify the oral lesions of pediatric patients with LCH.

Methods: Sixty two patients were recruited for oral evaluation. The patients with OL were classified according the affected tissues involved in: bone, mucosae, and bone/mucosae (tooth, bone and periodontal involvement).

Results: Of a total of 62 patients, 40% showed OL and 48.4% were associated to multisystem disease and 51.6 % were associated to single system disease (unifocal lesion 56.3% and 43.7% multifocal lesion). Forty seven and four percent had oral bone lesions, 10.5% had mucosae lesions and 42.1% had bone/mucosae lesions. Oral lesion observed in the unifocal single system involvement were exclusively osseous, in multifocal single system patients the involvement was bone and oral mucosae, meanwhile in a multisystem patients the lesions were osseous, mucosae, or osseous and mucosae lesions. The principal clinical features were: pain, swelling and osteolytic lesions founded in x-ray and CTscan of maxillary bones lesions. The principal features of bone/mucosae lesions were: gingival and mucosal enlargements with extensive bone loss, severe tooth mobility, localized bone loss, mobility of permanent teeth, early eruption of teeth and, insolated mucosae involvement were: palate reddish dots lesions, pericoronaritis in the first permanent molars, and erosive lesion in the labial mucosae.

Conclusions: Multidisciplinary team is a necessary approach to achieve an early diagnosis and adequate treatment of oral cavity by pediatric dentistry to improve the quality of life of patients with LCH.

Juvenile Xanthogranuloma, Neurofibromatosis Type 1, Mesenchymal Hamartoma of The Liver and Undifferentiated Embryonal Sarcoma in A Young Children. Case Report

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Purpose: Juvenile Xanthogranuloma (JXG) is an histiocytic disorder, that affects the skin and rarely others organs. Its association with Neurofibromatosis type 1 (NF1) has been established. We present a patient with this association and moreover with a mesenchymal hamartoma of the liver (MHL) with a foci of undifferentiated embryonal sarcoma (USL).

Methods: Case report. Chart and literature review.

Results: 26 months old male, without NF1 family history, began at 3 months of age with "cafe au lait" macules of different diameters in trunk and extremities associated with axillary/groin freckling. At about 18 months of age, he developed a localized followed by disseminated JXG confirmed by skin biopsy. A CT scan was performed and an abdominal mass was founded in the right flank. The mass (6.9 cm x 4.5 cm x 5.4 cm) was unique, polylobated and mainly cystic shaped with peripheral enhancement of the contrast. It was located at segment IV of the liver. Alpha fetoprotein dosage was normal. The mass was completely resected without complications and the pathology findings were: mesenchymal liver hamartoma with small foci (0.8×0.4 cm) of undifferentiated embryonal sarcoma of liver. The surgical margins were negative. The staging workup was performed and no evidence of extrahepatic disease was found. At 5 months of follow-up the patient is alive without evidence of recurrence. After the literature

review, we do not found the association of NF1 and JXG with MHL or USL.

Conclusion: Due to the low frequency, these diseases may deserve to be included in the international registry of rare histiocytic diseases of the Histiocyte Society, in order to increase the knowledge about these disorders. More data is needed to understand the pathogenesis of the association between NF1, XGJ, MHL and USL.

Clinical Research on Efficacy Comparison Between CHFU-LCH 2006 Protocol and 2012 Protocol for Childhood Langerhans Cell Histiocytosis

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Purpose: To compare the efficacy and adverse reaction of CHFU-LCH 2006 protocol (based on LCH-III protocol) and its updated version CHFU-LCH 2012 protocol.

Methods: Children diagnosed between January, 2006 and November, 2012 were managed under 2006 protocol. Children diagnosed later between December, 2012 and December, 2015, received 2012 protocol (removal of methotrexate and treatment for SS-LCH extended to 12 months).

Results: There were 96 patients enrolled in 2006 protocol and 86 patients enrolled in 2012 protocol. Among patients in the MS-LCH subgroup, there were 4 and 5 cases respectively in 2006 and 2012 group quitted the protocol and was transferred to other rescue protocols. There were 5 and 4 cases dead, respectively. There were totally 93 children categorized as MS-LCH in our study. The rate of EFS and OS among children with risk organ (RO) involvement children were also significantly lower than those without RO. The EFS rate was significantly lower in children who did not respond to the initial 6-week therapy than those who responded. The 5-year EFS for SS-LCH subgroup was (84.8±5.3)% and (86.7±5.6)% for the 2006 and 2012 group, the 5-year projected OS was 100% in both groups. The 5-year EFS for MS-LCH subgroup was (50.0±7.1)% and (53.2±10.0)%, the 5-year OS was (90.0±4.1)% and (90.6±4.5)% for the 2006 and 2012 group. The Grade 3/4 chemotherapy related adverse reactions occurred in 50.0% of patients with MS-LCH in the 2006 group, which was significantly higher than that in the 2012 group.

Conclusion: The 2012 protocol non-inferior to 2006 protocol with less adverse reaction to chemotherapy. However, the EFS of MS-LCH is still not satisfactory in both groups, the treatment strategy may be further modified to improve prognosis. Risk organs involvement and response to 6 weeks initial chemotherapy are the most important prognostic factors for MS-LCH.

Outcome of Children with Langerhans Cell Histiocytosis and Single-System Involvement: A Retrospective Study at a Single Center

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Purpose: To improve our understanding of patients with single-system LCH (SS-LCH), we did a descriptive review of the clinical patterns and outcome of children with SS-LCH treated at Shanghai Children's Medical Center.

Methods: From 2010 to 2014, 60 evaluable newly diagnosed patients with histiopathology-confirmed SS-LCH were enrolled. Systemic chemotherapy was given to all patients. Two protocols were used in our institution during the period of this study: DAL HX-83 study based protocol or LCH-II study based protocol. Treatment decision was according to the physician's own experience.

Results: Of the 60 patients (37 boys and 23 girls), the median age was 3.9 years (range, 0.3 to 15.3 years). Bone was the most frequently affected organ (56/60, 93.3%). Of the 56 patients suffered from SS-bone disease, 35 (62.5%) had unifocal disease and 21 (37.5%) had multifocal disease. CNS-risk lesions were seen in 9 patients (15%) at diagnosis. The 3-year event free survival (EFS) and 3-year overall survival (OS) for all cases were 100% and (79.1±5.4)%, respectively. The 3-year EFS of SS-LCH patients with unifocal disease at diagnosis was significantly higher than that of those with multifocal disease (94.7 ±3.6% vs. 51.3%±11.1%, p = 0.000). When stratified by initial number of sites involved (unifocal or multifocal), the differences in 3-year EFS were not statistically significant between the DAL HX-83 cohort and the LCH-II cohort (SS-LCH, unifocal group: 100% vs. 92.6±5.0%, respectively, P = 0.362; SS-LCH, multifocal group: 42.2±12.7% vs. 80%±17.9%, respectively, P = 0.144). Seven patients experienced 1 or 2 reactivations with first activation occurring 9 months after the diagnosis. All these 7 patients with disease reactivation were initially bone involvement. Only the number of initial sites was associated with an increased risk of reactivation.

Conclusions: To better care patients with SS-LCH, our next step is to optimize disease stratification and treatment modalities based on the current published evidence.

Bone Marrow is The Key Site of Excessive IFN Gamma Production in Primary Hemophagocytic Lymphohistiocytosis

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Purpose: IFNγ is a critical cytokine in the pathophysiology of primary hemophagocytic lymphohistiocytosis (HLH), but its cellular sources, the tissues driving the response and the kinetics of IFNγ production remain incompletely understood. This is relevant for understanding factors determining success and failure of anti-IFNγ therapy.

Methods: We used IFNγ Thy1.1KI reporter mice, adoptive transfer of luciferase transgenic T cells and TCR spectratyping in different tissues to characterize the localization, diversity and IFNγ production of pathogenic T and NK cells in HLH. Disease was induced in perforin-deficient (PKO) mice by lymphocytic choriomengitis virus (LCMV) and by a novel protocol with murine cytomegalovirus (MCMV).

Results: In response to the infectious triggers, T cells accumulated in lymph nodes and spleen in PKO and wt mice, but then rapidly spread to peripheral tissues in PKO mice. Excessive IFNγ production was observed in PKO CTL, less in CD4 T cells in both infection models. NK cells showed excessive IFNγ production after MCMV, but not after LCMV infection. The overall T cell response was less diverse in PKO mice and while the same oligoclonal pattern was observed in spleen, liver and bone marrow, organ-specific changes in the clonal hierarchies were noted. This was particularly pronounced in the bone marrow, where by far the most excessive IFNγ production could be demonstrated, independent of the viral load.

Conclusion: In primary HLH, oligoclonal T cell responses evolve in lymphoid tissues and then distribute to other organs, where they are further edited. The key site of CTL dependent excessive IFNγ production is the bone marrow, which is a major site of HLH pathology. Control of bone marrow driven cytokine production appears to be a key target of anti-inflammatory therapy.

Langerhans Cell Histiocytosis in The Emma Children's Hospital: Efficacy Of Treatment, Long-Term Survival, and Permanent Consequences in Patients Treated During The Vincristin-Cytarabin-Prednison Protocol Era

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Purpose: Langerhans Cell Histiocytosis (LCH) is a neoplastic disease that varies widely in clinical presentation. In the Emma Children's Hospital/Academic Medical Center (EKZ/AMC) a protocol with vincristine, cytarabine and prednisone (VCP) has been used in the past. Long-term efficacy of treatment and the occurrence of a selected number of permanent consequences have previously not been evaluated. Goal of the study was to measure outcomes in patients treated at our institution before the Histiocyte Society protocols were introduced.

Methods: A cohort of patients treated between 1962 and 2008 was identified from our patient-database. Clinical staging, treatment and response to treatment, reactivations and permanent consequences were evaluated.

Results: Of the 97 patients identified, 14 (14.4%) patients had multisystem LCH (MS-LCH) with risk-organ (RO) involvement, 17 (17.5%) MS-LCH without RO involvement, 27 (27.8%) multifocal bone/special site LCH and 39 (40.2%) unifocal LCH. Overall 11 patients (11.3%) died, 87 patients (95.6%) attained no active disease (NAD). Twenty MS-LCH patients started treatment on the VCP-regimen. Fifty-eight percent of the MS-LCH patients on the VCP-regimen survived (80% of the MS-RO+ patients). Of the 10 MS-RO+ patients 8 reached NAD, 3 after treatment intensification. Two other poor-responders died. Two experienced reactivation. All MS-RO- patients reached NAD, 4 after treatment intensification. Five out of 10 had a reactivation, 1 of the 5 died. Twenty-eight (28.9%) patients suffer from permanent consequences (PC), the most common Diabetes Insipidus (DI) in 9,3%. Of the multisystem patients 4 (12.9%) patients and of the single system patients 5 (7.6%) patients were found with DI.

Conclusion: Overall survival for MS-RO+ patients on the VCP-regimen is comparable to that of LCH-III, as is the incidence of DI in the whole cohort. Although the total number of patients on the regimen is limited, treatment results are comparable to those obtained in the JLSG-studies.

Non Resolving Lesion of Oral Cavity

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Purpose of this case report is to highlight the long duration before diagnosis of Langerhans cell histiocytosis can be made and also good response to therapy in these patients. 19 year old male, unmarried, student, presented with three years history of swelling of left soft palate. There was no history of skin rash, dyspnea or tachypnea, polydipsia and polyuria, bone pain, lymphadenopathy, weight loss, fever, gingival hypertrophy, ataxia, and memory problems. Examination revealed a fungating mass arising from left soft palate, extending to hard palate and gums of upper jaw. Tissue biopsy showed polymorphous infiltrates comprising of histiocytes, eosinophils and lymphocytes. Few of the histiocytes showed elongated nuclei with grooving. Immunohistochemical stains including CD1a, CD207 and S100 were positive consistent

with diagnosis of Langerhans type histiocytosis. Staging CT scan showed a soft tissue mass involving left middle cranial fossa abutting left temporal lobe encasing left internal carotid artery. It was eroding greater wing of sphenoid bone, sphenoid sinuses. Infiltrative soft tissue has nearly completely eroded hard palate, pterygoid plates, maxillary antrum walls, ramus, body, coronoid and condylar process of the mandible, infiltrating the bilateral masticator spaces and the infra-temporal fossae. There was bilateral level II cervical lymph nodes measuring 1.8 cm. MR brain showed approximately 9 × 7mm slightly lobulated well encapsulated suprasellar enhancing nodule which likely represent LCH related deposit or a synchronous infundibular primary lesion. Bone marrow biopsy was negative for disease involvement. Final impression was "high risk" multisystem Langerhans cell histiocytosis. He was started on CEPV chemotherapy regimen (Cytarabine, Etoposide, Prednisolone and Vinblastine). After first cycle of chemotherapy his swelling reduced significantly and he started to take orally. He has completed four cycles of CEPV and waiting for interim PET scan.

Braf V600E Mutation is Associated with A Cardiac and Neurological Phenotype but not Mortality in Erdheim-Chester Disease: Results from A Single-Center 165-Patient Cohort

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Erdheim-Chester disease (ECD) is an inflammatory myeloid neoplasm characterized by a heterogeneous phenotype. Between 60 and 75% of ECD patients carry the BRAFV600E mutation. We lack genetic-phenotype association studies. The BRAFV600E mutation was investigated in a large French ECD cohort. The association between the presence of this mutation and the clinical phenotype, as well as the overall mortality, was analyzed with uni- and multivariate analyses. A total of 165 patients (119 men, mean age at diagnosis 56.4 years) were included, and the BRAF status could be obtained for 133. The presence of the BRAFV600E mutation was significantly associated with cardiac (73 versus 27%, $p< 0.0001$) and cerebellar (23 versus 4%, $p = 0.007$) involvement, diabetes insipidus (35 versus 16%, $p = 0.03$) and retro-orbital infiltration (31 versus 11%, $p = 0.02$). Regarding heart involvement, cardiac right atrial pseudotumor was the cardiac localization that was most closely linked to BRAF status (univariate odds ratio (OR) 14.81, 95% confidence interval (95% CI) 4.87-44.97, $p<0.0001$). Survival was not different among the BRAFV600E and wild-type patients. As shown by the uni- and multivariate analyses, overall mortality was associated with age at diagnosis, retroperitoneal involvement (HR 3.85, confidence interval 1.68-8.83) and lung involvement (HR 2.74, confidence interval 1.38-5.43). The central nervous system was also confirmed to be an independent predictor of death. Interferon-alpha and targeted therapies were associated with better survival. The presence of the BRAFV600E mutation in ECD is associated with cardiac and neurological involvements but not mortality. Retroperitoneal and lung involvements are associated with worse survival.

Analysis of Somatic Mutations in Japaneses Patients with Langerhans Cell Histiocytosis

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Background: Mutually exclusive somatic mutations in mitogen-activated protein kinase (MAPK) pathway genes have been identified in about 75% of patients with Langerhans cell histiocytosis (LCH). In western countries, BRAF V600E mutation accounts for about 50%, followed by MAP2K1 mutations for about 25%. Previous reports from Japan, using relatively low sensitivity methods, showed the frequencies of BRAF V600E mutation was varied (21-59%).

Methods: Forty four Japanese patients with LCH were tested for BRAF V600E mutation in biopsied lesional tissues by using allele-specific real-time polymerase chain reaction-based assay kit (Entrogen, Woodkand Hills, CA). The detection limit of this assay kit is 1%. Seven of BRAV V600E negative patients were tested for MAPK pathway genes by next-generation sequencing (NGS).

Results: Of 44 patients, 40 were children and 4 were adults. The median age at diagnosis was 3 years-old (range: 4 months - 66 years). Eighteen patients had single-system disease and twenty-six patients had multisystem disease. Seventeen cases (38.6%) harbored BRAF V600E mutation and all of these were children. Among 7 of BRAF V600E mutation negative patients, NGS revealed that 5 (71.4%) were positive for MAP2K1 mutations and one of these was also positive for ERBB3 mutation.

Conclusions: Though the number of cases are small, in Japanese LCH patients, the frequency of BRAF V600E mutation might be low compared to Western countries, while that of MAP2K1 mutations might be high.

Sepsis As A Mimicker of HLH in A Pediatric Intensive Care Unit

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Purpose: Hemophagocytic Lymphohistiocytosis (HLH) is a disease of overwhelming inflammation with a high mortality rate. Early recognition and initiation of treatment may improve survival. This study examined five pediatric control patients without HLH in the pediatric intensive care unit (PICU) at Children's Mercy Hospital with clinical diagnosis of sepsis and one pediatric patient with HLH in the PICU for the diagnostic criteria and known and exploratory cytokine levels.

Methods: This prospective study was approved by the IRB at Children's Mercy Hospital Kansas City Missouri, USA. Chart review for clinical diagnostic criteria and additional blood sent for laboratory criteria of HLH. Cytokine levels on day 1 and 3 of PICU admission performed when samples available. Plasma cytokines were evaluated using magnetic bead immunoassay.

Results: Total of six patients enrolled on study; five that did not meet diagnostic criteria for HLH and one patient that did satisfy diagnostic criteria for HLH. The child with HLH had the most elevated serum ferritin of greater than 10,000 ng/ml compared with mean ferritin of 1574 ng/ml for non HLH patients. The patient with HLH also had the most elevated soluble interleukin 2 level of 8,039 U/ml compared to median value of 1551 U/ml for non-HLH patients.

Conclusion The use of serum cytokine levels may identify children with HLH and lead to earlier initiation of therapy.

Refractory Multisystem Langerhans Cell Histiocytosis with Marked and Durable Response to Dabrafenib

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Purpose: To evaluate the efficacy of the single agent oral B-RAF kinase inhibitor dabrafenib in a patient with refractory multisystem B-RAF V600E mutation positive Langerhans Cell Histiocytosis (LCH).

Methods: IRB approval was obtained for compassionate-use dabrafenib for a 2 year old with multisystem B-RAF V600E mutation-positive LCH, with gastrointestinal, skin, bone, lymph node and hepatosplenic involvement who was previously treated with multiple cycles of cytarabine, clofarabine, cytarabine/clofarabine combination therapy, and alemtuzumab, but with refractory disease after each agent. Biopsies of the gastrointestinal tract showed diffuse LCH involvement from the gastric mucosa to the rectum. She presented with knee & hip pain, abdominal pain, diarrhea, and massive hepatosplenomegaly extending to the pelvis. Dabrafenib mesylate was administered orally as a 10 mg/ml liquid formulation reconstituted from powder (5.25 mg/kg/day divided twice daily).

Results: This patient had a marked response to treatment with dabrafenib within 1–2 months with resolution of abdominal distension, diarrhea, lymphadenopathy, and hepatosplenomegaly. Dabrafenib was extremely well tolerated with adverse effects of only transient hypokalemia and intermittent skin rash. This remission has been maintained for 12 months to date.

Conclusion: This marked, prolonged response to an oral B-RAF inhibitor that was extremely well tolerated, shows proof-of-principle that the B-RAF mutation identified in LCH can drive proliferation, and that its inhibition has great therapeutic promise. Further studies are needed to see if dermatologic toxicities/skin malignancies are also seen in children, and if there are additional unanticipated toxicities unique to children, as well as to define the optimum duration of therapy.

Challenges of Haemophagocytic Lymphohistiocytosis Cases in Yangon Children Hospital

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Purpose: To evaluate the management and outcome of HLH cases in our center.

Methods: A retrospective study in a five year period (from 2012 January - 2016 December).Diagonsis is mainly based on the clinical criteria and haemophagocytosis in bone marrow morphology proposed by HLH2004 because of limited facilities to detect genetic defects of familiar HLH and modern technique to find the association with Epstein-Barr virus, serum markers of sCD25 and circulating sCD163. Treatment is according to HLH2004 for primary HLH and for those who can diagnose a secondary cause, treated according to the disease (without bone marrow transplant).

Results: Total 11 cases of HLH were diagnosed during five years. Majority of patients were girls (1:2.7), all cases presented with fever and hepatosplenomegaly however only 36%(4/11) presented with lymphadenopathy. Patients presented with skin eruptions, CNS abnormalities and DIC are 18%(2/11), 18%(2/11), 36%(4/11) respectively. Four cases have strong family history and treated as possible Familiar

HLH. Among the secondary HLH, the causes were tuberculosis(1/7), langerhan cell histiocytosis(1/7), Non Hodghkin Lymphoma (1/7), EBV infection (1/7) and unknown(3/7). Three cases 27% abandoned treatment, five cases 46% expired and three cases 27% were still on treatment. Two children relasped 6months and 4 months after offtherapy.

Conclusion: HLH, a severe rapidly progressive and lethal disease is not only difficult to get proper diagnosis but also encounter challenges in treatment with limited facilities.

HIF1A is A Critical Mediator for Primary and Secondary Hemophagocytic Lymphohistiocytosis

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Purpose: Although a steroid/etoposide-based regimen, as first-line therapy, is effective to treat hemophagocytic lymphohistiocytosis (HLH), it still has substantial morbidity. Thus, novel, less toxic therapies for HLH are needed.

Methods: We took bio-informatic approaches and re-analyzed published microarray data of patients with familial hemophagocytic lymphohistiocytosis (FHL) and patients with systemic juvenile idiopathic arthritis (sJIA), which is tightly associated with secondary HLH. To validate the human HLH data, HIF1A levels were measured in two established HLH mouse models by flow cytometry. Furthermore, to determine the role of HIF1A in HLH, a transgenic mouse line with inducible expressions of HIF1A/ARNT proteins in hematopoietic cells was generated and analyzed.

Results: Our transcription factor-target enrichment analysis predicted HIF1A as one of the common key transcription factors in both FHL and sJIA datasets; gene set enrichment analysis (GSEA) showed that the HIF1A signature is also significantly enriched in both datasets. Gene ontology analysis revealed that the common leading edge genes of the HIF1A signature are related to chemotaxis, glycolysis, and immune response. Consistent with human HLH data, elevated HIF1A protein levels were confirmed in both the Lymphocytic choriomeningitis virus infected Prf1-/- HLH model and the CpG-treated model. Moreover, hematopoietic specific expression of HIF1A/ARNT proteins in the C57BL/6 background caused lethal HLH-like phenotypes: severe anemia, thrombocytopenia, multi-organ failure, splenomegaly, and ferritinemia. Mechanistically, these mice showed type-1 polarized macrophages, reduced NK cells, and slightly changed dendritic cells, but unaffected T/B cell populations. Furthermore, the HLH-like phenotypes in this mouse model are independent on their adaptive immunity or IFN-Î[3] signaling, since induction of the HIF1A/ARNT allele resulted in similar phenotypes in the Rag1-/- and Ifngr-/- background.

Conclusion: Our data revealed that the HIF1A signaling pathway is a critical mediator for both primary and secondary HLH and could potentially be a therapeutic target for a broad spectrum of HLH.

A Phase II Trial of Lenalidomide in Adults with Histiocyte Disorders

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Purpose: Evaluate the efficacy and safety of lenalidomide in adult patients with Langerhans cell histiocytosis (LCH), histiocytic sarcoma (HS) and Erdheim-Chester disease (ECD).

Methods: Patients 18 or older with LCH, ECD or HS in need of systemic therapy are eligible. Initial lenalidomide dose is 10 mg daily on days 1–21 of a 28-day cycle. If no grade 3 or greater toxicity occurs during cycle 1 the dose is escalated to 25 mg. The primary endpoint is overall response rate (ORR) using MRI and PET/CT per International Working Group criteria. All patients were required to take aspirin 81 mg daily if not on systemic anticoagulation at baseline.

Results: Eleven of a planned 12 patients have been enrolled. Histologies were ECD (n = 3), HS (n = 2), and LCH (n = 6). BRAF mutations were identified in 2 of 11 patients. Seven patients received prior treatment. The number of treatments received were 1 (n = 1/7), 2 (n = 3/7), 3 (n = 2/7), and 7 (n = 1/7). Four of 11 patients have responded, all with LCH (2 CR and 2 PR). One LCH patient subsequently progressed after achieving CR. 2 additional LCH patients have had SD but improvement in symptoms. All 3 ECD patients had SD and both HS patients had PD as best response. Median duration of response has not been reached. One patient with HS died, all others remain alive. The most common toxicities were fatigue (n = 6), neutropenia (n = 5), nausea (n = 4), anemia (n = 3), thrombocytopenia (n = 3), and rash (n = 3). The only grade 3

toxicities were neutropenia and thrombosis (n = 1 each). No grade 4 or grade 5 toxicities occurred.

Conclusion: Lenalidomide has excellent activity in LCH though lesser activity in ECD and HS.

Clinical Study OF E-Chop Regimen as A Salvage Therapy for Childhood Refractory Hemophagocytic Lymphohistiocytosis

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Purpose: To investigate the efficacy with E-CHOP regimen as a salvage therapy for children refractory hemophagocytic lymphohistiocytosis(HLH).

Methods: Total 18 patients with refractory HLH were enrolled in this study.The efficacy of treatment with E-chop regimen after 2 and 4 weeks were evaluated according to the United States Midwest Cooperative HLH Group.

Results: Of 18 refractory HLH patients, 8 were males and 10 females.The median age was 4.5(1-11) years old.The overall response rate (ORR) was 83.3%(15/18),including 6 patients (33.3%) achieved complete remission (CR) and 9 patients (50%) achieved partial remission(PR).The underlying disease of HLH were identified in 17 patients,including 4 case of primary HLH(CR 3 cases,PR 1 cases),2 cases(PR) of tumor associated HLH and 11 cases (CR 2 cases,PR 6 cases)of EBV associated HLH.There were still one cases with unknown underlying disease.The 3 patients who had no response to E-CHOP died within 2 to 4 weeks after salvage therapy. Fifteen patients who achieved PR or CR survived to undergo allogenic hematopoietic stem cell transplantation (allo-HSCT) or splenectomy.

Conclusion: The study suggested that E-CHOP regimen appeared to be an effective salvage protocol for children patients with refractory HLH.

A Re-Examination of Murine HLH: Kinetics, Comparative Therapy, and Novel Combinations

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Purpose: The study of hemophagocytic lymphohistiocytosis (HLH) in mice has provided unique insights into immune regulation, defined disease pathophysiology, and suggested several strategies for the targeted therapy of human HLH. Multiple groups have studied the therapeutic potential of blocking interferon gamma (IFN-g), JAK/STAT signaling, and other conventional or novel approaches in mice. However, it is not clear how well therapy in these models reflects clinical realities in patients, or how these therapies may compare with, or complement each other.

Methods: We performed a stringent kinetic analysis of multiple markers of HLH or immune activation in LCMV-infected normal or perforin deficient mice, assessing: soluble CD25, serum granzyme B, IFN-g, CXCL9, ALT, LDH, ferritin, CBC indices, spleen size/inflammatory infiltrates, and a detailed clinical scoring system. We also examined select indices and survival after various therapeutic interventions.

Results: This kinetic analysis revealed that murine HLH may be divided into 3 phases: normal immune activation, pre-symptomatic immune hyper-activation, and the fully developed HLH clinical syndrome. Consequently, all therapeutic interventions may be categorized as pre-emptive, pre-symptomatic, or post-HLH, based on their timing. We observed that while many interventions were effective pre-emptively, therapy was much more challenging after HLH developed. Toxicities of etoposide and JAK inhibitors were substantial in this context and IFN-g blockade had incomplete efficacy. Gene expression and cell signaling studies revealed unique targets in these mice. Results of studies examining comparative, combination, and novel therapies will be presented.

Conclusion: A careful definition of the kinetics of HLH development is essential for interpreting the results of therapeutic studies in murine models. The context for specific therapies has a significant impact on both efficacy and toxicity, which may be relevant for clinical contexts. This approach is revealing innovative and potentially optimal combinations for targeted therapy of human HLH.

Targeted Therapy of Juvenile Xantogranuloma with BRAF V600E Mutation: A Report of Two Cases

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Purpose: To evaluate the efficacy of the BRAF inhibitor vemurafenib efficacy in the treatment of refractory BRAF V600E-positive juvenile xantogranuloma in children.

Methods and Results: Patient 1 (1.1 y.o. at the moment of diagnosis) was diagnosed with JXG with lesions of chest bones, shoulder gridle bones, femur, sacrum, multiple skull lesions with soft-tissue retrobulbar component, liver and spleen enlargement. Patient was treated with salvage regimen of LCH-IV (cytarabine + cladribine), but experienced severe

infectious complications. Computed tomography showed no improvement in any of the lesions. BRAF V600E was detected with Sanger sequencing in biopsied lesion. Considering this, vemurafenib was administered at 480 mg/day as monotherapy. After 6 months all skeletal lesions fully resolved except for skull lesions that resolved partially. Soft-tissue component resolved completely. Patient 2 (4.5 y.o. at the moment of diagnosis) was diagnosed with JXG of suprasellar and supraorbital region (soft-tissue component) and diabetes insipidus. IC-1 as first-line therapy was performed (vinblastine + prednisone), without effect. Cladribine was administered as a second-line therapy, but the tumor volume increased progressively (+27% of tumor size). Considering that and BRAF V600E mutation in tumor, vemurafenib was administered (480 mg/day). After 3 months of therapy a 1/3 reduction of tumor was registered with MRI scan.

Conclusion: vemurafenib may induce significant responses in BRAF V600E-positive non-LCH histiocytic disorders in children.

Profound Hyperferritinaemia is not so Specific for the Diagnosis of Haemophagocytic Lymphohistiocytosis in Asian Children - A Single Centre Study from Singapore

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Purpose: Profound hyperferritinaemia of >10,000 mcg/L has been shown to be highly specific for the diagnosis of haemophagocytic lymphohistiocytosis (HLH) in western literature but similar studies are lacking in Asian children.

Methods: We did a retrospective study over 6 years (2010-2015) and reviewed the records of all paediatric (up to 17yrs of age) inpatient admissions with a ferritin value of >500 mcg/L and identified children diagnosed with HLH as per the HLH 2004 criteria in this period. Children on regular transfusion, with incomplete work-up or with samples taken outpatient were excluded. Sensitivity and specificity of hyperferritinaemia were calculated at ferritin levels of > 500 mcg/L, >2,000 mcg/L and >10,000 mcg/L.

Results: Sixty children were identified with high ferritin of >500 mcg/L and only 9 were confirmed to have HLH. All children diagnosed with HLH had ferritin of >10,000 mcg/L. Sensitivity of hyperferritinaemia in the diagnosis of HLH was consistently high at all ferritin levels of >500 mcg/L, >2,000 mcg/L and 10,000 mcg/L (100% with Confidence Interval of 66.37-100%). However, specificity was found to be much lower, at 35.29% (95% CI of 22.43%-49.33%) for 2,000 mcg/L and at 76.47% (95% CI of 62.51%-87.21%) for 10,000 mcg/L. Twenty-one children had ferritin of >10,000 mcg/L of which 12 did not have HLH and had an alternative diagnosis mostly infection.

Conclusion: Profound hyperferritinaemia, although a useful screening test is not very specific for the diagnosis of HLH in Asian children. Macrophages in Asian populations are likely to be genetically more hyper-responsive and secrete more ferritin secondary to infective stimuli and may not have associated HLH. As HLH specific investigations like NK cell function, CD25 assay and genetic testing are not readily available in Asia, reliance on hyperferritinaemia in diagnosis of HLH should be used with great caution to avoid over-diagnosis and unnecessary treatment.

Severe Dengue (SD) Complicated by Reactive Haemophagocytic Syndrome (HS): Five Years Experience in a Tertiary Intensive Care Unit (ICU) in Malaysia

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Purpose: Malaysia recorded 215 dengue deaths from 108,698 cases in 2014. Johor state had second highest mortality, with 25 deaths from 6,323 cases reported. We observed rising incidence of HS with possible higher mortality. This study aimed to determine incidence and significance of HS, clinical features, associating factors and mortality rate in SD.

Methods: A retrospective cohort study of confirmed dengue cases in our 29-bedded adult (> 12 years old) ICU from 2010 to 2014. SD was defined using WHO 2009 classifications. HS was diagnosed clinically and scored using HLH-2004 diagnostic criteria (without molecular and immunological studies), proposed HLH diagnostic criteria 2009 (HLH 2009) and HScore. Univariable and multivariable logistic analyses were performed to identify associating factors.

Results: Among 8802 ICU admissions, 288 (3.27%) were dengue. After excluding 9 patients with missing medical records, we had 198 (70.97%) SD, 20.2% died. Severe leak, severe bleed, lethargy, hepatomegaly, APACHE, SAPS II and SOFA score, HS probability > = 0.7, maximum AST, ALT, LDH and ferritin were significant associating factors for SD mortality (p-value<0.05) though none were significant by multivariable logistic analysis. HS probability > = 0.7 were clinically consistent with HS (28 cases, mortality rate 39.3%). Median age was 33.5 years (IQR:16), 64.3% were female. Fourteen had bone marrow biopsy, 12 (86%) demonstrating haemophagocytic activities. Median duration of ICU stay was 3 days (IQR:5). Median duration from onset of dengue

symptoms to death was 9 days (IQR:8). Eight out of 11 (72.2%) patients with maximum ferritin > 100,000 microgram/L died. Severe organ involvement, lethargy, diarrhoea, hepatomegaly, SOFA score, HLH 2009, continuous veno-venous haemofiltration, intubation, maximum AST, ALT, LDH and lactate were significant associating factors to HS (p-value<0.05). Only maximum ferritin (p-value = 0.018) and HLH 2009 (p-value = 0.034) were significant associating factors after multivariable analysis.

Conclusion: HS was commonly associated with SD and had higher mortality.

Langerhans Cell Histiocytosis Presenting with Proptosis in A Child

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Background: Langerhan's cell histiocytosis (LCH) is a rare disease accounting for less then 2% of new cases enrolled each year in the Children's Hospital Lahore, it is the most common type of childhood histiocytoc disorder in children under 15 years of age. Purpose: to report an unusual presentation of LCH in a child.

Methods: We report the case of a 3 year old female who presented to our hospital with fever & exopthalmos of left eye,on complete blood count only mild anemia was present, skeletal survey revealed no bony lesion, vertebral bodies & intervertebral spaces were normal and so were joint spaces. Bone marrow examination & abdominal ultrasound also were normal, however CT scan revealed lytic lesion involving left temporal bone, floor of left orbit & left zygomatic arch with associated soft tissue component extending into Left pterygo palatine fossa & nasopharynx, minimal soft tissue component also seen in Left retro-orbital extraconal space with mild proptosis of Left eyeball. MRI revealed large hetrogenously enhancing mass with necrotic component within seen along Left infratemporal, Left parapharangeal space, basi-sphenoid & along Left globe suggestive of sarcomatous mass. Histological examination of the soft tissue mass biopsy revealed neoplasm composed of sheets of Langerhan's cells along with eosinophils, foam cells & lymphocytes. Immunohistochemistry revealed S100 & CD1a positivity in tumor cells,confirming LCH diagnosis. Induction vinblastine weekly 6mg/m2 for 6 weeks & prednisone. Repeat MRI after 3 months revealed significant interval change 7 × 6.6cm mass had shrunk to 3 × 2.3 cm.

Discussion: LCH has varied clinical manifestations depending on organs involved, bone involvement being commonest followed by lung,liver, lymph node & skin etc. Definite diagnosis depends on histopathology of tissue biopsy with immunohistochemistry. This is the only case of LCH presenting with proptosis & normal skeletal survey over a period of six years.

A Case of Severe CNS Involvement in Macrophage Activating Syndrome

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Introduction: Macrophage Activating Syndrome (MAS) is a rare but potentially fatal disease. MAS is currently classified among the secondary forms of hemophagocytic lymphohistiocytosis (sHLH). The reasons is that MAS shares clinical and laboratory features with primary genetic HLH (pHLH). The diagnosis of MAS is usually delayed due to the presence of non-specific symptoms at presentation and managed as in sepsis. All unfortunately progressed to multiple organs dysfunction and died. The underlying causes for MAS were considered to be juvenile rheumatoid arthritis. MAS is treated with NSAIDs, corticosteroids, DMARDs and biologic therapy.

Case and Review: A 12.3-year-old girl presented prolonged fever for more than 2 months. And confused mental status was developed 1 week ago. Her skin rash appeared on face and trunk for 1 year (wax and wane). She was admitted to tertiary hospital for full work-up of FUO. All cultures results were negative. Finally she was transferred to the university hospital due to the mental dysfunction. WBC 19,320/mm3, Hb 10.7g/dL, platelet 208,000/mm3, ESR 66mm/hr, CRP 18.8mg/dL, AST/ALT 1,373/374 IU/L, TBilirubin 2.2, BUN/Cr 53.7/2.53mg/dL, PT/aPTT 18.1/64.4sec, Fibrinogen 286, Ferritin 8,093ng/dL, CSF WBC 16/mm3, protein 68.3mg/dL, EEG : background suppression with no epileptic discharge. Brain MRI : focal high signal intensity in the right parietal deep white matter. r/o ADEM. Bone marrow biopsy : hemophagocytotic histiocytes are seen and immunohistiochemical stain for CD 68 was increased histiocytes. She received MPD pulse for 3days. After that the prolonged fever was disappeared.

Results: Her CNS condition and cytokine storm were improved after MPD pulse. After receiving MPD pulse for 3days, she took medicine of cyclosporiene A and Etanercept.

Conclusion: We should consider the possibility of CNS involvement in MAS who developed febrile seizure with FUO. We report a successful use of MPD pulse and cyclosporine A therapy of MAS in CNS involvement.

Ocular Juvenile Xanthogranuloma: A Report of Three Infant Cases Involving IRIS

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Introduction: Juvenile xanthogranuloma (JXG) is an uncommon non-Langerhans cell histocytic inflammatory disorder that generally presents in children as self-limiting skin lesions. Extracutaneous organ involvement is sometimes life-threatening and requires systemic therapy.

Case reports: Case 1. A 4-month-old boy presented with hyphema and glaucoma, and yellowish-white nodule was recognized on his left iris. Three months after a 6-week topical and systemic corticosteroids, local relapse occurred. Although successfully re-treated, his corrected visual acuity was 0.02 at the age of five. Case 2. A 3-month-old boy presented with right corneal opacity and glaucoma due to severe anterior synechia of the iris. He was successfully treated with 6-week topical and systemic corticosteroids followed by 3-month low-dose maintenance, but his right eye had no light perception. Case 3. A 19-day-old boy presented with right conjunctival injection and corneal opacity. Abnormal iris associated with white membranous tissue was noticed in. He is currently under low-dose maintenance following 6-week topical and systemic corticosteroids. In all cases, monomorphic histiocytic infiltration in the patient's iris was pathologically observed. The diagnosis of JXG was based on immunohistochemical staining of positive CD68 and CD163, and negative CD1a. There was no evidence of skin and other lesions. Topical and systemic steroid therapy was effective for all the cases, although an early recurrence occurred in Case 1.

Discussion and Conclusions: Ocular JXG is rare. It mainly occurs in the iris of young children. Topical, periocular, and systemic corticosteroids is effective, however, the prognosis of visual function remains poor. Our experience of an early relapsed case might indicate the necessity of a prolonged systemic therapy. Moreover, JXG should be included in a possible differential diagnosis when a patient present with conjunctival injection and/or glaucoma. Early diagnosis and intervention, together with close cooperation with ophthalmologists, may be critical for a better prognosis of visual function.

Phenotyping of Leukocyte Subsets in Patients with Hemophagocytic Lymphohistocytosis Associated with Hematological Malignancies

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Purpose: Hemophagocytic lymphohistiocytosis (HLH) involves uncontrolled activation of monocyte/macrophage system, with resulting hyperinflammatory syndrome, which in adults is often triggered by malignancy. The study was aimed to evaluate leukocyte immune profiles of patients with hematological malignancies and secondary HLH.

Methods: Tissue samples from 42 adult patients (15 women, 27 men; aged 26–85 years) with malignancy-associated HLH (22 lymphoid, 20 myeloid tumors) were investigated by flow cytometry. The patients were evaluated and treated at the Hematology Center between 2009–2016, and their samples analyzed routinely at the Department of Clinical Pathology and Cytology, both at Karolinska University Hospital. Samples were obtained prior to (31 patients) or after established HLH-diagnosis (24 patients), with paired BM samples in 13 subjects. Neoplastic clones were excluded from analysis. Results were compared to a cohort of 35 adult patients without malignancy and HLH.

Results: Non-neoplastic monocytes were increased in 40% patients with myeloid (M) and 31% subjects with lymphoid (L) tumors but a subset of patients had monocytopenia (10% M, 15% L). Aberrant lymphocyte marker expression could be found on non-malignant myeloid cells in all M-tumors. Lymphocyte subsets showed both quantitative and qualitative aberrations, with NK-cytopenia equally common before and after HLH diagnosis. Prior to HLH diagnosis, decrease in T-cells was found more often in M- than in L-tumors (60% vs. 25%), whereas the reverse was observed in established HLH. M-tumors were associated with increased CD4/CD8 ratio as compared to L-tumors, where the loss of T-cell markers was uniform. B-cell lymphopenia was prominent in the entire cohort, and progressed after HLH diagnosis (67% vs. 74%).

Conclusion: Shifts in leukocyte subsets and phenotypical changes occurred in both M- and L-malignancies, however with different patterns and to different extents. It remains to be clarified if those reflect impact of the underlying malignancy on BM microenvironment or the HLH-associated phenomena.

A 15- \Months\ Old Child Presenting with Highly Aggressive BRAF(+) Multisystem LCH with CNS Involvement: Arguments for use of Dabrafenib p.o.+ Low Dose Cytarabin I.V. as the Treatment of Choice

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Purpose: Our patient had a history of an unrecognized skin and bone Langerhans cell histiocytosis (LCH) for almost 4

months in a community hospital. On admission he looked critically ill, he had mutisystem involvement with risk organs (bone marrow infiltration of 60–70%, involvement of the liver and rapidly progressing neurologic symptomatology due to massive involvement of the splanchnocranium with direct involvement of the middle cranial fossa. Cytospin of the likvor did not show individual malignant cells, but has revealed positivity of BRAF 600/602StripAssay (on the 1% sensitivity level). Patient was rapidly worsening. Progression of neurologic symptoms and massive infiltration of bone marrow with LCH is known to be associated with inferior outcome with the use of standard regimens so upfront targeting the known driving mutation was considered here.

Methods: Dabrafenib was chosen here because of more favorable data from juvenile animal studies. Considering the massive CNS involvement the ARA:C 100mg/m2/day as i. v. infusion was added together with dabrafenib.

Results: The treatment response after two weeks (time the abstract is written): bone marrow cleared to maximally 30% not viably looking CD1a+, Langerin + cells, CSF positivity dropped to the level of 0.1% for BRAF 600/602 StripAssay only. Clinically we have observed marked clinical improvement with the possibility for out patient based care starting from the day 8. Child is able to walk, play, neurologic status has completely returned to normal. Next to activated the RAS-RAF-MEK-ERK pathway distinct plasma protein profiles exists in LCH suggesting that probably pathologic myeloid cells contribute to the inflammation of the disease (Daniel Zinn, USA, Texas). Proteomic analyses of the pretreatment plasma and CSF of our patient are pending.

Conclusion: we present rapid response of the neurologic status in a child with BRAFV600E mutated LCH to out patient treatment with dabrafenib and standard dose i.v. ARA-C.

Detection of Somatic Mutations by PCR-Based Next-Generation Sequencing from Fixed Clinical Specimens in Childhood LCH

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Purpose: Langerhans cell histiocytosis (LCH) is characterized by inflammatory histiocytic neoplasms that exhibit oncogenic constitutive activation of the RAS/MAPK pathway. Somatic mutations of BRAF and MAP2K1 have been identified in 70–80% of LCH cases. Recent studies have reported the clinical potential of targeted therapies using BRAF or MEK inhibitors. Sensitive molecular assays for detecting oncogenic mutations in LCH will enable the prediction of patient response to targeted agents. The aim of this study is to establish a method for detecting low frequency mutations from fixed LCH specimens by polymerase chain reaction (PCR) based on deep sequencing.

Methods: We extracted genomic DNA from formalin-fixed, paraffin-embedded clinical specimens from 20 patients with childhood LCH. Two samples were excluded because of low quality PCR amplification. We studied 18 cases using a PCR-based targeted next generation sequencing platform with custom designed primers that contained overhang adapter sequences. The amplicon libraries generated for MiSeq were analyzed for detecting mutations in Exon12, 15 of BRAF gene and Exon2, 3 in MAP2K1 gene.

Results: We detected somatic mutations in 17 of 18 samples (94%) at various allele frequency (3.3-30%, median 8.6%). BRAF was mutated in 15 of 18 samples (83%) and MAP2K1 was mutated in 5 of 18 samples (28%). BRAFV600E, the most frequent mutation in LCH, was identified in 9 of 18 samples (50%). In-frame deletions in exon 12 of the BRAF gene was found in 4 of 18 samples (22%).

Conclusion: We identified genetic alterations of BRAF or MAP2K1 in over 90% of childhood LCH cases at even low allele frequency. High resolution analysis using formalin-fixed, paraffin-embedded clinical specimens could be a reliable implement as companion diagnostics for clinical use.

Hematopoietic Stem Cell Transplantation in Children with Refractory Langerhans Cell Histiocytosis

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Purpose: Effect and indication of hematopoietic stem cell transplantation (HSCT) has been still undetermined for children with refractory Langerhans cell histiocytosis (LCH). We retrospectively analyzed 30 children with refractory LCH undergoing HSCT in Japan.

Methods: Total 30 children with refractory LCH who underwent an allogeneic HSCT were registered in the Transplant Registry Unified Management Program (TRUMP) database between 1996 and 2014.

Results: The male/female ratio was 18/12. At diagnosis of LCH, the median age was 10 months (range, 3–60 months), and 19 were positive for risk organ involvement, 6 were negative and 5 were unknown. The median age at HSCT was 24 months (range, 12–135 months), and the median interval between the diagnosis and HSCT was 349 days (range, 51–3,773 days). Eleven patients underwent HSCT using myeloablative conditioning (MAC) regimen, whereas 19 patients received reduced intensity conditioning (RIC) regimen. Donor sources were related donor in 9 patients and unrelated in 21 patients (cord blood 19 and bone marrow 2). Neutrophil recovery was observed in 24 patients and the median time to engraftment was 21 days. Acute GVHD of grade II - IV, chronic GVHD were observed in 6 and 4 patients, respectively. With median follow-up of 18 months after HSCT, 13 patients died and 8 of them within 3 months after HSCT. The overall survival (OS) was not different between RIC and MAC. In regard to disease status at HSCT, recipients with no active disease/partial response (n = 6) had better outcome than those with active disease-stable/ progressive (n = 19) (5-year OS 100% vs. 52.1%, p = 0.035) (5 data missing).

Conclusions: Of 30 HSCT-recipients for refractory LCH, 17/30 (57%) are alive while post-transplant death occurred in 13/30 (43%). Novel measures are required to stabilize the disease activity before HSCT.

When Langerhans Met Crohn: A Clinical Case Report and a First Three-Dimentional Reconstruction of Human Gut

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Purpose: Langerhans cell histiocytosis (LCH) affects 0.9 per 100,000 children per year, and is characterized by granuloma formation in affected organ systems. LCH has a wide range of clinical presentations, and gastrointestinal involvement has been known as a part of LCH clinical spectrum since an autopsy study by Harries and Keeling in 1973. Crohn's disease (CD) is a granulomatous disease primarily affecting the gastrointestinal tract, with the annual incidence of up to 4 per 100,000 children per year. The purpose of this study is to present a case report and discuss differential diagnostic challenges faced by clinicians taking care of LCH and CD patients. In addition, we take a deep dive in tissue immunopathological architecture and present the first three-dimensional visualization of human gut.

Methods/case report: A 16 months-old boy was diagnosed with multifocal bone LCH and responded well to chemotherapy. No other system was affected until the age of 10 years when he presented with gastrointestinal symptoms and CD1a-negative colonic granulomas typical for CD. He was diagnosed with CD and received anti-TNF therapy with good response. Despite recent advances in the field of genetics, the pathogeneses of these two granulomatous diseases remains to be elucidated. In order to gain further insights into the immunopathology at the site of inflammation we performed CLARITY, a method that makes tissue transparent, and analyzed complete intestinal biopsies using confocal microscopy.

Conclusion: The presented case report opens up for a discussion addressing diagnosis, monitoring and treatment of LCH and CD. Moreover, along with unique spatial insights into the human gut architecture, our work contributes to a better understanding of the inflammatory milieu in the three-dimensional perspective that may be important for immunopathology studies not only for LCH and CD, but also for other disorders with a chronic inflammatory component in the gut.

Vemurafenib in a Child with Life-Threatening Multisystem Langerhans Cell Histiocytosis

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Background: Most children with Langerhans cell histiocytosis (LCH) can be cured with conventional chemotherapy, but

children with risk-organ dysfunction not responding to first-line treatment still have a poor prognosis. The BRAF inhibitor vemurafenib might be a salvage option in these patients, but clinical experience of this drug is limited, in particular in children.

Case report: A febrile 2 year-old girl presented in poor clinical condition. Examination revealed cervical lymphadenopathy and hepatosplenomegaly. Laboratory assessment revealed pancytopenia (hemoglobin 7.1 g/dL, leukocytes 3.23 G/L, platelets 68 G/L) and low total protein (5.3 g/dL). The clinical condition rapidly deteriorated, and the girl needed regular transfusions of red blood cells and platelets. LCH was diagnosed in a biopsy of a cervical lymph node. Induction therapy with prednisone and vinblastine was started, but did not improve the clinical condition, as neither did the addition of etoposide. After the BRAF mutation V600E was detected, therapy with vemurafenib was started (20 mg/kg/day), which resulted in a rapid clinical improvement and hematologic recovery. Despite relatively high vemurafenib plasma levels, therapy is well tolerated with mild alopecia and photosensitivity of the skin as only side effects. After 16 weeks of treatment with vemurafenib, the girl is in good clinical condition. However, BRAF V600E alleles are still detected in the blood.

Perspectives: To date, the experience with vemurafenib in the treatment of children with LCH is scarce. Most of the reported patients showed a rapid response to vemurafenib, but optimal treatment duration is unclear. Potential side effects of vemurafenib include the development of skin cancer, but on the other hand, cessation of therapy is associated with a high risk of relapse. Therefore, we plan in our patient an overlapping chemotherapy with prednisone, vincristine, and cytarabine. Whether the assessment of BRAF allows valid monitoring of the disease needs to be established.

Transcriptional Profiles, Lineage Tracing with BRAF-V600E, and HLA-DQB2 Expression Support a Model of Blood CD1c+ Cells as Precursors To LCH Lesion CD207+ CELLS

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Purpose: Langerhans Cell Histiocytosis (LCH) is characterized by granulomatous lesions that include pathologic CD1a+/CD207+dendritic cells (DC). Activating somatic MAPK pathway gene mutations have been identified in hematopoietic stem cells and lesion DCs in patients with high-risk LCH, though the differentiation pathway remains uncertain. The purpose of this study was to define the origin of LCH lesion CD1a+/CD207+ cells.

Methods: We compared transcriptomes of LCH lesion CD1a+/CD207+ cells to established gene signatures from human peripheral blood and tissue myeloid populations including CD14+ monocytes, CD16+ monocytes, CD14+ DCs, macrophages, epidermal Langerhans cells, CD1c+ myeloid dendritic cells (mDCs) and CD141+mDCs. Additionally, quantitative PCR of BRAF-V600E and HLA-DQB2, and HLA-DQB2 surface expression were used to identify clonal lesion and peripheral blood monocyte and dendritic cell populations.

Results: When comparing lesion CD1a+/CD207+ cells to blood and tissue DC/monocyte populations, the CD1c+ mDC gene signature was most similar to gene expression profile of lesion CD1a+/CD207+ cells. In order to further test the hypothesis that LCH CD1a+/CD207+ cells arise from CD1c+ mDCs, we investigated subpopulations within the LCH lesions for the BRAF-V600E allele and found that, in addition to LCH CD1a+ and CD1a+/CD207+ DCs, BRAF-V600E was also identified in LCH lesional CD1c+ mDCs. Furthermore, HLA-DQB2, highly expressed in LCH CD1a+/CD207+ cells, was also expressed in LCH lesion CD1c+ cells, but not in any other lesion myeloid subpopulations. Furthermore, HLA-DQB2 was expressed only in peripheral blood of patients with active high-risk LCH, and HLA-DQB2+ CD1c+ blood cells were highly enriched for the BRAF-V600E allele.

Conclusion: These data support a model where blood CD1c+ mDCs with hyperactive ERK migrate to lesion sites and differentiate into LCH CD1a+/CD207+ cells. If differentiation from CD1c+ DCs to CD1a+/CD207+ cells is critical to LCH pathogenesis, blocking this process may represent a novel therapeutic opportunity.

High Prevalence of Lymphopenia in Langerhans Cell Histiocytosis: Correlation with Early Onset and More Severe Disease

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Purpose: Langerhans cell histiocytosis (LCH) is characterized by accumulation of immune cells in granulomas resulting in tissue destruction. The disease may be mild affecting a single system (most commonly bone or skin) or severe disseminated affecting at least two systems (multi-system), and it has been associated with genetic and immunological abnormalities. Since we often observed low lymphocyte counts in our LCH patients we decided to evaluate this in a systematic way and to investigate potential links to the disease pathogenesis.

Methods: Lymphocyte and monocyte counts at LCH diagnosis, at periods with and without treatment and correlation of the findings to disease category, age and organ involvement. Values in healthy children and adults served as controls.

Results: Immunophenotyping of blood cells in 19 patients showed lymphocyte counts below the lowest age-specific reference limit for 8/19 patients and close to the lowest limit for the rest. Decreased B-cell and NK-cell counts, as well as monocyte counts, were mainly observed in patients on treatment, but T-cell counts were significantly lower compared to controls regardless of treatment. Particularly affected of these abnormalities were patients with multi-system disease. To increase our study cohort, we reviewed the medical records of the LCH patients admitted in Astrid Lindgren Children's Hospital over a 15-year period. 7/40 treatment-naive patients at diagnosis were found to be lymphopenic and 4/7 had multi-system disease. 15/40 patients were lymphopenic at least once during periods without treatment and, most importantly, almost all patients had frequently lymphocyte counts close to the lowest age-specific reference limit even at periods without treatment.

Conclusion: We observed high prevalence of lymphopenia in LCH patients, associated with earlier disease onset and more severe disease course. This finding is likely related to LCH pathogenesis and may be a piece in the puzzle that may help improve understanding this intriguing disease.

Dramatic Efficacy of Dabrafenib in Langerhans Cell Histiocytosis Harboring the BRAF V600E Mutation: Two Cases Report

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Objective: To explore the effect of BRAF inhibitor Dabrafenib in Langerhans cell histiocytosis harboring the BRAF-V600E mutation.

Methods: After informed consent, In first patient, we executed the reduced intensity chemotherapy (Vindesine 3mg/m2, every two weeks for one times; oral Prednisone for 1 weeks, and 1 weeks off) combined BRAF inhibitor Dabrafenib oral treatment (50mg/d, twice). Serial 18-FDG PET-CT Scan was used to assess the efficacy and tolerability of the therapeutic regimen before and after treatment. In the second patient, we used single-agent Drabrafenib(50mg/d, twice) since the effect of chemotherapy was not obviously, and many complications such as bone marrow suppression, infection and gastrointestinal adverse reactions occurred in the treatment duration.

Results: In first case, Serial 18-FDG PET-CT scans after 5 months of treatment revealed marked improvement of the lymph nodes compared with the former, and the curative effect was significant. The detection of BRAF-V600E gene in this patient showed a markedly decrease in the abundance of mutation. In the second Case, there has been largely improved on rash, vulvar ulcer, lymph node, liver and spleen after 3 months treatment. The liver function and appetite of children were significantly higher than before.

Conclusion: The BRAF inhibitor Dabrafenib may become a potent approach either combined with reduced intensity of chemotherapy, or used as single-agent in refractory high-risk patient that harboring the BRAF V600E mutation. It may reduce the toxicity of chemotherapy and lower the risk of relapse.

Clinical Characteristics and Prognostic Factors of Epstein-Barr Virus-Associated Hemophagocytic Lymphohistiocytosis in Children

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Objective: To investigate the clinical features and prognostic factors of Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis (EBV-HLH) in children.

Methods: The clinical and laboratory data of children with EBV-HLH were retrospectively analyzed at a single institute in China from September 2015 to January 2017. Statistical analysis was performed using SPSS 20.0.

Results: A total of 76 cases of EBV-HLH were identified, accounting for 55.07% of HLH (138 cases), of which 4 cases were diagnosed as primary HLH. There were 31 boys and 45 girls in total and the median age of onset was 42 months. The clinical features of EBV-HLH were similar to other HLH, which were accompanied by multiple organ dysfunction. Only the differences of alanine aminotransferase and fibrinogen were statistically significant between 4 cases of primary HLH and cases without abnormal genetic changes ($P = 0.048, 0.043$). Compared with the state of remission, the median levels of serum ferritin (SF), serum EBV-DNA, soluble interleukin-2 receptor (sCD25), IFN-Î^3 and IL-10

during disease activity status and outbreaks were significantly increased (P<0.001). With median overall survival of 172 days, the overall survival rates in 1, 3, 6 and 12 months were 96.5%, 88.5%, 80.7% and 76.6%. Multivariate analysis showed the independent risk factors of poor prognosis included absolute neutrophil count (ANC)<0.5 × 109/L (HR = 0.200, 95% CI:0.031-0.789, P = 0.029), SF>2,000 ug/L (HR = 6.723, 95% CI:1.444-31.297, P = 0.015), serum EBV-DNA>1 × 105/ml (HR = 10.582, 95% CI:1.424-74.745, P = 0.011) and blood routine failed to recover in 2 weeks (HR = 9.681, 95%CI:1.249-35.034, P = 0.006).

Conclusion: Clinical characteristics and routine laboratory tests have little significance in telling whether EBV-HLH has potential primary causes. SF, sCD25, serum EBV-DNA and cytokine are sensitive indicators for monitoring HLH activity. Children with ANC<0.5 × 109/L, SF>2,000 ug/L, serum EBV-DNA>1 × 105/ml and blood routine failed to recover in 2 weeks are easier to continually uncontrolled or outbreak, and reactivate after complete remission or even cause death.

Langerhans Cell Histiocytosis in Adults Is Associated with Additional Solid and Hematologic Malignancies

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Purpose: The increased rate of additional malignancies in Langerhans cell histiocytosis (LCH) patients is a notable concern that has been previously observed. Reports in the literature have included mixed case reports of adults and children, or small case series focused on the pediatric LCH population. However, to our knowledge there has not been a large, single-institution study in consecutive adult LCH patients, nor a large recent study in the modern era after the widespread use of tumorigenic agents such as etoposide. Here we report our 25-year single institution experience of adult LCH patients with additional malignancies.

Methods: We identified 155 consecutive patients ≥18 years with histologically confirmed LCH (S100+, CD1a+) at our center between 1990–2015. Demographics and detailed oncologic history were recorded to identify patients with additional malignancies, excluding non-melanoma skin cancers. The Kaplan-Meier method was used to estimate overall survival.

Results: Of 155 adult LCH patients, 46 (30%) patients had an additional malignancy. Median age was 54 years (range 28–89) with a median follow-up of 3.7 years (0.1-22.2). Overall survival (OS) was 11.2 years, with 32 (70%) alive at last follow-up. There were a total of 61 non-LCH malignancies among the 46 patients, with 30 (49%) preceding LCH diagnosis, 10 concurrently (≤3 months; 22%), and 21 (46%) after. Ten patients presented with 2 malignancies in addition to their LCH diagnosis, and 2 patients presented with 2 malignancies. There were 45 solid tumors (74%), 9 lymphomas (15%), and 7 other hematologic malignancies (11%).

Conclusion: Our cohort of adult LCH patients demonstrates an exceptionally high number of additional malignancies, consistent with existing literature. However, our study includes predominantly malignancies diagnosed preceding or concurrent with LCH, suggesting a cause of malignancy independent of LCH treatment. Further exploration of the biology of this rare disease may elucidate the mechanism of increased additional malignancies.

Allogeneic Hematopoietic Stem Cell Transplantation Provides Cure for Adult Patients with Hemophagocytic Lymphohistiocytosis (HLH): A Retrospective Study of The Chronic Malignancies and Inborn Errors Working Parties (CMWP and IEWP) of The EBMT

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Purpose: Allogeneic stem cell transplantation (alloSCT) is indicated in familial, recurrent or progressive hemophagocytic lymphohistiocytosis (HLH; hemophagocytic syndrome). While data for alloSCT outcomes are available for the pediatric setting, information for adults is very limited.The aim of this study was to retrospectively analyze the information from the EBMT databases about adult HLH patients who underwent allogeneic stem cell transplantation.

Methods: We obtained data of 70 adult (≥18 years of age) patients transplanted due to HLH. Additionally, 33 responses from the clinical centers were received for an HLH-oriented questionnaire.

Results: Median age at transplantation was 28 (range:18-65). There was a slight male predominance 45/70 (64%). Reduced intensity conditioning was used in 22/67 (33%) of patients. The median survival time was 9.4months. The three year OS was 41% (95% CI 29–54%). For patients who survived until 3 months, this proportion was more favorable with an OS of 61% (95% CI 46–77%) at 3 years after transplantation. After 12 months no relapses of HLH were recorded : the cumulative incidence reached 15% (95% CI: 5–24%). The non-relapse mortality reached 35% (95% CI: 22–47%) after 15 months. Unlike the pediatric population, where reduced intensity conditioning (RIC) was associated with higher survival, in adult patients there was no difference between the conditioning types. Data from 33 questionnaires have confirmed clinical picture typical for HLH at the diagnosis: fever in 31/32 (97%), splenomegaly in 28/30 (93%), hemophagocytosis 26/30 (87%) and hyperferritinemia with median concentration of 6,102 ng/ml (range: 63–260,160). Gene with the most frequently found mutations was STXBP2 (6/15).

Conclusion: To our knowledge, this is the largest analyzed group of adult patients with HLH who underwent allogeneic stem cell transplantation. Relatively low relapse incidence confirms that alloSCT can effectively cure HLH. Patients who survive the first period after this procedure can expect a long disease-free survival.

HLH Diagnostic Criteria Evaluated on 83 Patients from The Polish HLH in Adults PALG Registry

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Purpose: The most widely used HLH-2004 criteria are frequently used for adults, but they were not validated in this group. Additionally, 2 out of these 8 criteria are rarely available (i.e. sCD25 concentration and NK-cell activity), what forces modification and inclusion of patients with 4 (instead of 5) criteria fulfilled when only 6 are tested and ferritin concentration exceeds 2,000 ng/ml. Recently a new alternative named HScore was proposed for adults. Diagnosis made above 169 out of 337 points and it does not require any specialized tests.

Methods: Data of patients from the Polish Registry of HLH in Adults (under the auspices of Polish Acute Leukemia Group) were analyzed. The median age of 83 patients was 38 years (range 18–82), with a 60% male predominance. Patients were diagnosed based on HLH-2004 criteria (including the above-mentioned modification) and a direct comparison of both diagnostic systems was made.

Results: All patients presented with hyperferritinemia (median 11,400 ng/ml) and some degree of cytopenia (with 75% fulfilling the duocytopenia criterion). Patients were almost uniformly febrile (99%; 82/83) and had splenoand/or hepatomegaly (93%; 77/83). In this group diagnosed with modified HLH-2004 criteria median HScore was 258. All patients were above threshold of 169 (range:169-337). It peaked in virus-associated HLH 286 ($p<0.01$; compared to the other triggering factors). Unexpectedly, hypertriglyceridemia (not hyperferritinemia) was responsible for the highest number of HScore points (50.2 vs 41.9; $p<0.0001$).

Conclusion: HLH should always be included in the differential diagnosis of febrile patients with organomegaly and cytopenia, especially when hyperferritinemia occurs. The use of HScore may allow diagnosis of more patients in an earlier stage than HLH-2004 criteria, but should be used with caution because it may also give more false positive results. Previous results of the patient should be analyzed to avoid including normal results among the HScore criteria.

Abdominal Findings in Erdheim-Chester Disease (ECD): MRI and CT Assessment on a Cohort of 61 Patients

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Purpose: To define variability of abdominal involvements in ECD as seen on Magnetic Resonance Imaging (MRI) and Computed Tomography (CT), and to investigate the correlation between BRAFV600E mutation and frequency of findings.

Methods: This single center prospective study was performed on 61 biopsy-proved ECD patients (46 men), who signed written informed consent. The MRI or CT images (45 MRI, 16 CT) were reviewed by two experienced radiologists in consensus. 58 patients had tissue samples available for BRAFV600 testing. The correlation between the mutations and frequency of involvements was analyzed using Fischer exact test; p-value ≤ 0.05 was considered significant.

Results: 52 patients had abdominal involvement, and 9 showed normal imaging findings. Perinephric fat involvement was the most common finding, seen in 41 (67%) patients. In 34 (56%) cases, perirenal involvement extended to renal sinuses. 37 (61%) patients had sheathing or stenosis of proximal ureters. Isolated calyceal ectasia and hydronephrosis were observed in 8 and 23 patients, respectively. 29 (48%) patients, had adrenal gland infiltration. Peri-vascular involvement was frequent (60%); described as sheathing or stenosis of renal artery (30 patients), periaortic infiltration (26 patients) and infiltration of other aortic branches (15 patients). Among patients with positive BRAFV600E results [54% (31/57)], significant positive correlation was found between the mutation and frequency of perinephric fat infiltration (p: 0.002), renal sinus involvement ($p<0.001$), sheathing or stenosis of proximal ureters ($p<0.001$), hydronephrosis ($p<0.001$), adrenal gland involvement ($p<0.001$), periaortic infiltration (p: 0.02), sheathing or stenosis of renal artery ($p<0.001$) and sheathing of other aortic branches (0.04).

Conclusion: Due to the high incidence and poor prognosis of retroperitoneal and abdominal peri-vascular involvements in ECD, abdominal MRI or CT imaging should be performed at the time of diagnosis and during disease monitoring. BRAFV600E mutation status could be helpful in evaluating frequency of involvements.

The Role of Regulatory T Cells in the Immune Regulation of Langerhans Cell Histiocytosis

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Purpose: Langerhans cell histiocytosis (LCH) is characterised by lesions containing CD1a+ and/or CD207+ dendritic cells as well as an inflammatory infiltrate including T cells. New treatment options are required for patients who remain unresponsive to current standards. FOXP3+ regulatory T cells (Tregs) are present in high numbers in LCH lesions (suggestive of an immune-suppressive environment), but the frequency and importance of other T cells with regulatory functions, such as gamma delta T cells, mucosal associated invariant T cells and type I natural killer T cells is for the most part not established. This project represents the most comprehensive analysis of unconventional T cells in patients with LCH.

Methods: We have analysed LCH tissue samples from blood and lesions stored in the Fiona Elsey Cancer Research Institute's Tissue Bank. Cells were characterized using 13-colour flow cytometry, and in vitro assays of T cell function.

Results: We report that the frequency and function of unconventional T cells are altered in patients with LCH compared to healthy donors.

Conclusion: These findings suggest that immune regulation is defective in LCH and that changes in these T cell subsets

may be important factors in LCH onset and progression. Targeting these factors could therefore be a promising avenue of investigation in the development of new immune based therapies.

Monocytopenia is Present in The Vast Majority of Hemophagocytic Lymphohistiocytosis (HLH) in Adults and Should be Used as a Sensitive Additional Diagnostic Criterion and its Response To Treatment Carries Poor Prognosis

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Background: HLH can cause life threatening cytopenia (s) and organ failure with very high mortality. Early diagnosis and management are essential for survival. The current HLH 2004 criteria are not optimal. Monocytopenia is not included in the diagnostic criteria although HLH is associated with activation, mobilization of and shift of monocytes to tissues where phagocytosis occurs.

Hypothesis and Goal: Monocytopenia could be a sign of "shift" and active tissue hemophagocytosis and treatment of HLH should induce reversal of monocytopenia.

Methodology: A retrospective analysis adults diagnosed by the hematology consult service at our institution and presented with fever and cytopenia(s) between August 1st, 2015 and June 30th, 2016 and were diagnosed with HLH based on at least 5 of 8 HLH2004- criteria. Moncytopenia was defined as the lowest absolute Monocytic count <0.2/ul within 2 weeks of diagnosis.

Results: A total of 29 patients (9 females) (16-82 Y) with diverse diagnoses. 27 (93%) were monocytopenic. 12 (41%) patients had active malignancies; the most common were lymphomas (7; 3 HD, 1 DLBCL, 1 Plasmablastic NHL, 1 T cell NHL and PTLPD). Four patients had myeloid malignancies (2 MDS, 1 AML after salvage chemotherapy with disseminated TB, and one with myelofibrosis). Six had transplantation (4 allo-, 1 Autologous HSCT, and 1 kidney transplantation presented with PTLPD), 1 ITP and AIHA (Evan's) presented with portal and superior mesenteric vein thrombosis, and one with Kostman Syndrome, 1 HIV on HAART therapy, 1 MethylMalonic Acidemia (MMA) and one with Adult Onset MAS. 2 patients had SLE and one had SCA misdiagnosed as hyperhemolysis or splenic sequestration. HLH accompanied the first presentation of all the 8 cases of lymphomas with (3) or without H1N1 (5) triggering. One patient had salvage chemotherapy for relapsing AML but failed to recover marrow was in remission but bone marrow culture.

H1N1 Virus Kills by Hemophagocytic Lymphohistiocytosis (HLH) and Immunosuppressive Therapy (IST) May Protect Against It in Non-Hematopoietic Stem Cell Transplant Patients

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Purpose: To study contribution of HLH to death after H1N1 infection in adults during an outbreak at a single institution and the impact of concurrent immunosuppressive therapy (IST) used in transplantation and other disorders on HLH.
Methodology: Retrospective data of adults with H1N1 presented to ER/OPC/ICU of KFSHRC in Riyadh, SA during an outbreak between August 20, 2015 and February 23, 2016. IST group were on one of the following drugs: CSA, Fk506, Mycophenolate or prednisone > 10mg/d or any combination. None-IST group was on no IST. H1N1 was tested via PCR of the nasopharyngeal swab or nasal or tracheal tube aspirates of the ventilated patients. Those with hematological manifestations were also followed prospectively. HLH was diagnosed based on HLH 2004 criteria.

Results: 136 patients (15-92y; median 37y) tested positive for H1N1 and presented mainly with flu/cough/fever/pneumonia. 25 patients were on IST: 10 allogeneic HSCT, 8 Solid organ transplants, and 7 had immune or allergic diseases (2 SLE, 2Br Asthma, 2Rheumatoid arthritis, and 1Crohn's. Total deaths = 7 (i.e. case fatality of H1N1 in this cohort was 7/136 or 5.1%). Seven (5.1%) patients developed HLH and an additional patient had suspicious HLH (fulfilled 4 criteria out of 4 tested included tissue biopsy). HLH occurred in 3 of the 10 Allo-HSCT on IST (30%); 2 died with HLH. None of the other patients who were on IST developed HLH or died (0% HLH and 0% Mortality). Of all the none IST group (n: 111), 4 fulfilled 5 criteria of HLH and 1 additional patient had suspicious HLH (4 criteria out of 4 tested) (4.5%). In none-IST group, 5 (4.5%) deaths occurred; 3 related to HLH and 2 were related to underlying disease or complication of H1N1 pneumonia. Additionally, 3 had life threatening HLH treated with steroids, IVIG and Etoposide and all recovered.

C-Reactive Protein and Bone Pain at Diagnosis Predict the Outcome of Pediatric Langerhans Cell Histiocytosis with Single-System Multifocal Lesions: Result of The Japan Langerhans Cell Histiocytosis Study Group-02 Protocol Study

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Background: Langerhans cell histiocytosis (LCH) with single-system (SS) multifocal bone (MFB) lesions is rarely fatal, but the patients may experience relapses and develop sequelae. The factors that associate with the post-treatment outcome of SS-MFB disease are not known.

Patients and Methods: We assessed the outcomes of all consecutive newly diagnosed pediatric patients with LCH with SS-MFB lesions who were treated with the Japan Langerhans Cell Histiocytosis Study Group (JLSG)-02 protocol in 2002–2009. The protocol consists of 6 weeks induction with cytarabine, vincristine, and prednisolone followed by maintenance therapy for 48 weeks. Events were defined as poor response to the induction therapy, relapse and any death.

Results: In total, 82 patients with a median follow-up duration of 8.0 years were eligible for analysis. At 6 weeks, 92.7% responded to the induction. However, 27.6% of the responders experienced relapses and 2.4% developed central diabetes insipidus. None of the patients died. The 5-year event-free survival (EFS) rate was 66.7%. Patients who had higher serum C-reactive protein (CRP) levels and bone pain at diagnosis had significantly lower EFS. Of 81 excluding one patient whose data was missing, 27 had normal CRP levels (≤0.3 mg/dL) and no bone pain at diagnosis (Group 1). Another 27 were either CRP-positive or had bone pain at diagnosis (Group 2). The remaining 27 were both CRP-positive and had bone pain (Group 3). Group 1 and Group 2 had similar average EFS (81.5% vs. 77.3%, $p = 0.810$). By contrast, Group 3 had a much lower average EFS (44.4%) than Group 1 and Group 2 ($p = 0.006$ and $p = 0.008$, respectively).

Conclusions: High serum CRP and bone pain at diagnosis were independent poor prognostic factors in pediatric patients with SS-MFB type LCH, who were treated with the JLSG-02 protocol.

Familial Hemophagocytic Lymphohistiocytosis: A Single-Center Experience

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Purpose: Primary hemophagocytic lymphohistiocytosis (HLH), also known as familial HLH (FHLH), is a very rare autosomal recessive immune disorder, characterized by hypercytokinemia and excessive T-cell and macrophage activation. Although it can develop at any age, the onset of the disease typically occurs within the first months or years of life.

Methods: We present three cases of FHLH diagnosed and treated in our center in the last twenty years.

Results: The first two patients were siblings with their parents coming from the same small Greek island. The first case was a 2.5-year-old girl that presented with anemia, thrombocytopenia, hepatosplenomegaly and fever. The bone marrow aspiration revealed high hypocellularity. The disease progressed rapidly and the child died within a few days after the diagnosis, before receiving chemotherapy. The second case was a 2.5-month-old boy that presented with pancytopenia, hepatosplenomegaly and sepsis. He was treated according to the HLH-94 protocol and four months after achieving remission, he underwent bone marrow transplantation (BMT) with an HLA-matched unrelated donor. Eventually, one month after the BMT, the disease relapsed and the child died. The third case was a 5-month-old girl from Syria with her parents being second cousins. She presented with fever, lymphadenopathy, pancytopenia and hepatosplenomegaly. She received treatment according to the HLH-94 protocol, she achieved remission but unfortunately, a compatible donor could not be found. The disease relapsed during the continuation therapy and the child died.

Conclusion: FHLH is a rare but potentially fatal disease, even after the BMT. It is characterized by a very rapid progression and thus more intensified treatment protocols are needed. Current treatment protocols achieve remission but recurrence can occur even during the continuation therapy. BMT prolongs event-free survival but does not increase the survival rate.

Evaluate the Outcome of HLH Treatment in Initial Therapy (8 Weeks) at National Children's Hospital

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Background: Hemophagocytic lymphohistocytosis (HLH) is characterized multisystem inflammation. HLH includes the great majority of patients with macrophage- relates disorders. The predominant clinical findings of HLH are fevers, hepatosplenomegaly and cytopenia. Other common findings include hypertriglyceridemia, coagulopathy with hypofibrinogemia, hepatitis, elevated levels of ferritin and

serum transaminases, and neurological symptoms. Survival as reported in the three largest reports on HLH from 5% (1- year) of Janka 1983 to 22% (5- year) of Arico 1996 and 55% (5- year) of HLH- 94 in 2002. The aim of this study was to evaluate the outcome after 8 weeks of initial therapy follow HLH-2004 protocol at National Children's Hospital, Vietnam.

Method: Retrospective study 53 patients with HLH characteristics were treated by initial therapy (8 weeks) of HLH-2004 protocol from Aug 2010 to Jul 2011. The patients were confirmed diagnosis HLH base on Henter 2004 of the Histocyte Society. Diagnostic criteria for HLH fulfilled (5 out of the 8 criteria): Fever, splenomegaly, cytopenias. Triglyceride ≥3.0 μmol/L and/or fibrinogen â‰¤ 1.5 g/L. Hemophagocytos is in bone marrow but no evidence of malignancy. Ferritin ≤ 500 μg/l, low or absent NK- cell activity, soluble CD 25 ≤ 2400 U/ml. HLH should be suspected in a patient when they have following signs and symptoms: hepatomegaly, lymphadenopathy, hyponatremia, hepatitis. Statistical analysis was performed with the SPSS program.

Results: The patients < 2 years old had 84.9% (< 1 year: 47.2%). The male to female ratio was 1,9: 1. There were 40/53 patients (75.5%) had EBV positive and 16/53 (30.2%) had CMV positive. 32.1% of patients had neurological signs such as convulsion 82.4%, cerebral nerve failure 23.5%. Respiratory failure had 37.7% and cardiac insufficiency was 41.5%. After 8 weeks of initial therapy, 22 patients (41.5%) achieved complete remission and continued therapy. In the first 2 months, mortality rate were 58.5% include 24 patients (45.3%) died from week 1–4 and 7 patients (13.2%) died from weeks 4- 8. The cause of deaths: multi-organs failure (68.8%), coagulopathy disorder (16.1%), septisemia (16.1%).

Conclusion: Children with HLH finished initial therapy of HLH- 2004 protocol were 41.5%. Our survival rate still lower than the other centers because delay treatment.

Clinical Presentation and Outcomes of Langerhans Cell Histiocytosis (LCH) in Yangon Children Hospital

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Purpose: Langerhans cell histiocytosis (LCH) is a rare disease, usually occurs in early age with varying clinical presentations. The aim of this study is to analyse the nature of the disease and outcomes of the treatment in our setting.

Methods: This was a retrospective study from 2012 to 2016 based on the medical records and imaging records of patients at the Hemato-Oncology Unit of Yangon Children Hospital.

Results: Twenty-nine patients were enrolled. There were 2 cases in 2012, 3 cases in 2013, 9 cases in 2014, 4 cases in 2015 and 11 cases in 2016. Male to female ratio was 1:2. Almost all were treated with LCH III treatment. According to their presentations, 4 (13.7%) had single system involvement, 9 (31%) had multi-system involvement and 16 (55.1%) had disease to the liver, spleen and haematopoietic system. Skin involvement, lytic lesions of the skull and hepatosplenomegaly occurred in almost all cases. Only 3 cases involved lymphadenopathy. 7 (24.1%) patients defaulted, 1 (3.4%) abandoned treatment, 11 (37.9%) were still on treatment, 7 (24.1%) were on follow up and 4 (13.7%), expired : 1 was due to drug toxicity, others - due to disease progression. There was only one case of LCH associated with diabetes insipidus (DI); the child had full recovery and was on regular follow up. In 7 follow up cases,1 case relapse and treated with relapse regimen. Patients on treatment received PO prednisolone, PO 6-mercaptopurine, and IV vinblastine.

Conclusions: Although LCH is a rare disease, we admitted 29 patients in 5 years. They presented with various symptoms. Almost all cases involved the bone, skin and extramedullary haemopoietic system. Even though there were defaulters due to transportation issues, achieving remission is possible.

Diabetes Insipidus with Decreasing Pituitary Stalk Widening but Metachronous Skull LCH Lesions

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Purpose: Diabetes insipidus (DI) can be the sole presenting sing of Langerhans cell histiocytosis (LCH), associated with hypothalamic/pituitary lesions that increase size wise, even with isolated/localized disease. We present a patient with DI and regressing pituitary lesion, whilst new LCH bone-disease evolved.

Presentation: A 2.5 year-old boy presented with polyuria/polydipsia of acute onset. DI diagnosis was established after a fluid deprivation and desmopressin substitution commenced. Thyroid function, cortisol, prolactin and IGF-I levels were normal. MRI demonstrated increased size of the pituitary (height of 6.5 mm), with superior protuberance. Additionally focal thickening of the pituitary stalk was noticed (3.4mm). The bright spot of the neurohypophysis was absent. Detailed evaluation for LCH and germ-cell tumor, including skeletal survey and serum/cerebrospinal fluid a-FP and hCG was negative. A suspicious

radiolucent right femoral lesion was biopsied and proved negative.

Follow-up: A 3-month follow-up MRI demonstrated normalization of the pituitary size (height of 2.4 mm), with homogeneous contrast uptake. In parallel, the desmopressin dose was decreased. Spinal MRI was normal. Three months later though, a soft, jellatinous, painless 2.5 × 2.5 cm lesion of the medial occipital area appeared, presenting features of an osteolytic lesion with mild contrast uptake on MRI. The lesion was curettaged with intralesional steroid infusion. Pathology was compatible with LCH (S100/CD1a/Langerin positive), no BRAF-V600E mutation was detected. Full disease reevaluation including a PET-CT scan proved negative, with further decreasing, normal appearing pituitary stalk (2 mm). With two system, low-risk disease the patient started treatment on LCH-IV Protocol (Stratum-I), with appropriate initial response.

Conclusion: Spontaneous regression of the pituitary lesion was seen in parallel with metachronous skull bone lesion evolution in a young BRAF-V600E negative LCH patient. End organ LCH lesions can have different timing and pacing of regression/ evolution. Careful monitoring and evaluation of the patients guides appropriate management and eventually cure.

Higher Metabolic Activity Seen on 18F-FDG PET/CT, in the Adrenal Glands of Patients with Erdheim-Chester Disease Harboring the BRAF V600E Mutation

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Purpose: ECD is known to affect several organs, including the adrenal glands. Aim of the current study was to investigate potential association between adrenal metabolic activity and the BRAF V600E mutation status.

Methods: Fifteen ECD patients (mean age at first ECD-manifestations: 53y.o.) were evaluated with whole-body 18F-FDG-PET/CT studies. Eleven patients harbored the BRAF V600E mutation, 4 were BRAF-negative, while in one patient the mutation status was not tested. PET-acquisition commenced 60 minutes after intravenous administration of 10-to-11mCi of 18F-FDG, while a non-contrast, low dose CT scan was performed for attenuation correction and co-registration. Metabolism in the adrenals was assessed by quantifying 18F-FDG uptake, using the MIM Vista workstation (version 6.5.9). A VOI encompassing both adrenal glands was drawn, and an automated SUVmax threshold-based approach was applied in order to include all 18F-FDG-avid regions of the adrenals, while excluding low-level background activity. The software enables automatic generation of separate VOIs encircling all areas above the SUVmax threshold set by the user (SUVmax threshold was set at 2). Afterwards, the following parameters were automatically obtained: SUVmax, SUVmean (average SUV), total 18F-FDG-avid adrenal volume (TV) and total glycolytic activity (TGA) of the adrenals, determined as the summation of the activity of each individual 18F-FDG-positive adrenal region, which is the product of each region's volume multiplied by its' SUVmean respectively. Finally, statistical analysis was performed using R software (version 3.3.3).

Results: Mann Whitney test revealed statistical differences ($p<0.05$) in TV and TGA values between BRAF-positive and BRAF-negative ECD patients, with mutation carriers showing significantly higher mean TV (11.33 vs 3.2) and TGA values (29.3 vs 7.58).

Conclusion: ECD patients who harbor the BRAF V600E mutation have hypermetabolic adrenals when compared to mutation-negative counterparts, implying increased susceptibility of BRAF-positive ECD-patients to adrenal involvement and potentially adrenal insufficiency.

A Genome-Wide Association Study Identifies Candidate Variants Associated with Increased Risk of LCH Relapse

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Purpose: Advances in treatment for Langerhans cell histiocytosis (LCH) have resulted in survival rates approaching 90% for high-risk cases; however, up-front chemotherapy fails to achieve cure for approximately 50% of all cases. While somatic BRAF-V600E mutation is associated with a 2-fold increased risk of relapse, other factors may also influence treatment failure. As treatment responsiveness is influenced by inherited genetic variation (IGV) in other malignancies, we hypothesize that IGV in LCH may also be impact outcomes for LCH patients. We therefore conducted a genome-wide association study (GWAS) to characterize the role of inherited genetic variants on LCH relapse.

Methods: LCH cases (n = 117) were recruited from Texas Children's Hospital, of which 52 patients experienced

relapsed LCH (44%). Genotyping was performed on the Illumina Omni5 Quad BeadChip. We evaluated the role of common variants (minor allele frequency >5%) on LCH relapse using multivariable logistic regression, adjusting for age at diagnosis, sex, and the top two principal components accounting for 81.67% and 3.01% of variation, respectively. We applied a significance level of $P<1.0 \times 10–5$, and all statistical analyses were conducted using PLINK.

Results: We identified a cluster of loci within an intron of the non-coding RNA gene LOC100506532 on chromosome 9 associated with LCH relapse (top hit odds ratio = 0.16; 95% confidence interval: 0.07-0.35; $P = 6.31 \times 10–6$). All hits within the cluster were $P<1.0 \times 10–5$.

Conclusion: In this genome-wide assessment of inherited genetic variation and LCH relapse, we identified a genomic region that may be associated with LCH relapse. While it is necessary to validate these findings in an independent replication set, our results provide initial evidence to suggest inherited risk factors influence LCH disease severity, and may have the potential to be used in clinical risk stratification strategies.

Evaluation of Maternal and Perinatal Characteristics and Risk of Langerhans Cell Histiocytosis in Texas, 1995–2011

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Purpose: Langerhans cell histiocytosis (LCH) is a myeloid neoplasia with a median diagnosis age of 30 months. In studies of pediatric malignancies, maternal and perinatal characteristics have been successfully evaluated to determine the impact of inborn variation on disease risk. We have reviewed registry data to determine if maternal and perinatal characteristics influence LCH development.

Methods: Information on Texas-born LCH cases (n = 164) for the period 1995–2011 was obtained from the United States (U.S.) Texas Cancer Registry. Birth certificate controls were randomly selected at a ratio of 10:1 for the same period matched on birth year. Unconditional logistic regression was used to generate adjusted odds ratios (aOR) with 95% confidence intervals (CI).

Results: Our findings indicate specific characteristics influence LCH risk. Non-Hispanic Black mothers were suggested as less likely to give birth to offspring who developed LCH compared to non-Hispanic White (NHW) mothers (aOR: 0.50; 95% CI: 0.24-1.03). Hispanic mothers were at increased risk of giving birth to offspring who developed LCH compared to NHW mothers (aOR: 1.58; 95% CI: 1.07-2.35). Children born from two Hispanic parents experienced an increase in LCH risk compared to children born from two NHW parents (aOR: 1.83; 95% CI: 1.15-2.90). Mothers born in Mexico versus the U.S. were suggested as less likely to give birth to offspring who developed LCH (aOR: 0.66; 95% CI: 0.41-1.07). Mothers who resided along the U.S.-Mexico border at time of infant birth were less likely to give birth to offspring who developed LCH (aOR: 0.54; 95% CI: 0.29-0.98).

Conclusion: Maternal and parental race/ethnicity were strongly associated with LCH risk. Further, mothers who resided along the U.S.-Mexico border at time of infant birth, and Mexico-born mothers, were less likely to give birth to offspring who developed LCH. These findings highlight novel risk factors that warrant assessment in future studies.

Effective Second Line Treatment with Cytarabine in a Patient with Refractory Multisystem Langerhans Cell Histiocytosis (LCH) Complicated with Macrophage Activation Syndrome (HLH-MAS)

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Purpose: To present the use of intermediate dose Cytarabine-only as a second-line treatment in a child with refractory multisystem LCH.

Methods: Single case presentation.

Results: A 12-month-old male toddler was admitted to our hospital due to prolonged febrile episodes, pancytopenia and hepatosplenomegaly with significant abdominal distention. Scalp, retroauricular and abdominal skin rash was noticed. Biopsies of the scalp and abdominal skin were performed and established the diagnosis of LCH, positive for BRAF-V600E by PCR. Further clinical, laboratory and imaging studies did not reveal lymphadenopathy, definitive bone disease, lung infiltration, brain/pituitary infiltration or diabetes insipidus. Bone marrow (BM) biopsy revealed decrease cellularity, heterogeneous hemophagocytosis and rare CD1a/Langerin+ cells, negative for BRAF-V600E by PCR. The IC-1 course (Vinblastine/Prednisone) of LCH-IV protocol (Histiocyte Society) was started. Skin cleared; there was very mild improvement of the hepatosplenomegaly, while the pancytopenia and febrile courses remained. Due to the persisted hemophagocytosis in BM and the clinical suggestion of HLH-MAS he was started on HLH-2004 protocol with dexamethasone/etoposide/cyclosporine for 4 weeks, with no significant improvement. Due to refractoriness of LCH, he was started

on second line treatment with Cytarabine monotherapy at 500mg/m2 q12 hours for 10 doses every 4 weeks. The patient showed clinical improvement after the first course, with gradual recovery of the pancytopenia and the hemophagocytosis and attained clinical remission (CR) after the 2nd course. Clinical, laboratory and imaging reevaluation after 3rd and 5th course (including PET-CT) confirmed remission. The patient received a total of 5 well-tolerated courses of Cytarabine monotherapy, and afterwards he was started on continuation treatment (Part2) with Vinblastine/Prednisone/6-Mercaptopurine/Methotrexate according to LCH-IV protocol. He is in CR and good clinical status on 4 months of Part2 treatment.

Conclusion: Intensive Cytarabine monotherapy could be a successful and well-tolerated second line treatment for patients with refractory multisystem LCH and concurrent HLH-MAS.

A Decade with Hemophagocytic Lymphohistiocytosis(HLH)- A Single Centre Experience From India

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Purpose: Isolated case reports of HLH from India have been published for the last 20–25 years. We, in our hospital diagnosed the first case of Infection associated HLH(IAHLH) in 2007 and the first case of Familial HLH(FHL) in 2012.We analyzed the clinical and laboratory characteristics of all the diagnosed HLH cases from our center for the last 10 years and tried to come to a conclusion.

Method: Clinical records of children fulfilling the 2004 diagnostic criteria of HLH admitted at Institute of Child Health, Kolkata during the time period of November 2016 to April 2017 were reviewed. Clinical and laboratory parameters along with treatment and outcome have been noted down.

Result: A total of 105 cases has been identified as HLH(Male 63,female 42) with the frequency being 0.3/1000 hospital admission. The age of presentation varies from 32 days to 15 years with a median age of 39 months. Among these 105 cases of HLH, IAHLH were 93 where FHL being 7 and HLH associated with immunedeficiency(HLHAI) were 5. Highest number of patients were found in 2012(24) and lowest number in 2015(7).Dengue was the most common infectious agent identified that caused HLH followed by EBV and Salmonella. Most of the patients of IAHLH has been treated with steroid only protocol with a survival rate of 82%, where FHL and HLHAI had a mortality of 100% despite combined chemotherapy and IVIg.

Conclusions: 1. IAHLH has a favorable outcome with minimal immunesupressive therapy. Risk stratification scoring and risk guided individualized therapy is helpful. 2. FHL and HLHAI are fatal. 3. The number IAHLH has been decreased (possible due to identification of lots of rickettsia associated HLH that has been successfully treated with antibiotics alone) and number of FHL and HLHAI have been increased(possibly due to better identification).

Unmanipulated Haploidentical Hematopoietic Stem Cell Transplantation Using Reduced-Intensity Conditioning for Paediatric Patients with Familial Hemophagocytic Lymphohistiocytosis: A Single Center Study

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Reduced-intensity conditioning (RIC) based unmanipulated haploidentical (HID) hematopoietic stem cell transplantation (HSCT) in the treatment of paediatric familial hemophagocytic lymphohistiocytosis (FHLH) is rarely reported. We conducted a retrospective study of five patients including three of PRF1, two of XIAP. Four of five donors have heterozygous mutation. Conditioning regimen was fludarabine/ cyclophosphamide/ antithymocyte globulin with or without low-dose irradiation. Mobilised marrow and blood stem cells were used as the grafts. All five patients achieved engraftment. All patients have been alive and achieved complete remission (CR) without any serious regimen-related toxicity with a median of 12 months follow-up time (range, 6–18 months) after HSCT. Four of five patients have mixed chimerism ranging from 17% to 87% but remain free of disease. One patient Loss of donor chimerism to 1% and relapsed, and no improvement in donor chimerism was seen following DLI. He underwent stem cell boosts, the donor chimerism increased to 99% and achieved CR2. Three patients developed acute graft-versus-host disease (GVHD) I~II°. One patients developed IV° GVHD after DLI. The HID HSCT with RIC regimen is an effective treatment for patients with FHLH. The safety of this regimen requires long-term follow-up and more prospective studies.

Prediction Models in Hemophagocytic Lymphohistiocytosis: Do We Need More?

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Purpose: Hemophagocytic Lymphohistiocytosis (HLH) comprises a spectrum of conditions characterised by hyper-inflammation triggered by infection, malignancies or

autoimmunity. HLH is still diagnosed based on criteria that were suggested more than a decade ago. There are hardly any markers that would help the clinician differentiate primary HLH which would need immunosuppression and hematopoietic stem cell transplant from "secondary" HLH triggered by easily treatable infections. Various prognostic scores have been evaluated in HLH addressing various aspects of the disease and its management. We evaluated all the prognostic scores studied in HLH as part of the present study.

Methods: A systematic search of Pubmed/Pubmed-central/Google-scholar databases were made for keywords containing HLH and scoring. All English language articles published till 30th April 2017 were included.

Results: 14 studies satisfied the criteria and were involved in the analysis. Prognostic scores or prediction models were identified in 8 of 14 studies. In HLH, scoring systems have been developed to address three issues in diagnosis and management. Firstly, in order to hasten the investigations including bone marrow a score was created called BM score. Leukoerythroblastosis on blood smear was found to be significant. The second question was if primary could be differentiated from secondary HLH. The HS score was developed for this purpose and was subsequently validated. The prognostic aspect of HLH or the treatment was been barely evaluated on the other hand. This was addressed by a score prepared by the same author which is presently being completed. Coagulopathy and raised ferritin were also noted to be prognostic of poor outcome in HLH in several studies.

Conclusion: There are few scores to address various ambiguities of the disease. The need of the hour would be a collaborative data on the disease profile and biomarkers at onset that can give better discriminative and prognostic information.

Autoimmunological Disorders, Neoplasms, and Mycobacterial Infections in Patients with PLCH

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Purpose: Pulmonary Langerhans cell histiocytosis (PLCH) is a disease, which can potentially lead to the development of cancer, autoimmunological diseases, and opportunistic infections. The aim of this study is to present the frequency of these conditions. Material and

Methods: Over a period from 2000 to 2015, 90 patients with PLCH were admitted to our Department. The mean age of patients was 35.78 ± 13.24 years, and 97% of them were cigarette smokers. The mean observation time was 62.9 ± 48.61 months.

Results: Three patients (3%) developed neoplastic diseases. One, 58-year-old man with regression of pulmonary lesions was diagnosed with cholangiocarcinoma after 5 years of observation, a 52-years-old woman developed chronic myelogenous leukaemia after 5 courses of cladribine treatment, and one woman 26-years-old had ovarian hamartoma, 4 years after chemotherapy. Systemic lupus erythematous was noticed in a 36-year-old man 2 years after chemotherapy, a 48-year-old woman was diagnosed at the same time with PLCH and biliary cirrhosis, and two men with psoriasis. Three (3.3%) patients had pulmonary mycobacteriosis, one patient Pneumocystis jiroveci pneumonia, and 9(10%) patients had frequent severe pulmonary infections. All patients, who experienced frequent respiratory infections were treated with corticosteroids.

Conclusions: Despite that, patients with PLCH were smokers, the non-smoking related malignancies were observed. Autoimmunological disorders were more frequently noticed than in general population. Severe pulmonary infections were mainly observed in patients who were under immunosuppressive treatment.

Risk Factors for Diabetes Insipidus in Pediatric Patients with Langerhans Cell Histiocytosis Treated with Cytarabine-Based Chemotherapy: Results of the JLSG-96/02 Study

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Purpose: Central diabetes insipidus (CDI) is the most frequent central nerve system (CNS) related permanent consequence in children with Langerhans Cell Histiocytosis (LCH). The lesions at the craniofacial bones, CNS, ear, and eye are defined as "CNS-risk lesions" in the development of CDI. However, the therapeutic effect on the development of CDI in relevance to "CNS-risk lesions" remains unknown. We tested if the development of CDI related to "CNS-risk lesions," is modified after treatment. Other factors affecting the post-treatment development of CDI, such as age, risk organ and relapse were also examined.

Methods: We conducted a retrospective analysis of total 307 patients (111 patients with multifocal bone (MFB) and 196 with multisystem (MS)) treated with JLSG-96/02 protocol from 1996 to 2009. Regimens of Japan LCH Study Group (JLSG) protocols contained Cytarabine, vincristine and

prednisolone. Statistical analysis was made by chi-square and Kaplan-Meier method with log-rank test. Cox proportion hazards regression was used for multivariate analysis.

Results: Overall 49 of 307 patients (MFB: 3, MS: 46) had CDI with median follow-up of 10.9 (range 1.9 : 21.1) years. Twenty five of the 49 CDIs were present already at the time of LCH diagnosis while 21 newly developed after treatment. "CNS-risk lesions" were positively correlated in the pretreatment CDI cases (19/25; p = 0.0247), but not in the post-treatment CDI cases (12/21; p = 0.49). In the latter group, relapse was a significant risk factor of CDI development (No 6/117 vs. Yes 15/53, p<0.001). For multivariate analysis, relapse was the only significant factor associated with CDI development (hazard ratio 7.80, 95%CI, 2.82-21.56, p<0.001).

Conclusions: After treatment with JLSG-96/02 protocols, "CNS-risk lesions" were no more a risk factor and relapse became a new risk factor in the development of CDI. Thus, we suppose that Cytarabine based- chemotherapy abolished the correlation of "CNS-risk lesions" and CDI development.

Clinical Features and Therapeutic Results in a 35-Year Cohort of Children and Adults with Langerhans Cell Histiocytosis Managed at a Single Institution

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Purpose: The aim of this study was to analyze clinical features and therapeutic results in children and adults with Langerhans cell histiocytosis (LCH) according to different disease classifications.

Methods: One-hundred-twenty-two patients (median age: 26.4 years) with LCH were considered. Fifty-one patients (19 children and 32 adults) were treated according to the same protocols (AIEOP 83, AIEOP-IX 89, LCH-I, LCH-II) from 1981 to 2000; 54 adults and 17 children were treated according to the GIMEMA LCH 2001 guidelines and to the LCH-III and LCH IV studies, respectively, from 2001 to 2015. Patients were divided according to: a) the Histiocyte Society criteria (two groups: single system, SS-LCH, and multisistem,MS-LCH); b) a disease score utilized in the GIMEMA LCH 2001 guidelines: Group 1 (unifocal SS), Group 2 (multifocal MS), Group 3 (MS without bone involvement), Group 4 (MS with bone involvement); Group 5 (honey combing pulmonary involvement).

Results: A single-site involvement was recorded in the majority of children (58%), while a multi-system involvement was more frequent in adults (54%). Children with SS-LCH had a significantly better response than those with MS-LCH (95% vs 67%, p = 0.023), while there was no difference between subgroups when the GIMEMA LCH 2001 guidelines score was utilized. Group 1 and Group 4 adults of have a significantly higher response rate than those of other groups (72% vs 38%, p = 0.001), but no difference was observed between patients with SS-LCH and those with MS-LCH. The overall progression-free survival (PFS) at 200 months was significantly better in SS-LCH patients compared to those with MS-LCH (48% vs 31%, p = 0.02). No difference in PFS was observed in adults, while children with SS-LCH showed a significantly better PFR compared to those with MS-LCH (83.5% vs 37.5%, p = 0.002).

Conclusion: Our experience suggests that the HS criteria developed for children could be not appropriate for LCH adults.

Notch Signaling Induces a Langerhans Cell Histiocytosis Gene Expression Signature in Human Monocytes

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Langerhans cell histiocytosis (LCH) is a histiocytic disorder characterized by the accumulation of CD1a+ langerin+ cells of unknown origin in different tissues. We have previously shown that Notch signaling is active in LCH lesions and that stimulation of the Notch pathway can induce CD1a and langerin expression in human CD14+ monocytes. Here we provide evidence that Notch signaling induces primary human CD14+ monocytes to acquire an LCH gene signature in vitro. In contrast, langerin+ cells derived in vitro from CD1c+ DCs or IL4-stimulated CD14+ monocytes differ in their gene expression signature from primary LCH cells. Inhibition of Notch signaling using a gamma secretase inhibitor abrogated the capacity of CD14+ monocytes to differentiate into langerin+ cells, whereas CD1c+ DCs were not affected. Additionally, JAG2 enhances the promoter activity of a RBPJ luciferase gene reporter construct thereby demonstrating Notch pathway activation in CD14+ monocytes. Chromatin immunoprecipitation using antibodies against RBPJ and histion marks for chromatin activation (H3K4me3) and repression (H3K27me) revealed that promoter regions of bone fide Notch target genes were bound by RBPJ and showed enriched binding of H3K4me3 upon differentiation of CD14+ monocytes towards CD1a+ langerin+ cells, while at the same time binding of H3K27me decreases. These data lead us to propose a model

in which CD14+ monocytes are the precursors of the LCH cells and in which JAG2 mediated activation of the Notch pathway initiates a differentiation of monocytes towards LCH cells in selected niches and thus contributes to LCH pathogenesis.

PCR-Based Detection of Langerhans Cell Histiocytosis (LCH) Molecular Signal in Cell-Free DNA from Patient Cerebrospinal Fluids (CSF)

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Purpose: Langerhans cell histiocytosis (LCH) is an orphan disease that predominantly affects children. LCH is difficult to diagnose due to non-specific presenting symptoms. However, early diagnosis is critical for effective treatment, particularly if disease involves the central nervous system (CNS). Recently, the presence of tumor cell-free DNA (cfDNA) in blood and cerebrospinal fluid (CSF) has been used for the detection of malignancies, including those affecting the CNS. Since most LCH lesions carry a characteristic BRAF V600E (c.1799T>A) mutation, we examined the feasibility of detecting this mutation using CSF-derived cfDNA.

Methods: With local REB-approval and informed consent, CSF was collected from two pediatric patients with known LCH. The CSF was centrifuged and pelleted cells were used for whole exome sequencing (WES) and cfDNA was isolated from the supernatant (2 mL) using a modified protocol and the QIAmp Circulating Nucleic Acid kit (Qiagen). Exon 15 of BRAF containing the c.1799T>A mutation was amplified using isolated cfDNA and a modified PCR protocol. Purified DNA products were Sanger sequenced.

Results: Purified cfDNA was successfully isolated from human CSF (0.15 and 0.16 ng/ÂμL for samples 1 and 2, respectively). Our modified PCR protocol successfully identified the c.1799T>A mutation in both samples. WES did not detect the mutation in the pelleted cells despite coverage depth >1100 reads.

Conclusion: Pure cfDNA can be successfully isolated from small volumes of CSF obtained from children with LCH. A modified PCR protocol successfully detected the c.1799T>A mutation in both CSF cfDNA samples while standard WES of the cellular material did not. Our protocol represents a potential liquid biopsy for the diagnosis of LCH affecting the CNS.

Clinical Features and Prognostic Significance of Liver Involvement in Patients with Langerhans Cell Histiocytosis

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Objective: To analyze the clinical manifestations, treatment and prognosis of LCH patients with liver involvement.

Methods: We conducted a retrospective study to evaluate the clinical datas of 110 LCH patients. From April 2011 to April 2015, newly diagnosed patients with histopathologically confirmed LCH were enrolled in this study. The patients were classified into two groups according to whether existed liver involvement. The clinical manifestations and efficacy of treatment were compared between two groups. The patients were followed up for 1–5 years, and the overall survival and disease-free survival were analyzed by log-rank test.

Results: The median age at diagnose was 5 years (range 2–204 months). Among them, there were 68 cases of male and 42 cases of female patients. The symptoms of 43 cases of hepatic LCH included with liver biochemistry abnormalities (16), hepatomegaly (22),liver biochemistry abnormalities(5).16 cases were diagnosed as sclerosing cholangitis by abdominal CT scan or liver biopsy. Standard chemotherapy protocol was used in all the patients, however, salvage therapy were used in 20 cases who were poor response to the standard treatment. The total effective rate of treatment was 77.3%, and the effective rate was about 51.2% in hepatic LCH patients. Liver transplantation was performed in 4 patients and 5 patients died during the follow-up.

Conclusion: There is a higher incidence of liver involvement in children LCH, and clinical prognosis of LCH with liver involvement is poor. The effect of regular conventional chemotherapy on hepatic LCH is not obviously and the salvage therapy should be considered seriously with the treatment related toxicity and side effects. Once LCH with sclerosing cholangitis was diagnosed, we can only improve the survival of patients through liver transplantation at the early timing.

A Novel CTL-Based Functional Assay Reveals a Strong Correlation between the Pathogenic of an UNC13D Variant and the Instability of its Translated MUNC13-4 Protein: MUNC13-4 Protein Expression Assay is a Reliable Method for Identification of Patients

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Purpose: Familial hemophagocytic lymphohistiocytosis (FHL) is a fatal syndrome of immune dysregulation and hyper-inflammation. Biallelic loss-of-function mutations in UNC13D gene encoding Munc13-4 protein cause FHL type 3 (FHL3). All the reported FHL3 cases evaluated for protein expression levels are shown to have significant reduction of Munc13-4 protein expression levels regardless of types of UNC13D mutations. We have previously reported that FHL3 can be rapidly screened by detecting intraplatelet Munc13-4 expression, but its reliability has not been evaluated. The purpose of this study is to elucidate the pathogenicity of a given UNC13D mutant and to evaluate the reliability of Munc13-4 expression assay as a FHL3 screening method.

Methods: We first determined the effectiveness of intraplatelet Munc13-4 expression assay by summarizing the result of FHL screening performed at our laboratory from 2011 to 2016. Next, we picked up 13 reported pathogenic missense UNC13D mutations and evaluated their influence on Munc13-4 protein expression, as well as on degranulation and cytotoxic function by transiently expressing cDNA constructs in human FHL3 model cell lines.

Results: Munc13-4 protein expression levels of the 14 FHL3 patients diagnosed at our laboratory were all significantly reduced regardless of their types of mutation, and flow cytometric detection of intraplatelet Munc13-4 protein identified these patients with high sensitivity and specificity. Eleven out of 13 reported UNC13D missense mutations caused significant reduction of Munc13-4 protein expression and functional defects in the transfected cell lines. The remaining two reportedly pathogenic UNC13D mutants did not cause reduction of Munc13-4 protein expression. Moreover, the degranulation and cytotoxic function of model cell lines transfected with these mutants were normal.

Conclusion: The pathogenicity of an UMC13D variant strongly correlates with the instability of its translated Munc13-4 protein, and FHL3 patients are likely amenable to rapid detection by Munc13-4 expression assay.

Characterization of a Large UNC13D Gene Duplication in a Patient with Familial Hemophagocytic Lymphohistiocytosis Type 3

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Purpose: Familial hemophagocytic lymphohistiocytosis (FHL) type 3 accounts for 30 to 40% of all FHL cases. More than 100 mutations in UNC13D gene throughout the 32 exons have been described to date and those affecting mRNA splicing are the most common molecular defect. The purpose of this study is to present the first case of FHL3 carrying intragenic duplication of UNC13D gene.

Methods: A one-month old boy with positive family history of hemophagocytic lymphohistiocytosis had a defect in NK cell degranulation and absent Munc13-4 protein expression. Conventional sequencing of genomic DNA demonstrated a heterozygous deep intronic mutation: c.118-308C>T derived from his father, while no mutation in UNC13D gene was identified in maternal allele. We therefore analyzed complementary DNA (cDNA) to identify an additional aberration in UNC13D gene.

Results: PCR detected an insertion, approximately 500 base pairs in length, between exon 6 and 17 of UNC13D gene transcript. PCR amplification using a forward primer in exon 11 and a reverse primer in exon 9 produced an abnormal product in the mother and the patient. Sequencing of the product revealed that exon 12 was followed by exon 7. We amplified patient's genomic DNA using a forward primer in exon 11 and a reverse primer in exon 8 and obtained a 2.5kbp long product. Direct sequencing of the product revealed that 5'-end of intron 12 was fused with 3'-end of exon 7 sharing 23bp homologous sequences within an AluSx element in intron 6 and an AluSz element in intron 12.

Conclusion: The first FHL3 case with intragenic duplication of the UNC13D gene is reported. We propose that a screening for FHL3 with NK cell degranulation and Munc13-4 protein expression assays are useful not to overlook these patients.

Pattern of Bone Recurrence in Pediatric Langerhans Cell Histiocytosis

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Introduction: Bone is the most frequent recurrent site in patients with Langerhans cell histiocytosis (LCH). To describe characteristic patterns of bone recurrence, clinical course of the relapsed cases were analyzed.

Patients and Methods: A retrospective review was conducted for the 70 children with LCH treated at our institution between 2002 and 2017. Among these cases, ten (5 males and 5 females) with bone involvement both at diagnosis and relapse and with full imaging studies were analyzed.

Results: Median age at diagnosis was 2.0 years (0.8-10.8) and median follow-up time was 7.5 years (5.2-12.8). Five cases initially presented with multi-focal bone involvement, and 5 with multisystem disease including one with risk organ involvement. Seven cases experienced multiple recurrences ranging 3 to 6 times. Median period from initial diagnosis to the last relapse was 4.3 years (0.8-6.4), and median follow-up time from the last relapse was 4.0 years (0.4-8.5). Number of bone relapse had reached to 28 in total; twelve solitary and sixteen multifocal, for which the sites were variable. Four cases relapsed at the same bone; one at the same mastoid bone10 months after the therapy completion. Another three relapsed after 3 to 7 year interval in the same bone (vertebrae, femur and scapula), however all of the recurrent sites changed their position subtlely from its original sites.

Discussion and Conclusion: Recurrence of LCH occurs in various places and quite unpredictably, even after a long interval. As presented in our cases, relapses may occur at the same bone, but often with site changes. Such features may derive from the fact that LCH is a neoplasm of myeloid origin that could change its lesions anywhere. Thus, therapy and follow-up aiming a localized control does not fit for the patients with multifocal LCH, rather a systemic approach is mandatory.

Real-World Outcomes of Treatment for Adult Hemophagocytic Lymphohistiocytosis: Retrospective Study of 148 Patients Over 8 Years in a Tertiary Hospital in China

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Purpose: Hemophagocytic lymphohistiocytosis (HLH) is an increasingly recognized hyperinflammatory disorder in adults, with high morbidity and mortality. There is currently no standard treatment for adult HLH patients. HLH-94 and HLH-2004 protocols, which have been developed for pediatric HLH, are widely used in practice for adult HLH. However, the efficacy and toxicities of these protocols have not been strictly evaluated and the relevant reports are lacking. Our study aims to investigate the effects of different protocols on the clinical outcomes of adult HLH patients in a real-world setting.

Methods: We performed a retrospective study in the largest tertiary hospital in Southwestern China over an 8-year period. We identified 148 adult patients fulfilling the HLH-04 diagnostic criteria, and analyzed etiology, clinical features, treatment regimens, and clinical outcomes.

Results: The average age was 35 years (16-83 years) and 89 patients were male. Underlying diseases included malignancy (66.2%), infection (18.2%), autoimmune disease (1.4%), iatrogenic disease (0.7%), and etiopathic HLH (13.5%). Patients were treated with HLH-94 based regimen (25.7%), HLH-2004 based regimen (24.3%), and other therapies (physician choice, 50.0%) respectively. The HLH-94 regimen achieved better response rate (68.4%, $P = 0.001$), as compared with HLH-2004 (33.3%) and other therapies (34.7%), and showed less toxicity. The median overall survival was 55.11 days. The HLH-94 treated group had better short-term survival profile, i.e. on day 30 ($P = 0.201$) and day60 ($P = 0.008$), compared with other two groups, but there was no benefit in long-term survival ($P = 0.201$). In multivariate analyses, the Hodgkin lymphoma subtype of malignancies and the use of chemotherapy were associated with better outcomes.

Conclusion: For adult HLH patients, the HLH-94 regimen might be associated with better treatment response, less toxicity, and higher short-term survival rate when compared with HLH-2004 and other therapies, but there is no long-term survival benefit. Continuing research effort remains necessary for optimizing treatment for adult HLH.

Monoallelic Mutations in Genes Related to Familial Hemophagocytic Lymphohistiocytosis (FHL): Report from the Italian Registry

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Purpose: To describe clinical, functional and genetic features of patients with monoallelic mutations in FHL-related genes.

Methods: Patients with complete or partial HLH-2004 diagnostic criteria and monoallelic mutations in at least one of the FHL-related genes were selected from the Italian HLH Registry. Perforin expression and NK-cell degranulation were

performed by flow-cytometry. Molecular analysis was performed by Sanger or Next Generation sequencing.

Results: Of the 600 patients reported to the Registry, 54 (9%) had monoallelic mutations in FHL-related genes. Median age: 5 years. 29/54 patients (54%) fulfilled at least 5/8 diagnostic criteria: fever (n = 49/52; 94%), splenomegaly (n = 37/50; 74%), cytopenia (n = 43/50; 86%), hypertriglyceridemia (n = 28/47; 60%), hypofibrinogenemia (n = 1/46; 0.02%), hyperferritinemia (n = 47/50; 94%), hemophagocytosis (n = 28/45; 62%), neurologic involvement (n = 12/41; 29%). Five patients died; of 8 who reactivated, 4 were transplanted. An associated/underlying disease was reported in 34/54 (63%): rheumatologic/autoimmune disorder, 22 (11 Juvenile Idiopathic Arthritis, 2 Kawasaki disease, Systemic Lupus Erythematosus, Cogan Syndrome, Colitis ulcerosa; 6 undefined); lymphoproliferative disease, 5 (2 acute lymphoblastic leukemia, 2 non-Hodgkin, Hodgkin lymphoma), infectious diseases, 6 (2 EBV, CMV, parechovirus, osteomyelitis, myocarditis), 1 pigment deficiency disease. Functional tests were performed in 32/54 (60%), showing impaired degranulation in 42% (n = 13/31) and defective perforin expression in 43% (n = 16/37). The genetic study revealed 31 monoallelic variants in PRF1 (n = 10), UNC13D (n = 8), STXBP2 (n = 8), STX11 (n = 3), LYST (n = 1) and Rab27A (n = 1). Four variants were reported as polymorphism. Of the 27 remaining, 25 were missense (14 predicted as probably damaging), 1 STOP, 1 frameshift. Two patients had mutations in 2 different genes.

Conclusions: 9% of patients reported to the Italian HLH Registry carries monoallelic variants in at least one FHL-related genes, including 52% being probably damaging. Altogether these patients are characterized by later onset, partial/milder disease, and partial functional defect. Thus, monoallelic mutation in one FHL-related gene defines a predisposing factor for HLH.

The Use of Whole Body Magnetic Resonance Imaging for Skeletal Staging of Childhood Langerhans Histiocytosis: Results of a Retrospective Cohort Study

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Purpose: Whole-body magnetic resonance imaging (WB MRI) can be used for staging of several paediatric malignancies. In the past, two small studies have been performed using WB MRI for staging of LCH. The aim of this study was to assess the role of WB MRI during staging at diagnosis and to compare WB MRI to plain radiography in the cohort of the Emma's Children Hospital.

Methods: A retrospective cross-sectional cohort study was performed. Patients diagnosed with LCH were eligible. For this study, patients were included if they had total body MRI at baseline and if targeted comparative imaging of MRI-positive lesions was available. All imaging (both WB MRI and plain radiography) was reassessed by two pediatric radiologists independently and scored for presence of lesions. For this study, plain radiography was regarded as the reference standard.

Results: Twelve patients were included for this study. A total of 55 lesions were detected and for 48 lesions comparative imaging was available. Thirty lesions were detected by plain radiography and 45 lesions were detected on WB MRI. Sensitivity of WB MRI was 90%.

Conclusions: WB MRI seems promising as a whole-body technique during staging of pediatric LCH patients with sensitivity of 90% in our cohort. In our study, WB MRI detected additional lesions, but the clinical relevance of these lesions are under debate. Future research should therefore focus on the value of WB MRI and plain radiography in a prospective setting.

Salvage Hematopoietic Stem Cell Transplantation for Advanced Hemophagocytic Lymphohistiocytosis

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Background: The therapy protocol of hemophagocytic lymphohistiocytosis-2004 was effective. However,there were still some refractory and recurrent cases could not achieve remission though by the other second-line treatment such as DEP protocol, CHOPprotocol, targeted drug etc. So these advanced cases recieved salvage hematopoietic stem cell transplantation.

Purpose: We reported that 24 cases advanced hemophagocytic lymphohistiocytosis recieved salvage hematopoietic stem cell transplantation.

Patients and Methods: 24 consecutive advanced cases from June 2015 to April 2017 in our center were analyzed. The median age of patients was 5 (1-11) years old. Patients with haploidentical transplantation received unmanipulated combined marrow and peripheral blood stem cells for transplant and patients with unrelated donor transplantation received peripheral blood stem cells. We used the conditioning with etoposide, busulfan or maphalan and fludarabine plus antithymocyte globulin (ATG) added cyclophosphamide and cytosine arabinoside or not. 15 patients were with haploidentical donors, and unrelated donors in 9 cases.

All the patients were positive with EB viral before the HSCT.

Results: There were two patients died before the hematopoietic reconstitution. Durable hematopoietic reconstitution was seen in 90.9%(20 of 22 patients) of recipients.With the median follow-up of 10 (1-22) months, 1-year overall survival (OS) rates for all patients were 66.7%. The event of death of 8 cases occurred within 6 month after transplantation.

Conclusions: Under appropriate protocol, the good outcome could be acquired though with the advanced stage of HLH.

Successful Secondary Hematopoietic Stem Cell Transplantation for Primary Graft Failure in Two Pediatric Cases with Hemophagocytic Lymphohistiocytosis

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Purpose: Graft failure(GF) is a fetal and life-threatening complication in HLH with allo-HSCT, the standard treatment has not been established.

Methods: we summarize two challenging case of HLH who experienced GF following first HSCT and successfully engrafted following salvage secondary HSCT.

Results: One case is a 8-year-old boy diagnosed with EBV-HLH, treated with the haploidentical HSCT following the conditioning regimen consisted of VP-16/Bu/Flud/ATG. The patient presented the clinical manifestation resembling those of immune encephalitis on the day 3 after the first HSCT. The methylprednisolone pulse therapy was administered immediately and the clinical symptoms improved. But Primary GF occurred and was diagnosed on day 24.The EBV-DNA load increased, subsequently the patient developed HLH. Fortunately, the patient underwent the secondary unrelated allo-HSCT following the conditioning regimen consisting of VP-16/Ara-C/Bu/CTX/ATG, GVHD prophylaxis consisting of FK506 and MMF. Consequently, the patient achieved neutrophil engraftment on day 14 and 100% donor chimerism after the second HSCT. During the clinical course of the second HSCT, the main complication was poor platelet graft function, and platelet engraftment on day 84. Until now, the patient was disease-free for 26 months. The other case is a 3-year-old boy diagnosed with primary HLH, treated with unrelated allo-HSCT following the conditioning regimen consisted of VP-16/Bu/Flud/ATG. The patient presented the clinical manifestation resembling those of acute liver failure on the day 12 after the first HSCT. The methylprednisolone pulse therapy and plasma exchange were administered immediately, and the clinical symptoms improved. But Primary GF occurred and was diagnosed on day 21. Therefore, the patient underwent the secondary unrelated allo-HSCT following the conditioning regimen consisting of VP-16/Mel/Flud/ATG, GVHD prophylaxis consisting of FK506 and MMF. Consequently, the patient achieved neutrophil engraftment on day 12, platelet engraftment on day 10 and 100% donor chimerism after the second HSCT. During the clinical course of the second

The Successful Use of Ruxolitinib for Refractory Hemophagocytic Lymphohistiocytosis (HLH)

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Purpose: To describe a case report utilizing Ruxolitinib for refractory HLH.

Methods/Results: An 11yo boy, from Burma, was admitted with myalgias, and spiking fevers to 40oC. He developed liver dysfunction, respiratory failure, and acute renal insufficiency. An extensive infectious work-up was negative and he deteriorated despite antimicrobials. Rheumatologic, immunodeficiency and oncologic etiologies were negative. Due to persistent fevers, multi-system organ dysfunction, and ferritin >20,000 ng/mL, he met criteria for HLH and was started on Dexamethasone and Etoposide per HLH-94. Initially, he showed a clinical response and within 72 hrs, was extubated, weaned off of ionotropic support with improvement in his coagulopathy and renal function. However, he had persistent fevers, splenomegaly, and laboratory criteria for HLH. After 10 days of HLH therapy he acutely deteriorated and received Anakinra. Despite that treatment, he developed respiratory failure, hemodynamic instability, and worsening liver and renal dysfunction. A bone marrow biopsy at that time demonstrated significant hemophagocytosis demonstrating refractory HLH. Alemtuzumab was not available for 72 hours and as the patient rapidly deteriorated, the decision was made to give ng Ruxolitinib in addition to Dexamethasone. He was started on Ruxolitinib 2.5 mg bid based on pediatric dosing used for graft-versus-host disease treatment. Within 24 hours, our patient became afebrile with rapid improvement in respiratory, liver, and hemodynamic function, inflammatory markers and decrease in transfusion requirements. He no longer required ionotropic support after 24 hours and was extubated within 3 days. As he was refractory to Etoposide, this medication was discontinued. Genetic testing was negative for HLH mutations. The patient is currently well, continuing to tolerate Dexamethasone weans and twice daily Ruxolitinib without any significant side effects and is currently undergoing evaluation for a planned BMT for refractory HLH.

Conclusion: Ruxolitinib should be investigated in clinical trials for the treatment of HLH.

MAPK Mutations Negatively Affect Lesional CD8+ T-Cell - LCH-Cell Ratios

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Purpose: Neoplastic Langerhans Cell Histiocytosis (LCH)-cells express constitutively active MAPK proteins. It is, however, unclear whether (neo)-peptides, derived therefrom, can trigger immune cells. We collected data on mutational status, Human Leukocyte Antigen (HLA) genotype and lesional immune signature to assess how each factor affects LCH presentation and outcome.

Methods: A Dutch-Canadian cohort, containing both pediatric and adult LCH patients, was established for this study. Blinded tissue biopsies from first disease onset were analyzed for BRAFV600E expression, by microdissection and PCR, and in some cases also for other LCH-related mutations. T-cell numbers and phenotype were assessed in the same biopsies by triple immunofluorescent staining and ImageJ software.

Results: Clinical data on 163 LCH patients, with a median follow-up time between date of biopsy and last hospital visit of 7.84 years, were collected. Among these patients, 152 biopsies were analyzed for T-cell numbers, and 137 for BRAF mutational status. HLA genotype was determined for 102 patients. Most patients presented with SS-LCH (n = 116). Remaining patients were diagnosed with MS-RO- (n = 34) or MS-RO+ (n = 13) disease. The incidence of BRAFV600E mutation in this cohort is 50.7%. Remarkably low CD8+ T-cell numbers were found in first onset LCH biopsies, with a median of 0.02 CD8+ T-cells per 1 LCH-cells (range 0.00-4.96). Stratification revealed that BRAFV600E lesions displayed a significantly lower number of CD8+ T-cells per LCH-cell ($p<0.0001$), with a median of 0.02 CD8+ T-cells per 1 LCH-cells (range 0.00-1.25). The same holds true for MAP2K1 mutation-bearing lesions ($p = 0.045$). BRAF mutation status, but not CD8+ T-cell: LCH-cell ratio, had a negative impact on event-free survival.

Conclusion: These data show that LCH lesion-infiltrating CD8+ T-cells do not have major impact on disease outcome. Our observation that CD8+ T-cells are clearly outnumbered by LCH-cells suggests that the constitutively active MAPK pathway somehow drives immune evasion.

Diagnostic and Management Guideline for Patients (<19Y) with a Thickened Pituitary Stalk and/or Central Diabetes Insipidus

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Purpose: To develop a guideline for the investigation and management of children and young people up to the age of 19 years with a thickened pituitary stalk (TPS) and/or central diabetes insipidus (CDI) where the aetiology is not apparent at presentation.

Methods: The guideline development group (GDG) identified the objectives and the clinical questions which needed to be addressed. These were reviewed by guideline stakeholders and used to direct a systematic literature search. Published evidence was appraised using the GRADE system. Where the literature search identified evidence to answer the clinical questions, the GDG made a guideline recommendation. Where there was insufficient evidence, the GDG drafted recommendations based on their expert opinion and reviewed these using a formal Delphi consensus process.

Results: The literature search identified 568 full text articles requiring grading covering the period Jan 1990 : Feb 2017. The search demonstrated that the most commonly reported causes of TPS and CDI in children are Langerhans Cell Hystiocytosis (LCH) and Germ Cell Tumours (GCT). The average prevalence of LCH and GCT in 11 case series (including a total of 741 patients) was 14% and 12% respectively. Common causes of pituitary stalk lesions in adults, metastatic tumours and neurosarcoidosis, do not form part of the differential diagnosis in children. High quality evidence was lacking for the majority of the clinical questions and two rounds of Delphi consensus were undertaken. A decision-making flowchart has been developed and will accompany the guideline.

Conclusion: The likely aetiology of TPS and CDI in children differs from that in adults and justifies the development of age appropriate guidelines for the investigation and management of these conditions. This will form the basis for future audits of practice and outcomes and is intended to improve the quality of care of children and young people with TPS and CDI.

Female with Diabetes Insipidus, Growth Hormone Deficiency and Slowly Progressive Pituitary Stalk Thickening: Use of Oral Prednisolone Treatment as Tool for Histiocytosis Diagnosis

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Purpose: To present the use of oral prednisolone treatment for therapy and probable diagnosis of isolated Langerhans Cell Histiocytosis (LCH) pituitary lesion.

Clinical presentation: A prepubertal 5 11/12 year-old girl presented with polyuria, polydipsia and decreased growth velocity. Height was on 10th-25th %ile, weight on 50th-75th %ile. Laboratory evaluation revealed central hypothyroidism (TSH:2.7μIU/ml, FT4: 0.84ng/dl), low basal FSH and LH, normal cortisol, ACTH and prolactin levels (baseline and stimulated). Serum and CSF a-fetoprotein and βHCG were negative. Skeletal survey was normal. MRI disclosed a slight hyperintense signal of the posterior pituitary gland and round thickening of the middle pituitary stalk d:4mm with avid contrast enhancement. The pituitary gland was of normal size 4.5 × 13 × 8.5mm Repeat MRI 10 months later showed augmentation of the pituitary stalk thickening (6mm) which further increased to 6.9 mm six months later. The bright signal of the posterior pituitary was absent. Growth hormone deficiency was diagnosed. She received PDN 40 mg/m2/daily for 2 months with pituitary stalk decrease to 4.6mm. She then received 5-day pulses q2 weeksx7 and q3 weeks for a total of 12 months treatment. At 12 months, the pituitary stalk measured <3 mm and remains so, with normal appearance 21 months off-treatment. Patient is 26 months off-treatment on follow up.

Results: There was appropriate response to PDN-treatment with normalization of the pituitary stalk, highly suggesting the diagnosis of histiocytosis. The patient continues to be on replacement therapy (desmopressin/thyroxine), and she was started on growth hormone therapy six months ago, resulting in growth acceleration. MRI remains unchanged.

Conclusion: Biopsy driven, pathology can lead to definitive diagnosis. Alternatively, a less invasive approach, is careful PDN LCH-type treatment. Prompt response, evident by decreasing thickening of pituitary stalk as observed in our patient, is highly suggestive but not confirmatory for LCH diagnosis. Careful follow-up is warranted.

Clinical and Laboratory Signs of Hemophagocytic Lymphohistiocytosis in Pandemic Influenza A (H1N1) Infection Patients Needing Extracorporal Membrane Oxygenation

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Purpose: Severe pandemic influenza A infection has been related to a high fatality rate with an unexplained peak in young adults. Interestingly, severe influenza has been associated with the hyperinflammatory condition secondary hemophagocytic lymphohistiocytosis (HLH). For a better clinical and biological understanding of influenza-associated HLH, we aimed to describe a cohort of severely ill patients with 2009 influenza A (H1N1) infection requiring ECMO and correlate the clinical, laboratory and histological findings to those seen in HLH.

Methods: Adult patients with AH1N1-infection requiring ECMO, from July 2009 to January 2010 in Stockholm, were included in the study. HLH-related clinical and laboratory data was retrieved from medical files. Soluble CD25 (sCD25), bone marrow aspiration, lymphocyte cytotoxicity function tests and genetic sequencing were performed when possible, especially in those with suspected HLH.

Results: Eleven patients, including several healthy young adults, with AH1N1-infection required ECMO, inotropic support and renal replacement therapy. All survived. Four male patients developed HLH according to HLH-2004 criteria and HScore. Patients with HLH showed signs of more severe (hyper)inflammation and organ dysfunction with higher serum ferritin and alkaline phosphatase (ALP) levels and a trend towards more abnormal lactate dehydrogenase levels and liver function tests, and more marked thrombocytopenia and splenomegaly. Moreover, 75% required conversion to veno-arterial ECMO. Bone marrow aspiration performed on patients with HLH showed signs of hemophagocytosis. Abnormal lymphocyte cytotoxicity (Lytic Units <10) was observed in 3/4 patients with HLH that also showed lower proportion of NK-cells. Treatment of HLH varied from no HLH-directed therapy to cytotoxic therapy.

Conclusion: Patients, including healthy young adults, with AH1N1-infection requiring ECMO may develop HLH and

should be monitored for signs of hyperinflammation and increasing organ dysfunction, since HLH-directed therapy may be beneficial.

Multivariate Analysis of Prognosis for Patients with Natural Killer/T Cell Lymphoma-Associated Hemophagocytic Lymphohistiocytosis

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Objective: A major cause of Hemophagocytic lymphohistiocytosis (HLH) is malignant neoplasms of the blood system, among which NK/T cell lymphoma is one of the most common risk factor. Patients with NK/T cell lymphoma hemophagocytic lymphohistiocytosis (NK/T-LAHS) have a worse prognosis and higher mortality. We aimed to explore the factors that affect the prognosis of NK/T-LAHS.

Methods: Clinical data of 42 patients with NK/T-LAHS diagnosed by Beijing Friendship Hospital from June 2008 to June 2016 were analyzed retrospectively.

Results: The survival time was counted until August 1, 2016. For the 42 NK/T-LAHS patients, 1-month survival rate was 48.9%, 2-month survival rate was 36.7%, 3-month survival rate was 28.8%, 6-month survival rate was 23.0%, and 12-month survival rate was 15.4%. NK/T-LAHS patients who underwent allogeneic hematopoietic stem cell transplantation (Allo-HSCT) (P = 0.000), exhibited peripheral blood Epstein-Barr virus (EBV)-positivity (P = 0.004), and achieved overall response (OR) remission after initial induction therapy (P = 0.007) had statistical significance.

Conclusion: NK/T-LAHS is a disease of poor prognosis and high mortality. NK/T-LAHS patients who achieved OR remission after the initial induction therapy had better prognosis than non-remission patients and Allo-HSCT was an effective way to prolong the survival of NK/T-LAHS patients. However, EBV positivity in peripheral blood was a poor prognostic factor in NK/T-LAHS patients.

Central Nervous System Involvement in Adult Hemophagocytic Lymphohistiocytosis: Our Experience in Diagnosis and Treatment

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Purpose: Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening clinical syndrome which involves excessive hyperplasia of activated lymphocytes and macrophages, great inflammatory cytokine production and destructive multiorgan infiltration. Central Nervous System(CNS) involvement is a severe complication, which can lead to poor prognosis. CNS-HLH has been reported and studied in children, but the clinical information of adult CNS-HLH is still lacking.

Methods: A retrospective analysis of 96 adult patients with HLH and combined with CNS involvement during November 2009 and December 2016 was conducted. CNS involvement was defined as the presence of neurological signs/symptoms or neuroimaging abnormalities or evaluation of cerebrospinal fluid cells and/or proteins. Clinical features, cerebrospinal fluid features, neuroimage changes, therapeutic outcomes and survival statistics were analyzed.

Results: Among the 96 patients, infection-associated HLH is the most common subtype in CNS-HLH(55.2%). 86 patients(89.6%) had various CNS symptoms, including consciousness disorders, seizures and so on. 39 performed cerebrospinal fluid examination and 10 had abnormal findings. There was 71.4% (50/70) patients suffers from image changes, and the commonest form of involvement was multifocal and bilateral abnormalities in brain white matter (36%). Most of the patients were treated with HLH 94/04 protocols, while 33 patients (34.4%) were also treated with intrathecal injection. The overall mortality was 56.3%, which was higher than the other adult HLH patients without CNS involvement (P = 0.000). In the multiple factors analysis of survival time, we found out that intrathecal injection was a protective factor for the prognosis of CNS-HLH (P = 0.014, Exp (B) = 0.469).

Conclusions: CNS involvement in adult HLH may have different kinds of CNS symptoms. The image changes may not be specific but can prompt the severity of CNS involvement and the outcome of CNS-HLH. can leads to poor prognosis. CNS-HLH had a poor prognosis and the intrathecal injection is an effective way to treat it.

Clinical Features of 52 Patients with HLH Accompanied by Gastrointestinal Bleeding

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Objective: To explore the clinical features of patients with hemophagocytic lymphohistiocytosis accompanied with gastrointestinal bleeding.

Methods: The clinical data of 52 patients diagnosed HLH accompanied with gastrointestinal bleeding in our hospital from January 2015 to March 2017 were analyzed retrospectively.

Results: For the 52 HLH patients with digestive tract hemorrhage, 1-month survival rate was 74.7%, 3-month survival rate was 53.8%, 6-month survival rate was 32.9%, and 12-month survival rate was 23.3%. Thrombocytopenia (P = 0.036), other sites of bleeding (P = 0.030) ï¼Œallogeneic

hematopoietic stem cell transplantation (P = 0.026) had significant impacts on patients.

Conclusion: HLH accompanied with gastrointestinal bleeding was considered a threaten of life, which indicate poor prognosis of patients. "Thrombocytopenia" and "other sites of bleeding" shorten the survive time of patients, "allogeneic" hematopoietic stem cell transplantation" improve the survive time of patients.

Clinical Research: Treatment of Hemophagocytic Lymphohistiocytosis with Third-Party Mesenchymal Stromal Cells

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Mesenchymal stromal cells (MSCs) represent an attractive tool for HLH therapies on grounds of their immunomodulatory and regenerative properties. Here, we retrospectively analyzed 12 cases of refractory/relapsed hemophagocytic lymphohistiocytosis (HLH) presented at our institution from April 2016 to April 2017 treated with MSCs after the combination of chemotherapy; and consecutive 25 cases of refractory/relapsed HLH in our hospital during the same time period but without MSC therapy were as control group. The median age was 24 (9-59) years, The median disease course was 10.4 (3.9-53.7) months. The median time to neutrophil recovery time was similar between two groups (10 days vs. 14 days, p = 0.414), but platelet recovery time was slower in MSCs group (11 days vs. 15 days, p = 0.075). ALT was comparable in MSCs and control groups (p = 0.143), AST was comparable in MSCs and control groups (p = 0.0829), TBIL was comparable in MSCs and control groups (p = 0.474), DBIL was comparable in MSCs and control groups (p = 0.542).Significant difference was found in Fbg between MSCs and control groups (p = 0.0238). Significant difference was found in MIP-1 alpha,IL-8,IFN-g and TNF-a between MSCs and control groups (p<0.05). 5 cases with severe multiple organ damage (liver function, blood coagulation function) were not improved in the initial treatment, but 2 cases were improved significantly after infusion UC - MSCs. Our hypothesis was that the application of MSCs could be effective in the treatment of HLH, since MSCs possess a broad repertoire of immunomodulating mechanisms impacting both innate and adaptive immunity pathways. Further studies are necessary to answer these questions.

Clinical Research of Pediatric Langerhans Cell Histiocytosis with Craniofacial Bone Involvement

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Purpose: The objective of this article was to elucidate the clinical characteristics and prognosis of pediatric Langerhans cell histiocytosis (LCH) with craniofacial bone involvement.

Methods: A retrospective analysis was performed in LCH patients registered between January 2007 and July 2013 at a single institute. They were stratified and treated according to Histiocyte society LCH-III protocol.

Results: A total of 145 patients with craniofacial bone involvement were analyzed out of 232 LCH patients (62.5%). Among the 145 patients, there was 104 cases with CNS-risk bone involvement (CNS-risk group) and 41 cases without CNS-risk bone involvement (non-CNS-risk group). The age of patients in CNS-risk group (median age 25.5 months) was significantly lower than the non-CNS-risk group (median age 58.0 months), P<0.01. The rate of patients classified as LCH-III Group 1 in CNS-risk group (70.2%) was higher than the non-CNS-risk group (34.1%), P<0.01. The relapse rate in CNS-risk group (44.2%) was higher than the non-CNS-risk group (14.6%), P<0.01.The 3-year event-free survival rate(EFS) in non-CNS-risk group was higher than that in CNS-risk group,(P<0.01). The incidence of diabetes insipidus in CNS-risk group (21.2%) was higher than the non-CNS-risk group (12.2%), while without statistical significance. In addition, this research didn't find significant differences in gender, additional bone (other than craniofacial bone) involvement between the two groups.

Conclusions: The incidence of craniofacial bone involvement in LCH patients was high. Moreover, the children with CNS-risk bone involvement were mainly infants and young children, with a more serious clinical manifestation, a lower EFS and a higher relapse rate.

Outcome of Children with Hemophagocytic Lymphohistiocytosis with HLH-2004 Protocol in Japan

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Purpose: Prognosis of hemophagocytic lymphohistiocytosis (HLH) in children has varied from spontaneous regression to fatal. Protocols with intensive chemo- and immune-therapy, such as HLH-94 or HLH-2004, have improved the outcome of HLH, with heterogeneity among different subtypes. In western countries, most children with HLH have the primary form (mainly familial HLH, FHL), whereas in Eastern Asia including Japan, the secondary form (mainly EBV-associated HLH) is more prevalent. Therefore, it may be useful to establish the next strategy by the evaluation of outcomes among different HLH subtypes in Japan.

Methods: Ninety patients with HLH of less than 18 years old were registered to the HLH-2004 protocol in Japan from February 2007 to November 2011. Out of 82 eligible patients, nine patients were excluded from the efficacy analyses: three long-term outlook, one withdrawal before the trial treatment, and five protocol violation. Forty-one (56.2%), 9 (12.3%), and 23 (31.5%) patients had EBV-HLH, FHL, and HLH of unknown etiology, respectively. Patients with resistant or relapsed disease after the treatment with HLH-2004 and those with FHL received hematopoietic stem cell transplantation (HSCT).

Results: The 5-year overall survival (OS) rate was 73.9% (95% CI, 62.2%:82.5%). Induction rate after initial therapy was 58.9%. OS rates significantly differed among HLH subtypes: 85.3%, 66.7%, and 56.2% for EBV-HLH, FHL and unknown etiology, respectively ($p = 0.028$). Other clinical features including central nervous system involvement and laboratory findings at onset were not associated with the outcome. Of 17 patients undergoing HSCT, the 5-year OS of patients with ($n = 6$) and without remission before HSCT ($n = 11$) were 83.3% and 54.5%, respectively ($p = 0.273$).

Conclusion: Outcome of children with HLH, who were treated with the same protocol, differs among different subtypes. Therefore, the strategy for different subtypes including FHL or EBV-HLH should be established as a next study.

Comprehensive Strategy to Establish the Clinical Diagnosis for Patients with Hemophagocytic Lymphohistiocytosis

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Purpose: Hemophagocytic lymphohistiocytosis (HLH) is a heterogeneous disorder. Defects in at least 14 genes (AP3B1, BLOC1S6, CD27, ITK, LYST, MAGT1, PRF1, RAB27A, SH2D1A, SLC7A7, STX11, STXBP2, UNC13D (MUNC13-4), XIAP (BIRC4)) have been associates with familial HLH and associated conditions. A timely and cost effective diagnosis strategy is needed to support a more personalize treatment plan to achieve a better outcome.

Methods: We developed a comprehensive testing strategy including a 14 gene Next Generation Sequencing (NGS) testing panel and reflex to long PCR and exon centric copy number variation (CNV) analysis, and complemented with epigenetic and protein expression analysis to evaluate a comprehensive testing algorithm for the diagnosis of HLH and associated disorders.

Results: By reviewing the testing results in the first 1460 clinically suspected HLH patients, we found 125 patients to have single or bi-allelic mutations in HLH related genes, 204 have variants with unknown clinical significance, 69 patients carried variants in more than two genes. In addition, gross deletions and duplications have been identified in 10 patients in seven (SH2D1A, XIAP, MAGT1, RAB27A, STXBP2, LYST, SLC7A7) HLH genes. Interestingly, one female patient with one nonsense mutation in BIRC4, also affected by X inactivation abnormalities. For the non-genetic studies, perforin expression analyses detect 78% of patients and carriers with PRF1 variants. XIAP and SAP protein analyses by flow cytometry found 87% and 95% of patients with likely pathogenic variants in SH2D1A and BIRC4 genes respectively.

Conclusion: To achieve a definitive diagnosis in patients with HLH, a comprehensive approach is desired which includes sequencing, CNV and epigenetic studies as well as immunological work up. This comprehensive testing strategy provide cost effective testing platforms with reasonable clinical sensitivity for patients with HLH.

Salvage Treatment of Pediatric Refractory Epstein-Barr Virus-Associated Hemophagocytic Lymphohistiocytosis with L-DEP Protocol

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Background: Epstein:Barr virus-associated hemophagocytic lymphohistiocytosis (EBV-HLH) is more prevalent in Asian population. Previous studies have shown that more than 30 % patients with EBV-HLH do not respond to standard therapy. In this study, efficacy and safety of L-DEP (PEG-aspargase-doxorubicin-etoposide-methylprednisolone) protocol as a salvage therapy for pediatric refractory EBV-HLH was evaluated.

Methods: From January to December of 2016, 18 patients with refractory EBV-HLH were treated with L-DEP protocol at Beijing Children's Hospital. Treatment efficacy and adverse events were evaluated at 2 and 4 weeks post L-DEP treatment.

Results: Over all response rate (ORR) was 72.2% (13/18), among which there were 38.9%(7/18) complete response (CR) rate and 33.3% (6/18) partial response (PR) rate. Two patients had familial HLH (FHL) triggered by EBV. The patient with FHL-2 achieved CR and the other one with FHL-3 had no treatment response and died. The All 6 patients with chronic active EBV infection-associated HLH had treatment response with 3 CR. Five patients without treatment response died 10 to 20 days post L-DEP protocol. Ten of 13 patients with treatment response underwent allogeneic hematopoietic stem cell transplantation (allo-HSCT), among which 6 were alive. Compared with the level of serum EBV-DNA before salvage treatment (median 2.7 × 104 copies/mL), that levels at 2 weeks (median 1.46 × 103 copies/mL) and 4 weeks (median 7.27 × 102 copies/mL) after receiving the L-DEP regimen were significantly lower ($P = 0.028$ all). All patients had I-II degree bone marrow suppression and one patient had reversible pancreatitis due to PEG-aspargase.

Conclusions: This study suggests that L-DEP is a safe and effective salvage therapy prior to allo-HSCT for refractory pediatric EBV-HLH.

Keywords: PEG-aspargase, liposomal doxorubicin, Epstein:Barr virus, Hemophagocytic lymphohistiocytosis, Pediatric

Clinical Features of Perinatal Stage Related Hemophagocytic Lymphohistiocytosis

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Purpose: Hemophagocytic lymphohistiocytosis (HLH) is a rare and severe clinical syndrome characterized by a dysregulated hyperinflammatory immune response. Hemophagocytic lymphohistiocytosis manifesting during pregnancy/postpartum continues to be a rare entity. From the clinical observation of case in our center, we found out that many the cases of the pregnancy/postpartum related HLH was in the perinatal stage. This study was to analyze the clinical features of perinatal stage related-HLH.

Methods: A analysis of 11 patients with HLH who were in the perinatal stage during January 2011 and October 2016 was conducted. Obstetric materials, clinical features, associated disease/factors and therapeutic outcomes were analyzed. Perinatal stage is the period between 28th week of pregnancy to one week after delivery.

Results: Among the 11 patients, nine of them were primipara. As for the onset time of HLH, five were during pregnancy and six were during postpartum. Six of these patients was complicated with other associated disease/factors, and infection was the commonest (5/11), while the other five had an unclear etiology. Four patients who were in pregnancy were treated with HLH-94/04 protocols after the cessation of pregnancy, while the six who were in postpartum were also treated with HLH-94/04. The overall response rate was 63.6% (9/11). Two patients in postpartum died of HLH.

Conclusions: Perinatal stage HLH is commonly observed in the pregnancy/postpartum HLH. As for the possibility of immune disorders when maternal is in perinatal stage, the pregnancy/postpartum itself can lead to the onset of HLH. Infection is still the commonest associated factors, which may be related to the imbalance of Th1/Th2. HLH-94/04 protocols after the cessation of pregnancy may be effective, but the cessation of pregnancy itself may not be enough for the perinatal stage related-HLH. The perinatal stage related-HLH still has a better outcome than the other subtype of secondary-HLH.

Haemophagocytic Lymphohistiocytosis: A Decade of Experience in a Paediatric Centre in South-East Asia

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Purpose: Haemophagocytic lymphohistiocytosis (HLH) is a rare syndrome of hyperinflammation. If recognized early, it can be effectively controlled to allow definitive diagnosis and therapy according to underlying aetiology. We aim to share a decade of clinical experience in a paediatric center in South-East Asia (SEA).

Methods: We retrospectively reviewed patients who met the diagnostic criteria of HLH-2004 from Jun 2005 to Dec 2016.

Results: Eighteen patients with a diagnosis of HLH were screened. Of these, 9 fulfilled HLH-2004 diagnostic criteria. Mean age at diagnosis was 39 (4 to 117) months. Fever, splenomegaly and hyperferritinaemia were consistently found in all 9 patients. Majority presented with cytopenia (7 of 9) and haemophagocytosis (8 of 9). Hypertriglyceridemia and/or hypofibrinogenemia were seen in a third of the patients. The

underlying etiologies of HLH included suspected/ confirmed primary HLH (n = 7) and cancers (n = 2). Majority (7 of 9 patients) were tested positive for EBV infections. Of 7 patients with suspected primary HLH and had research tests done in Karolinska Institute, Sweden; only 2 patients were found to have an associated mutations (UNC13D and XIAP). Majority (8 of 9) of patients responded to HLH 1994/2004 based therapy. Haematopoietic stem cell transplants were performed in 4 with confirmed/ suspected primary HLH. At a median follow-up of 3.3 (range, 0.2 to 11.8) years; 7 of 9 patients are alive and cured/ well. Of the 2 patients who died, 1 died progressive malignant disease and another of transplant complication.

Conclusion: Majority of patients in our small series survived HLH. The high survival rate is likely related in part to early treatment. A significant number of patients with clinically suspected primary HLH lack a genetic diagnosis when screened for associated mutations. There is a possibility that novel mutations specific to South-East Asians remains to be discovered.