Histiocytic Disorders in Pediatric Hematopathology

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Introduction

- Brief overview/classification of histiocytic disorders
- Focus on the most common entities
- Format: real case presentation
- Emphasis on issues relevant to our daily practice
- Briefly touch on recent biologic and genetic insights
Immune function and phenotype of histiocytes

- Derived from bone marrow hematopoietic stem cells
- Part of the immune system with two main functions.
  - Antigen processing and presentation
  - Phagocytosis
- Two subtypes:
  - Dendritic cells - antigen processing and presentation
    - (CD1a+, CD14-)
  - Monocytes/macrophages - phagocytosis
    - (CD1a-, CD14+ CD68+, CD163+)
Classification of Histiocytic Disorders

- **Class I - Dendritic cell related**
  - Langerhans cell histiocytosis (LCH)
  - Secondary dendritic cell processes
  - Juvenile xanthogranuloma (JXG)
  - Solitary histiocytomas of various dendritic cell phenotypes

- **Class II - Monocyte/macrophage related**
  - Hemophagocytic lymphohistiocytosis (HLH)
  - Rosai-Dorfman Disease
  - Solitary histiocytoma
  - *Lipid Storage Disorders (storage histiocytosis)*

- **Class III - Malignant histiocytic disorders**
  - Monocytes – related: AML M4/5, CMML
  - Dendritic cell related histiocytic sarcoma
Case 1

- A male infant delivered by C-section at 35 weeks
- At birth, had hepatosplenomegaly and mild ascites.
- Shortly developed thrombocytopenia and anemia, required multiple transfusions
- FH: a F. sibling died of fulminant liver failure of unknown etiology on DOL 17
Extensive infection and other work up were all negative

- Blood and stool cultures for bacterial pathogens
- Serology workup for viral infections and others
  - CMV, Rubella, herpes I and II, parvovirus B19, Toxoplasma gondii
- Anticardiolipin antibodies
- Lupus anticoagulative antibodies
- Metabolic screen
- Urine for bile acid synthesis
- Hereditary tyrosiemia
Had two biopsies:

- liver biopsy
- bone marrow biopsy
Bone marrow aspirate smear-patient
Bone marrow aspirate smear - patient
Liver biopsy-patient
Liver biopsy - patient

CD 68
Case 1

- Dx of HLH was highly suspected.
- Studies for natural killer (NK) and cytotoxic T-cell function and perforin expression were initiated.
- The patient deteriorated rapidly with metabolic acidosis, fulminate liver failure and DIC.
- Died on DOL 25. An autopsy was performed.
Autopsy liver-patient
Autopsy brain-patient
Systemic lymphohistiocytic infiltrate with remarkable hemophagocytosis, organs involvement:

- Leptomeninges
- Liver
- Spleen
- Lymph nodes
- Bone marrow
- Lungs
- Kidneys
- Adrenal glands
- Pancreas
- Testicles
Further evaluation and follow up

Patient:
- lack cytolytic function in NK cells (0, NL > 3.2 LU).
- Normal NK cell number with NL perforin expression.
- No mutation detected by PRF1 gene sequencing.

Mother:
Decreased perforin expression and cytolytic function in cytotoxic T cells, and normal in NK cells.

Father: Normal
Further evaluation and follow up

F. Sibling:

- hydrops fetalis at birth
- Hepatosplenomegaly
- Pancytopenia
- Triglyceride 184 mg/dl (normal <150 mg/dL)
- Liver transaminases (AST 8897 IU/L; ALT 1770 IU/L)
- Ferritin level 63100 ng/ml (normal 3 to 244 ng/ml)
Autopsy liver - sibling
Case 1 – Diagnosis

Familial hemophagocytic lymphohistiocytosis

Pediatr Dev Pathol. 2006; 9(3):239-44.
Case 2

- A 22 y/o F. had kidney transplant 5 years ago due to focal segmental glomerulosclerosis (FSGS)
- On immunosuppression with stable renal function.
- Presented with fever, headache, vomiting and back pain for 1½ months.
- Extensive workup, all negative
  - Negative Blood PCRs for EBV, CMV, adenovirus, parvovirus, enterovirus, and BK virus.
  - No organisms isolated from cultures.
  - Normal chest X-ray.
  - Normal renal ultrasound
  - Empirically given antibiotics, but with persistent daily spiking fevers
- Due to her persistent fever and progressive pancytopenia, a bone marrow specimen was obtained.
Case 2

- **LAB tests:**
  - CBC: wbc3.8, Hg8.5, Plt 93
  - Fibrinogen 135 (normal 150-300mg/dL)
  - Triglycerides: 512 (normal <199mg/dL)
  - Ferritin: 14,094 (normal 3-105ng/mL)
  - Soluble IL-2 receptor: 28,245 (normal 45-1105 unit/mL)
  - No HLH associated genetic defects were detected.

- **Diagnosis:** Secondary HLH associated with histoplasmosis

- Treated with antifungal agent; in a few days patient’s fever and hematologic abnormalities were resolved.

*American Journal of Transplantation, 2010, 10(3): 687-91*
Hemophagocytic lymphohistiocytosis (HLH)

- A rare, potentially fatal clinical syndrome caused by hyperinflammatory reaction due to **defective cytotoxic function**
  - Excessive activation of cytotoxic T lymphocytes and histiocytes
    - Overproduction of inflammatory cytokines
      - IL-1, IL-2Rs(sCD25), IL-6, TNFα, and IFNγ.

- **Main clinical features**
  - fever
  - hepatosplenomegaly
  - pancytopenia
  - coagulative abnormalities
  - neurological symptoms
  - skin rash
Hemophagocytic lymphohistiocytosis (HLH)

- Familial or primarily HLH (~25%)
  - Due to genetic defects
  - Mostly in infancy and early childhood (70-80% cases, < 1 year old)
  - First case reported by Farquhar and Claireux in 1952

- Sporadic or secondary HLH (~75%)
  - Associated with predisposing factors/underlying conditions
  - Affects any age, tends to occur in older children and adults.
Genetic (primary)HLH

FHLH, subtype and associated genes

FHLH1: Gene unknown, 9q21.3-22
FHLH2: PFR1 (perforin), account for ~20%-30% worldwide, 1999
FHLH3: UNC13 D (Munc13-4), account for ~20%-30% worldwide, 2003
FHLH4: STX11 (syntaxin), ~20% of Turkish/Kurdish families, 2005
FHLH5: STXBP2 (syntaxin binding protein 2)/UNC18-2, 2009

Inherited immune disorders associated HLH

- X-linked lymphoproliferative disease (XLP)
  - SAP deficiency-SH2D1A mutations.
  - XIAP deficiency-XIAP (aka BIRC4) mutations
- Chediak-Higashi syndrome (CHS)
  - CHS1 mutations
- Griscelli syndrome type 2 (GS2)
  - RAB27A mutations
- SCID, Wiskott-Aldrich Syndrome
Impaired cytotoxic pathway in genetic HLH

Secondary HLH

Underline conditions associated with secondary HLH

- Infection
  - Virus: EBV, CMV, HHV6, HHV8, HSV
  - Bacteria, fungi, parasites
- Autoimmune disorder:
  - macrophage activation syndrome (MAS) associated with systemic juvenile rheumatoid arthritis (sJRA)
- Malignancy
  - Leukemia/lymphoma
  - solid tumors (Ewing Sarcoma, RMS, mediastinal germ cell tumor)
- Immunodeficiency
  - Transplant, chemotherapy…
Diagnostic Criteria for HLH- 2004

A. Familial disease/known genetic defect.
   or
B. Clinical and laboratory criteria (fulfill 5/8 criteria)
   1. Fever
   2. Splenomegaly
   3. Cytopenias (affecting 2 of 3 lineages in the peripheral blood):
      Hemoglobin <90 g/L (in infants <4 weeks: hemoglobin <100 g/L)
      Platelets <100 x 10⁹ /L
      Neutrophils < 1.0 10⁹ /L
   4. Hypertriglyceridemia (fasting ≥265 mg/dl) and/or hypofibrinogenemia(≤1.5 g/L)
   5. Hemophagocytosis in bone marrow, CSF, lymph nodes, or liver
   6. Low or absent NK-cell activity
   7. Ferritin ≥ 500 mg/L
   8. Soluble CD25 (soluble IL-2 receptor) ≥ 2,400 U/ml
HLH-Morphological evaluation

- **Hemophagocytosis**—the morphological hallmaker of HLH
  - Missed in 40% of cases at first examination

**Tips in morphologic evaluation**

- Be alert, keep a high index of suspicion
- Preferred material/tissue: bone marrow, liver tissue
- Look for **clues** other than hemophagocytosis
  - Active looking histiocytes
  - “Friendly” histiocytes
  - Small sneaky histiocytes
  - Immunohistochemical staining CD163 can be helpful.
HLH-Morphological evaluation
Bone Marrow
Active looking histiocytes

- Irregular cytoplasmic contours
- Cytoplasmic vacuoles, blebs, projects
- Free fragments
“Friendly” histiocytes

- kissing, hugging, wrapping “target” cells
- perinuclear halo
- bag of cells
Small, “sneaky” hemophagocytic histiocytes
Mimics of hemaphagocytic histiocytes
CD163 can be helpful
Case 3

- A previous healthy 9 year old girl presented with recurrent epistaxis
- Lab: Leukopenia and thrombocytopenia
- Hematology consultation: A bone marrow sample was obtained
Case 3

- Molecular study detected two different mutations in Acid-Glucosidase (GBA) that are associated with Gaucher disease.

- Dx: Gaucher disease
Case 4

- 3 yr old boy was found splenomegaly on routine well check up.
- He was asymptomatic and followed for 1.5 years
- A BM sample was obtained.
Case 4

- Two mutations in NPC1 gene detected by molecular study

- DX: Niemann–Pick disease type C (subacute/juvenile)
Lysosomal storage diseases

- Both Gaucher and Niemann-Pick diseases belong to a group of lysosomal storage disease.
  - GD, deficiency of glucocerebrosidase
  - NPD, deficiency of sphingomyelinase

- Rare genetic metabolic disorders with lysosomal enzymes deficiencies caused by gene mutation

- A diverse group of approximately 50 diseases
A 2 year old boy with skin rashes, multiple lytic bone lesions, and abnormal bone marrow signals

A bone marrow specimen was obtained
Langerhans cell histiocytosis

Other names

- Histiocytosis-X
- Eosinophilic granuloma (unifocal)
- Hand-Schüller-Christian syndrome (multifocal)
- Letterer-Siwe disease (disseminated)
Langerhans cell histiocytosis

- One of the most common dendritic cell disorders in children
  incidence: 3-5/million children
- Average age at presentation: 2.4 yrs
- M:F=1.3:1
- Characterized by the pathologic accumulation of LC and other inflammatory cells in organs. Birbeck granules in EM study
- Single lesion, multiple focal, or systemic disease
  - Organs commonly involved: skin, bone, liver, lungs, bone marrow, and brain.
- Epidemiology: increased incidence in patients with thyroid/autoimmune disease in family
LCH - Etiology and pathophysiology

Reactive or neoplastic?
New insights into pathogenesis of LCH

- Badalian-Very G. and colleagues reported the first recurrent mutation of LCH in 2010.
  - BRAF V600E mutation detected in 35 of 61 (57%) LCH.
- Brown NA et. al reported mutations in MAP2K1 in 27.5% of LCH cases
- Both BRAF mutations and MAP2K1 mutations are part of the RAS-MAPK pathway, they are mutually exclusive.
- Strongly support: LCH is neoplastic
- Clinical implication: BRAF-directed therapies, especially in the systemic and aggressive form of LCH

Case 6

- 2 year old boy presented with a two weeks history of fever and cough.
- On further evaluation, he had WBC 67000, mediastinal mass, and hepatosplenomegaly.
Case 6

- Immunophenotyping by flow cytometry:
  - Positive for CD2, surface CD3, cyt CD3, CD5, CD7, CD11b, and T-cell receptor γδ.
  - Negative for CD1a, CD4, CD8, CD25, CD34, TdT, and B or myeloid markers.

- Cytogenetics: 46,XY,t(8;14)(q24;q11.2),der(12)t(12;20)(q11;q13.3), der(20)t(12;20)(q21;q13.3)[9]/46,XY[5]

- FISH: MYC (8q24) rearrangement in 13% of cells

- Dx: T lymphoblastic leukemia with t(8;14)(q24;q11)

- T(8;14)(q24;q11) only detectable in ~1% of T-ALL, a highly aggressive type leukemia

- Refractory to standard therapy AALL0434, switch to AALL0031

- Received MUD cord blood transplantation five months after diagnosis

- At day +110 post-BMT, a routine BM was obtained
MYC (8q24) rearrangement in 15.6% of the cells on direct BMA smear.
Juvenile Xanthogranuloma

- A non-Langerhans cell dendritic histiocytic disorder
- Occur in all ages, but mainly affects infants and young children
- Mostly present as a single skin lesion
- Biological behavior
  - usually benign and self-limiting, especially solitary skin lesions
  - systemic JXG
    - can virtually involves any organs
    - may cause serious morbidity or even death
- Etiology: unknown.
- Well-described association with neurofibromatosis, juvenile chronic myeloid leukemia.
JXG and related hematopoietic neoplasms

Dr. W. Klapper et al (2011) reported a very similar case as this case

- A 5-year-old F. with diagnosis of T-ALL
- Five months later presented with an aggressive systemic JXG
- Clonal relationship between T-ALL and JXG
  - T-cell receptor gamma rearrangement in T-ALL blasts
  - Micro-dissected histiocytes from JXG lesion in a lymph node revealed an identical bi-allelic TCR-γ rearrangement

Pediatr Blood Cancer 2011;56:859–862
Histiocytic disorders and related hematopoietic neoplasm and clonality

In 2010 Dr. Ronald Jaffe, et al reviewed 15 patients who had histiocytic lesions followed ALL

- All patients were in ALL remission while developed histiocytic lesions (5 JXG, 1 LCH, 4 Langhans’ cell sarcoma, 1 Rosai-Dorfman disease, 4 histiocytic scarcoma)
- Clonal relationship with leukemia (Ig H or monoclonal TCR γ gene rearrangements)
- The post ALL histiocytic lesions are more aggressive than their native lesions
- Generally favorable prognosis
  (4 died of progressive histiocytic lesion, 1 died of recurrent ALL, 10 survived)
Case 6

- Follow up CT/PET scan showed disseminated JXG in his marrow, spleen, and mediastinum.
- Treated per LCH III with partially response
- Switch to thalidomide treatment
- Patient eventually died of disseminated JXG
Histiocytic Disorders in Pediatric Hematopathology

- Monocyte/macrophage related
  - Hemophagocytic lymphohistiocytosis (HLH)
    - Primary HLH
    - Secondary HLH
  - Lipid Storage Disorders (storage histiocytosis)
    - Gaucher disease
    - Niemann–Pick disease
- Dendritic cell related:
  - Langerhans cell histiocytosis (LCH)
    - BRAF gene mutation
    - MAP2K1 gene mutation
  - Juvenile xanthogranuloma (JXG)
    - Associated disorders and clonality
- Class III - Malignant histiocytic disorders