HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

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August 26, 2022

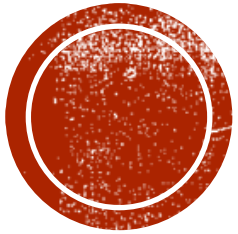
OBJECTIVES

- Define HLH
- Discuss pathophysiology
- Name predominant triggers
- Recite the diagnostic criteria
- Understand the clinical features
- Describe elements of the work up
- Appreciate the challenges of identification and successful treatment
- Pledge to be HLH Ambassadors

HLH SYNDROMES

- Primary HLH
  - Caused by **genetic mutations** impairing the cytotoxic function of natural killer (NK) cells and cytotoxic T cells
  - Typically presents in **infancy and childhood** (average age 10 months)
  - Familial HLH (autosomal recessive), or other inherited immunodeficiency syndromes such as Chediak-Higashi, Gricelli, and type II Hermansky-Pudlak syndromes
- Secondary HLH
  - Not genetic
  - Often called **reactive** because something triggers it, a predisposing condition such as **viruses**, autoimmune conditions, **malignancies**, or **post-transplant** (solid and stem cell)
  - Macrophage activation syndrome (MAS) is a term applied when HLH disease is associated with rheumatological disease such as juvenile idiopathic arthritis and systemic lupus erythematosus

Sandler et al, Frontiers in Immunology 2020
Daver et al, Cancer 2017



HLH: DEFINITION

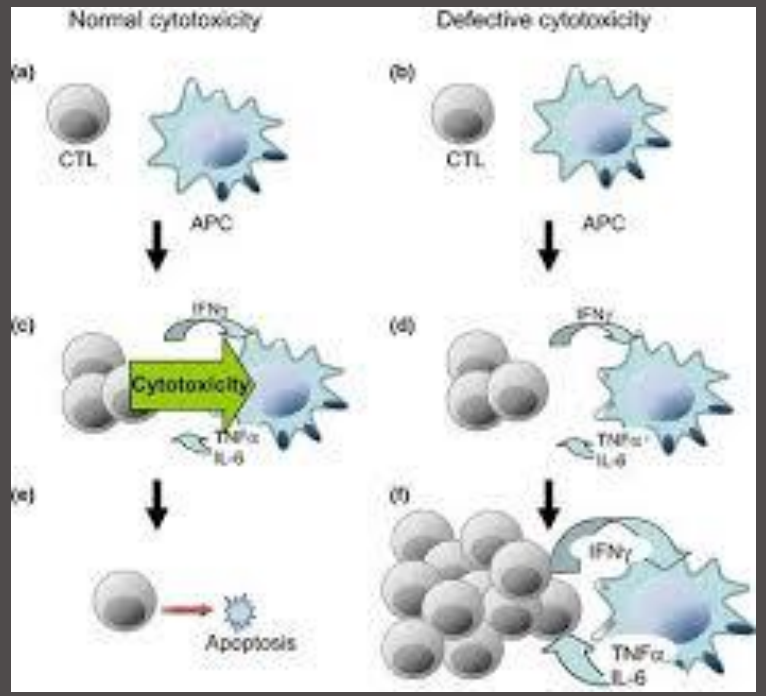
HLH – HEMOPHAGOCYTIC LYMPHOCYTOSIS

- Definition: HLH is a syndrome of **severe** immune activation and deregulation characterized by hyperactive macrophages and lymphocytes, proinflammatory cytokine hypersecretion, tissue infiltration, hemophagocytosis, and organ damage. This syndrome can be classified as primary and secondary HLH.
- Macrophages: professional antigen presenting cells (APCs), derived from monocytes
- Natural killer (NK) cells: lymphocytes that eliminate damaged, stressed, or infected host cells (such as macrophages)
- Cytotoxic lymphocytes (CTLs): activated T lymphocytes which lyse autologous cells such as macrophages bearing foreign antigens
- Histiocytosis - A generic name for a group of syndromes characterized by an abnormal increase in the number of certain immune cells called histiocytes. These include monocytes, macrophages, and dendritic cells

Sandler et al, Frontiers in Immunology 2020
Henter et al, Pediatr Blood Cancer 2007

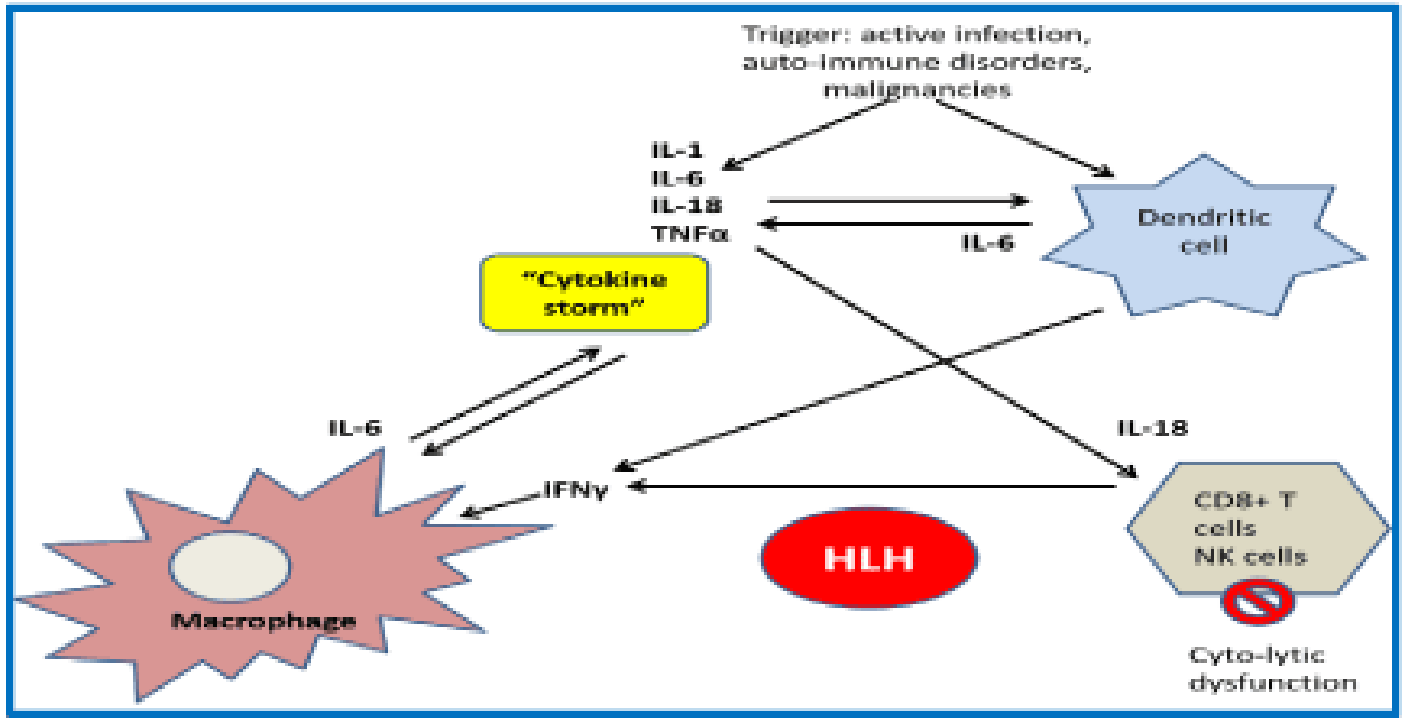
Cytokines of innate immunity.

Cytokine	Principal cell source(s)	Principal cellular targets and biologic effects
Tumor necrosis factor (TNF)	Macrophages, T cells	Endothelial cells: activation (inflammation, coagulation) Neutrophils: activation Hypothalamus: fever Liver: synthesis of acute phase proteins Muscle, fat: catabolism (cachexia) Many cell types: apoptosis
Interleukin (IL-1)	Macrophages, endothelial cells, some epithelial cells	Endothelial cells: activation (inflammation, coagulation) Hypothalamus: fever Liver: synthesis of acute phase proteins
Chemokines	Macrophages, endothelial cells, T cells, fibroblasts, platelets	Leukocytes: chemotaxis, activation
Interleukin-12 (IL-12)	Macrophages, dendritic cells	NK cells and T cells: IFN-γ synthesis, increased cytolytic activity T cells: T_H1 differentiation
Interferon-γ (IFN-γ)	NK cells, T lymphocytes	Activation of macrophages Stimulation of some antibody responses
Type I IFNs (IFN-α, IFN-β)	IFN-α: Macrophages IFN-β: Fibroblasts	All cells: anti-viral state, increased class I MHC expression NK cells: activation
Interleukin-10 (IL-10)	Macrophages, T cells (mainly T_H2)	Macrophages: inhibition of IL-12 production, reduced expression of costimulators and class II MHC molecules
Interleukin-6 (IL-6)	Macrophages, endothelial cells, T cells	Liver: synthesis of acute phase proteins B cells: proliferation of antibody-producing cells
Interleukin-15 (IL-15)	Macrophages, others	NK cells: proliferation T cells: proliferation
Interleukin-18 (IL-18)	Macrophages	NK cells and T cells: IFN-γ synthesis



CYTOKINE STORM

- The persistent activation of macrophages, T cells, and CTLs in patients with HLH leads to excessive cytokine production (cytokine storm) by all these cell types
- This phenomenon leads to multiorgan failure and mortality

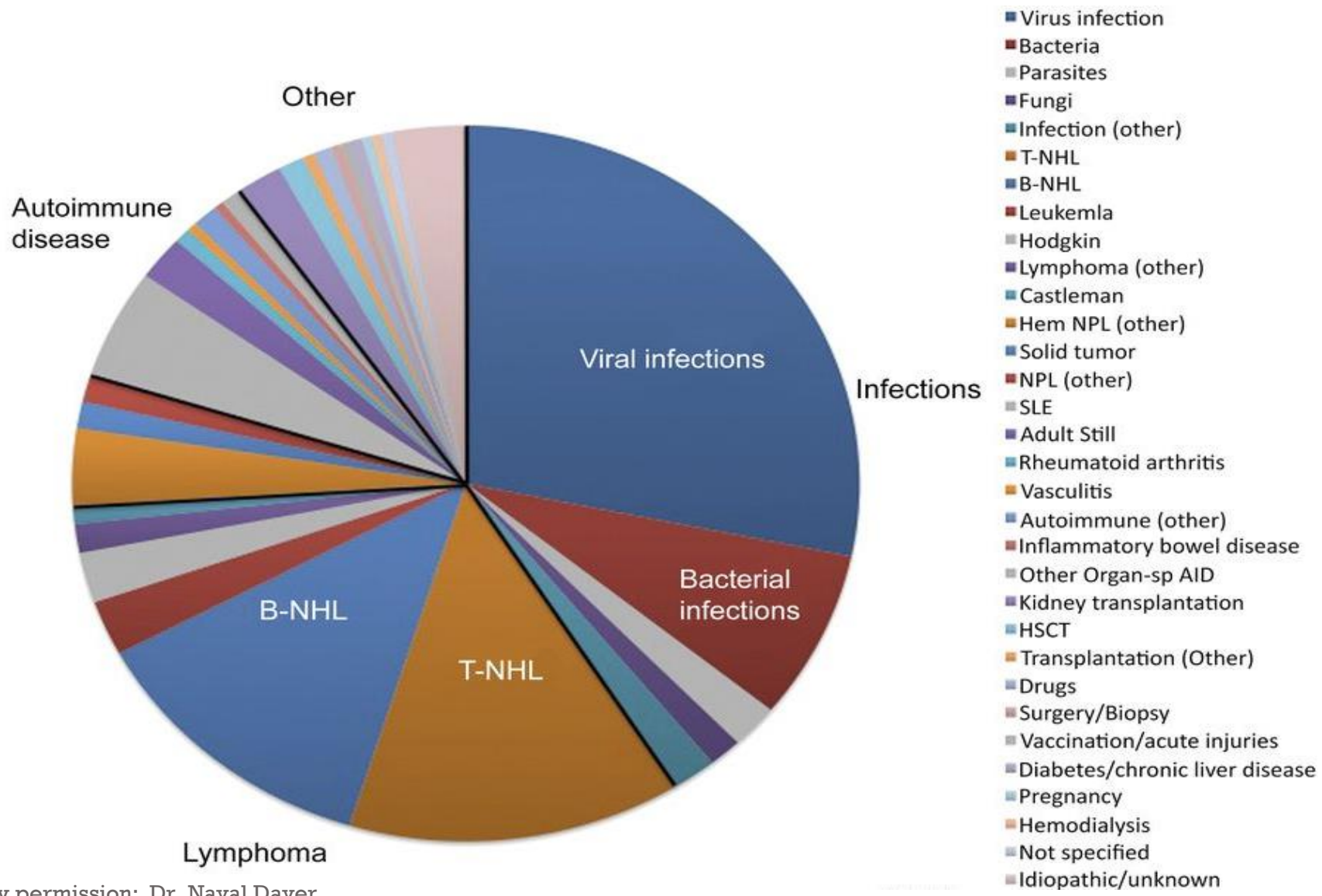

Trigger: active infection, auto-immune disorders, malignancies
IL-1
IL-6
IL-18
TNFα
IL-6
Dendritic cell
"Cytokine storm"
IL-6
IL-18
IFNγ
Macrophage
HLH
CD8+ T cells
NK cells
Cyto-lytic dysfunction

SECONDARY HLH CAUSES

Two Broad Categories

- Triggers that cause immune activation
- Triggers that lead to immune deficiency

Secondary HLH: Causes



By permission: Dr. Naval Daver

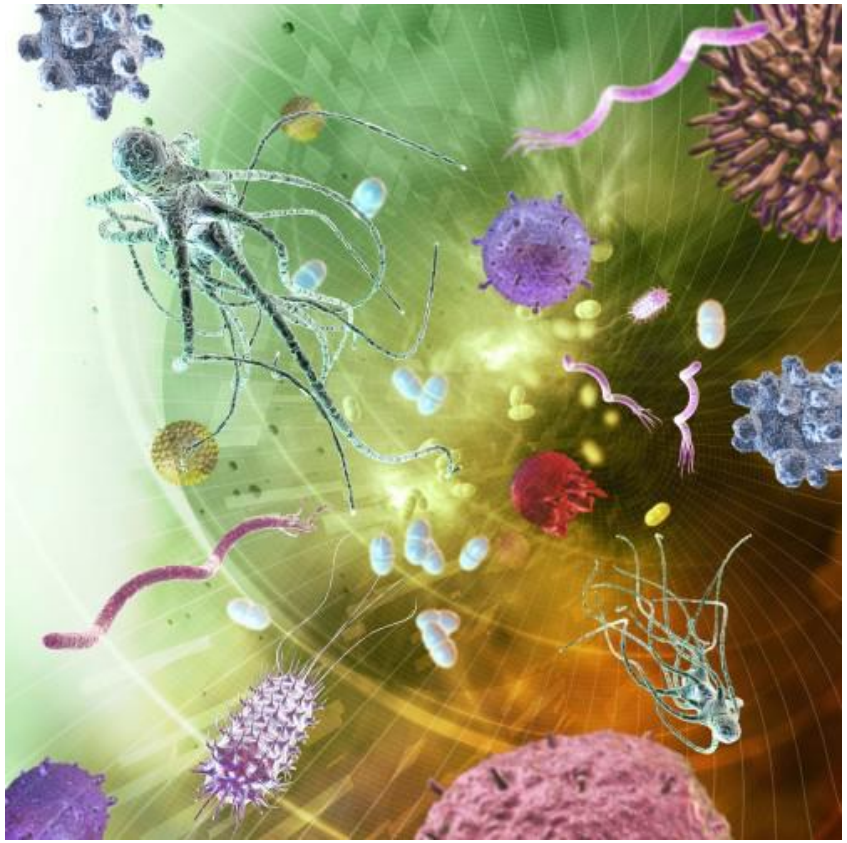
INFECTIONS AND HLH

- Viral
  - EBV
  - CMV
  - Parvovirus
  - HSV
  - VZV
  - HHV6
  - Measles
  - Influenza
  - HIV
  - Covid-19

Bacterial
- Brucella
- GNRs
- TB

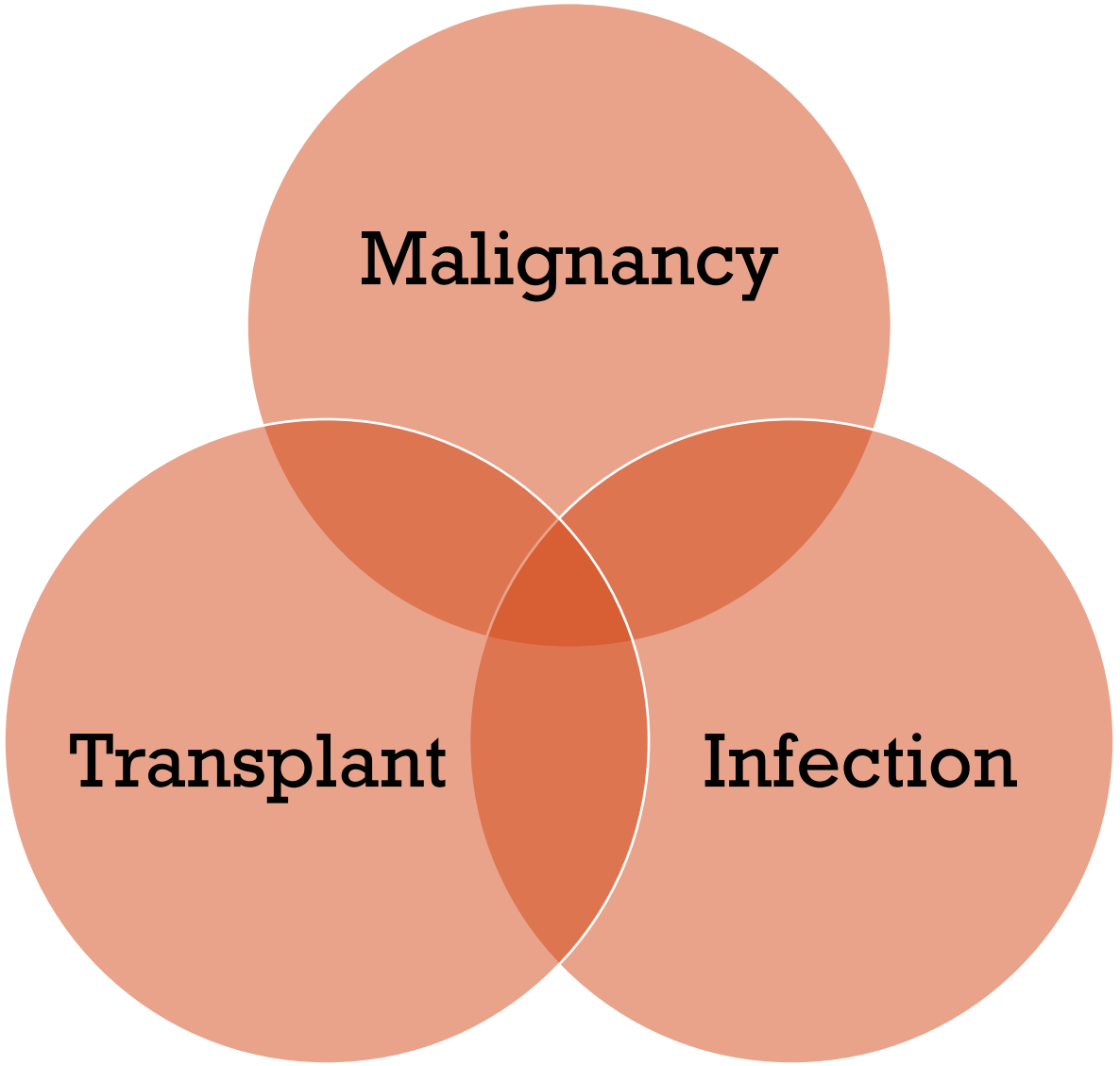
Parasites
- Leishmaniasis
- Malaria

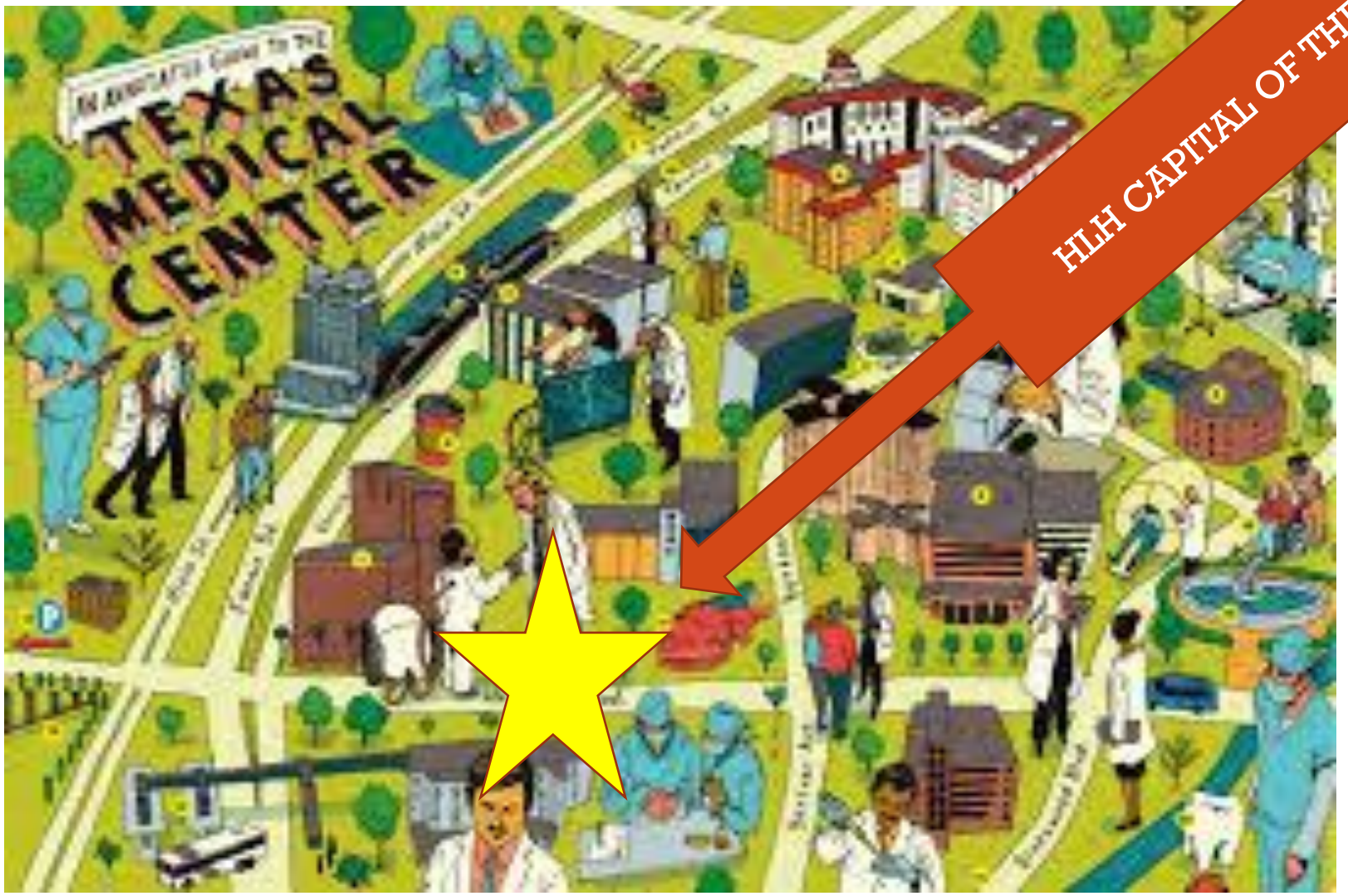
Fungal



Oztrock et al. Am J Hematol 2015.


GEORGE
CLOONEY
MARK
WAHLBERG
THE
PERFECT STORM


Malignancy
Transplant
Infection


TEXAS
MEDICAL
CENTER
HLH CAPITAL OF THE WORLD

EPIDEMIOLOGY

- HLH is primarily a pediatric syndrome, with highest incidence < 3 months of age
- Incidence is 1: 100,000 children
- 25% of HLH cases are familial
- Male-to female ratio1:1 in children, but in adults males have a slightly higher rate
- Adults: review of 2197 cases reported worldwide: 50% were from Japan; supporting an ethnic predisposition of malignancy-related HLH

Ferreri et al. Haematologica 2007.

HLH DIAGNOSTIC CRITERIA

HLH CLINICAL PRESENTATION

- Presents as a febrile illness associated with multiple organ involvement
- Initial signs and symptoms can mimic common infections, fever of unknown origin, hepatitis or encephalitis
- Patients may have already experienced a prolonged hospitalization or clinical deterioration without a clear diagnosis

Table 1. Diagnostic criteria for HLH used in the HLH-2004 trial*

The diagnosis of HLH† may be established:
A. Molecular diagnosis consistent with HLH: pathologic mutations of *PRF1*, *UNC13D*, *Munc18-2*, *Rab27a*, *STX11*, *SH2D1A*, or *BIRC4*
or
B. Five of the 8 criteria listed below are fulfilled:
1. Fever ≥ 38.5°C
2. Splenomegaly
3. Cytopenias (affecting at least 2 of 3 lineages in the peripheral blood)
Hemoglobin < 9 g/dL (in infants < 4 weeks: hemoglobin < 10 g/dL)
Platelets < 100×10^3/mL
Neutrophils < 1×10^3/mL
4. Hypertriglyceridemia (fasting, > 265 mg/dL) and/or hypofibrinogenemia (< 150 mg/dL)
5. Hemophagocytosis in bone marrow, spleen, lymph nodes, or liver
6. Low or absent NK-cell activity
7. Ferritin > 500 ng/mL‡
8. Elevated sCD25 (α-chain of sIL-2 receptor)§

Bergsten et al, Blood 2017

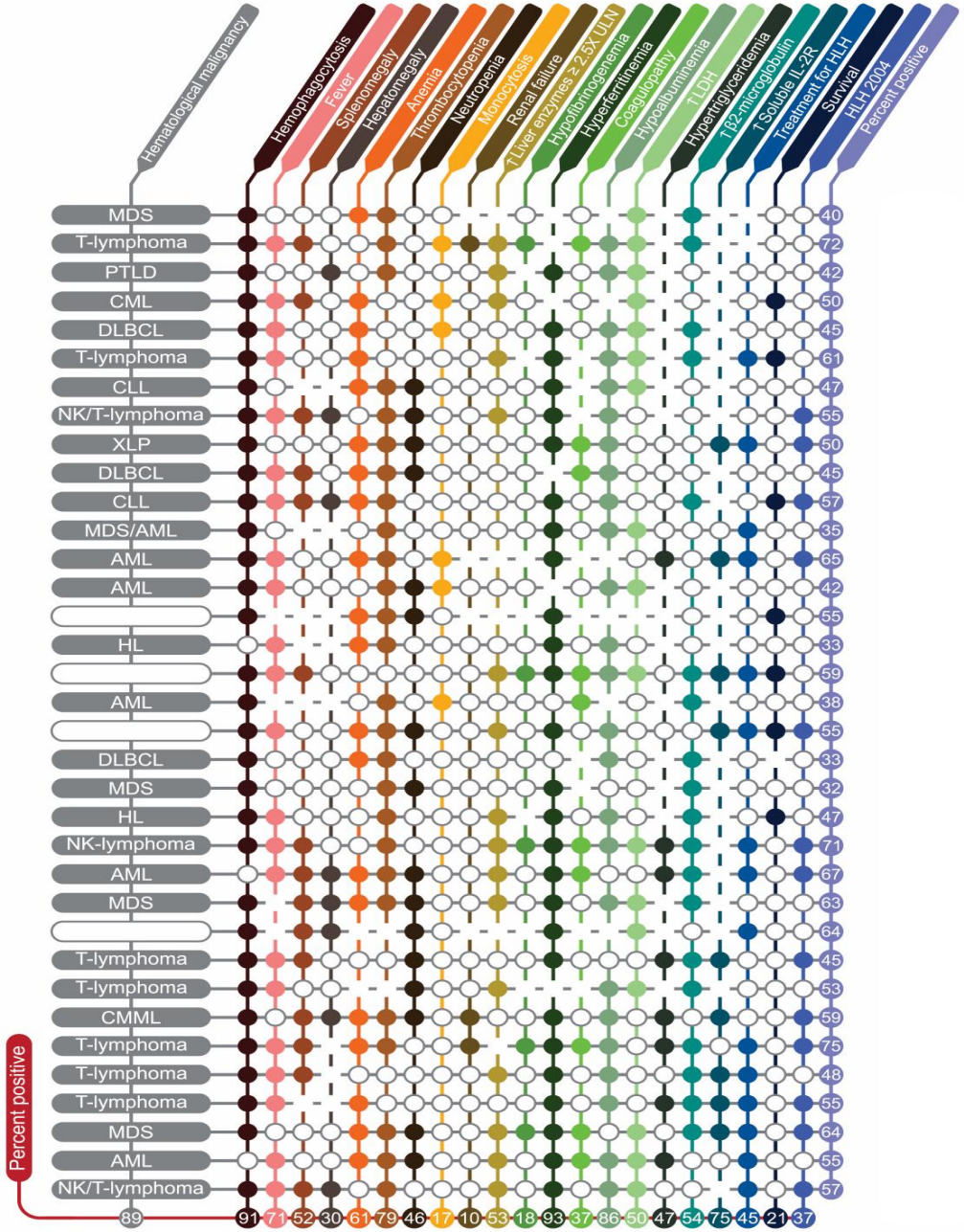
HLH Criteria: HLH-2004 Criteria

HLH-related Criteria	N (%) / Median [range]
Fever	33 (92)
Splenomegaly	26 (72)
Ferritin > 500 ng/mL	36 (100)
Median	52987 [3059 – 471540]
≥ 10,000	30 (83)
≥ 25,000	26 (72)
≥ 50,000	18 (50)
New ≥2 cytopenias	26 (72)
Triglycerides	310 [65 – 929]
Triglycerides ≥ 265 mg/dL	23 (64)
Fibrinogen	200 [27 – 702]
Fibrinogen ≤ 150 mg/dL	16 (44)
Serum IL2R (32/36; 89%)	7010 [749 – 127000]
>2400 U/mL	23/32 (72)
NK function (decreased-profoundly decreased)	12/13 (92)
Hemophagocytosis (18/36; 50%)	14/18 (78)
Bone marrow	12 (86)
Spleen and/or Liver	3 (14)
Molecular (17/36; 47%)*	4/17 (24)

PRESENTATION IN ADULTS - HLH

- Febrile illness
- Multiple organ involvement
- Hepatitis
- Encephalitis
- Bicytopenia (anemia, thrombocytopenia)
- Hypertriglyceridemia
- Hypofibrinogenemia
- Hemophagocytosis
- Low NK cell activity
- Ferritin >500
- Splenomegaly

Extended (Satellite) criteria for M-HLH: N=35



By permission, Dr. Daver.

Characteristics by type of underlying malignancy

Characteristics	Myeloid Malignancies (N=23)	Lymphoid Malignancies (N=11)	p-value
Age	66 [33 – 84]	47 [19 – 59]	**<0.001**
Male	15 (65)	10 (91)	0.21
Fever	21 (91)	10 (91)	>0.99
Splenomegaly	15 (65)	9 (82)	0.43
Hepatomegaly	11 (48)	4 (36)	0.71
CNS symptoms	16 (70)	10 (91)	0.22
Coagulopathy	17 (74)	7 (64)	0.69
Ferritin	75000 [5038 – 471540]	34100 [3059 – 232300]	0.11
LDH	**11480 [1594 – 42000+]**	**4300 [833 – 42000+]**	**0.17**
sIL-2R	5800 [749 – 127000]	16730 [6510 – 93800]	0.38
Fibrinogen	196 [41 – 615]	200 [27 – 325]	0.97
Triglycerides	290 [69 – 788]	418 [65 – 929]	**0.03**
Low NK cell activity	8/8 (100)	3/4 (75)	**0.02**
Clinical hepatitis	16 (70)	11 (100)	**0.06**
Nephritis	16 (70)	3 (27)	**0.03**
IFN-g	6 [0.3 – 340]	43 [9 – 262]	0.13
TNF-a	25 [2.8 – 288]	50 [6.8 – 454]	0.21
CRP	177 [18 – 338]	89 [38 – 232]	0.20
Hemophagocytosis	7/9 (78)	5/7 (71)	>0.99
Response			0.39
CR+PR	5 (22)	3/8 (38)	
NR	18 (78)	5/8 (62)	

By permission, Dr. Daver.

WORK-UP - HLH

WORK UP - HLH

- Ferritin
- IL2-R
- Triglycerides
- CBC
- LFTs
- Fibrinogen
- Bone marrow biopsy
- Viral studies

Jordan et al. 2011.

Why don't you start by ordering a ferritin level?

Ferritin and HLH

- Ferritin levels change dramatically in HLH; they usually do not behave this way in other conditions
- Rate of ferritin decline is a PROGNOSTIC MARKER
  - > 96% drop in 2 weeks
    30% mortality
  - < 50% drop
    68 % mortality

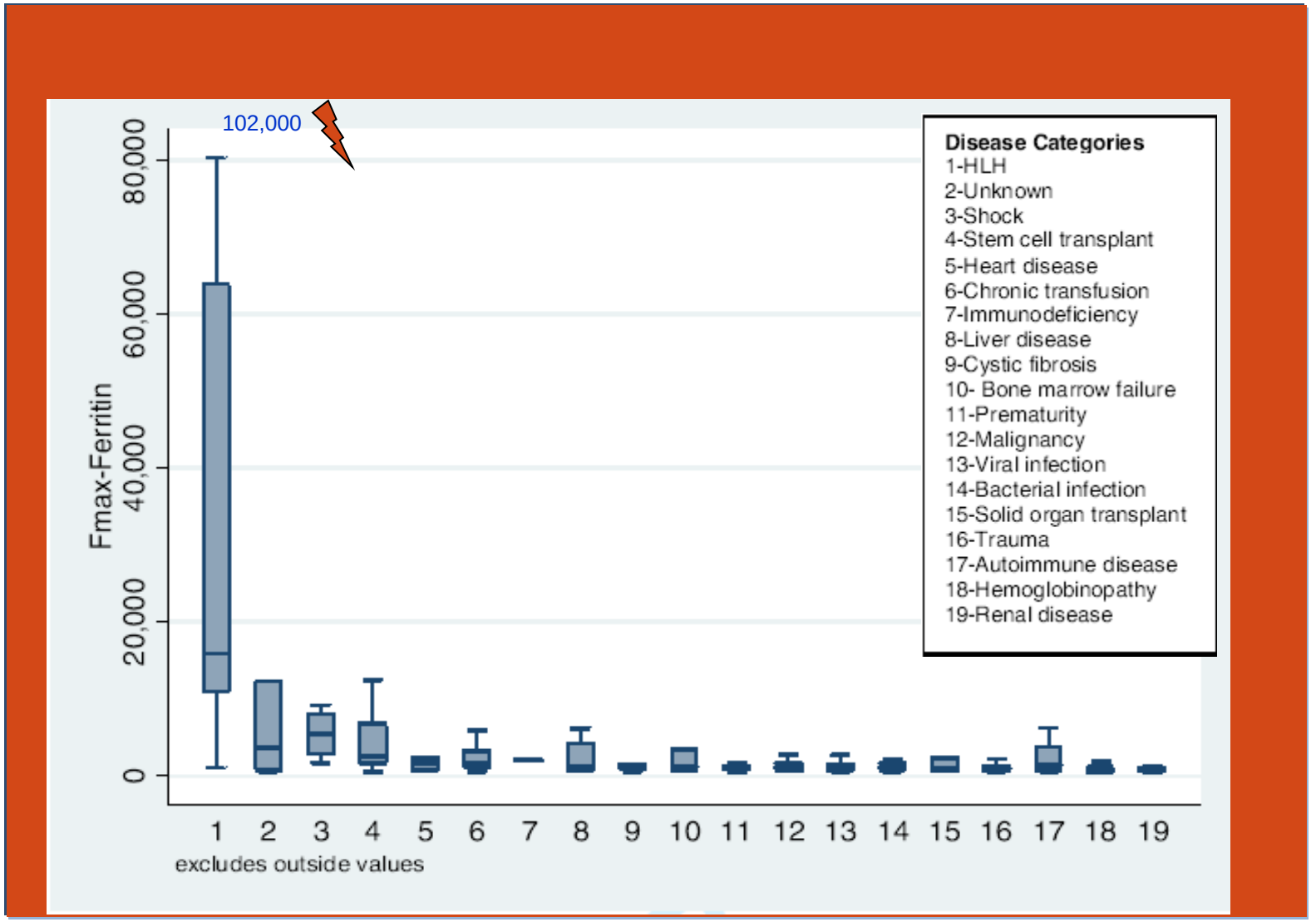
SERUM FERRITIN LEVELS IN HLH

- Very high serum ferritin is common in HLH
- In pediatric patients, >10,000 level was found to be 90% sensitive and 96 % specific

- Macrophages are a primary source of ferritin, which may account for the association between HLH and high ferritin levels.
- A protein responsible for iron hemostasis, called growth differentiation factor 15, is dramatically upregulated in patients with HLH and is responsible for increased serum ferritin

WU et at. Pediatr Blood 2013.

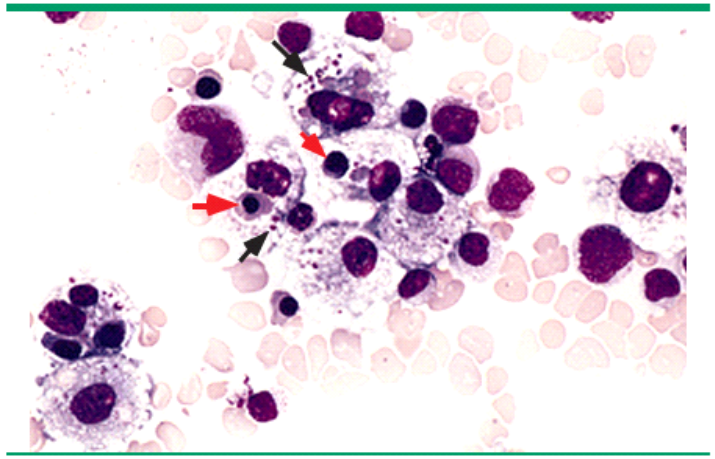
HLH-FERRITIN STUDY (PEDIATRICS)



Allen et al, 2007

BONE MARROW INVOLVEMENT

Infection-associated hemophagocytic syndrome



Bone marrow from a child with hemophagocytic syndrome, secondary to Epstein-Barr virus infection. Reactive histiocytes show phagocytosis of nucleated red blood cells (red arrows) and platelets (black arrows). Wright-Giemsa stain.
From: Brunning RD, McKenna RW. Tumors of the bone marrow. Atlas of tumor pathology (electronic fascicle), Third series, fascicle 9, 1994, Washington, DC. Armed Forces Institute of Pathology.

UpToDate®

Brunning et al, Atlas of Tumor Pathology 2004.

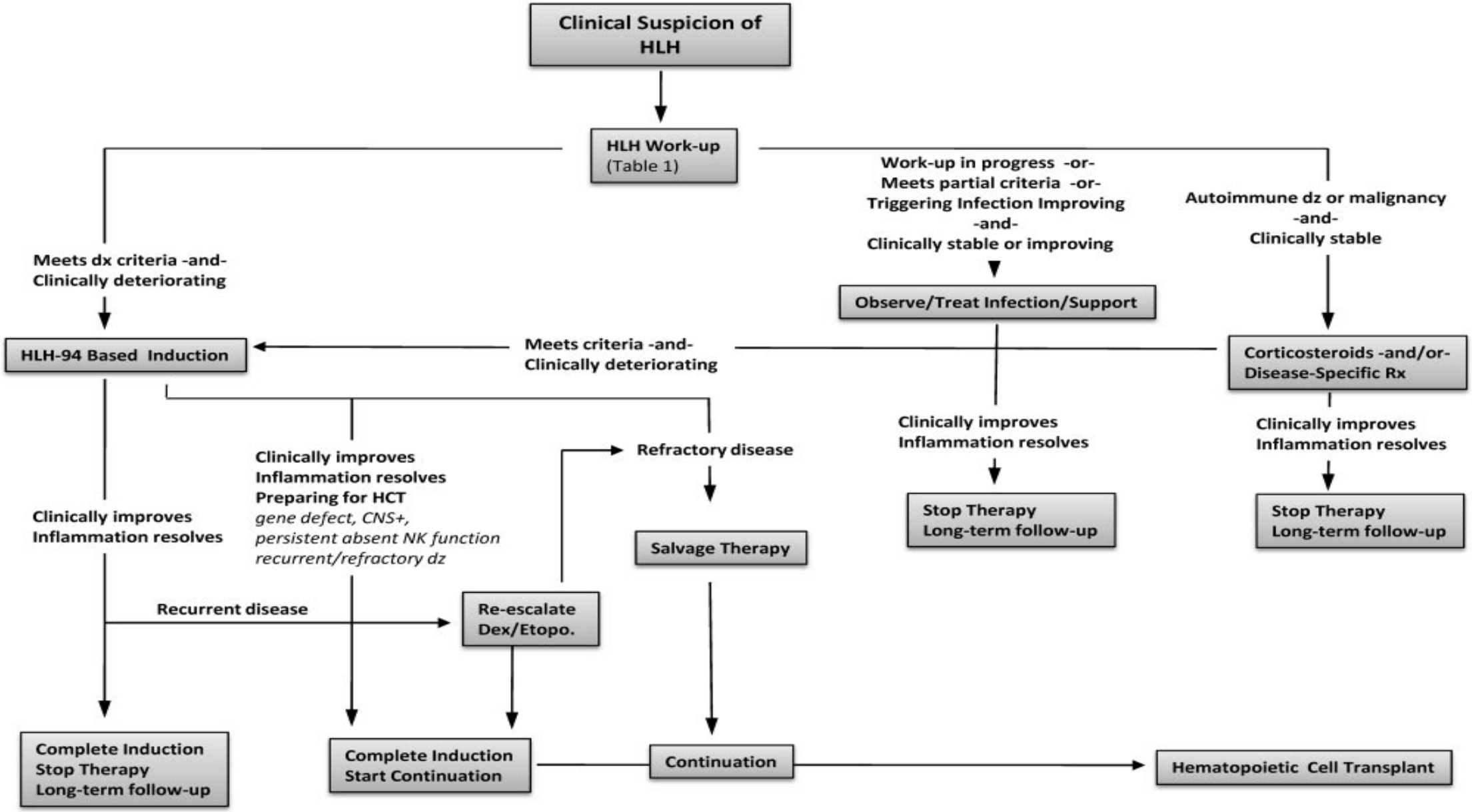
TREATMENT - HLH

Sandler et al, Frontiers in Immunology 2020

TREATMENT - HLH

- HLH-94 Protocol
  - Eight weeks of etoposide and dexamethasone
  - Intrathecal therapy (MTX) for those with CNS involvement
  - +/- cyclosporin (CSA) at week nine
  - SCT
- Trials/other regimens
  - HIT-HLH
  - NCT01104025
  - EURO-HIT-HLH
  - HLH-2004 (etoposide, dexamethasone and CSA initially
  - ATG, steroids, CSA, IT MTX
  - Cyclophosphamide, doxorubicin, vincristine, prednisone (CHOP)
  - Doxorubicin, etoposide, methylprednisone (DEP)

Sandler et al,Frontiers in Immunology 2020



Jordan et al. Blood 2011.

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Emapalumab in Children with Primary Hemophagocytic Lymphohistiocytosis

F. Locatelli, M.B. Jordan, C. Allen, S. Cesaro, C. Rizzari, A. Rao, B. Degar, T.P. Garrington, J. Sevilla, M.-C. Putti, F. Fagioli, M. Ahlmann, J.-L. Dapena Diaz, M. Henry, F. De Benedetti, A. Grom, G. Lapeyre, P. Jacqmin, M. Ballabio, and C. de Min

Emapalumab is a human anti–interferon-γ antibody that binds free and receptor-bound interferon-γ and neutralizes its biologic activity

Administered in Phase 2/3 trial along with dexamethasone.

Conclusions

- Neutralizing interferon-γ and controlling hyperinflammation with emapalumab were efficacious in previously treated children with primary HLH.
- Most patients who received emapalumab in the study proceeded to allogeneic hematopoietic stem-cell transplantation.
- Emapalumab was effective with a low level of toxic effects in patients with primary HLH.
- The study provides support for further investigation of emapalumab in patients with secondary forms of HLH.

Locatelli at al. NEJM 2020.

EMAPALUMUMAB

- On November 20, 2018, the Food and Drug Administration approved emapalumab (GAMIFANT, Novimmune SA), a monoclonal antibody that binds and neutralizes interferon gamma, for adult and pediatric (newborn and older) patients with primary hemophagocytic lymphohistiocytosis (HLH) with refractory, recurrent or progressive disease or intolerance with conventional HLH therapy.

FDA.gov

PROTOCOLS AT MDACC

- 2019-0362: Phase 2/3 Open-label Single Arm, Multicenter Study to Evaluate Emapalumab (monoclonal antibody that binds and neutralizes interferon gamma) in Patients with HLH

- 2014-0989: Phase 2: Alemtuzumab (monoclonal antibody that binds to CD52+) Etoposide & Dexamethasone

- Contact Dr. Naval Daver @ NDaver@mdanderson.org

MDACC HLH monitoring on therapy

Pre-Therapy/Within 3 days of diagnosis	**Within 7 days**	**During Therapy**	**Daily**	Weekly
Ferritin	EBV/CMV PCR	WBC	WBC	LP + CSF studies
CBC	HHV6 PCR	Hgb	Hgb	Cytokine 12
PT	Adenovirus PCR	Plt	Plt	Blood PCR (CMV/EBV)
PTT	CMV Serology	ANC	ANC	Lymphocyte subsets
Fibrinogen	Leishmania Serology	AST	AST	Blood Cx
D-Dimer	HSV PCR	ALT	ALT	Immunoglobulins
AST	VZV Serology	Bili	Bili	Repeat Abnormal Imaging
ALT	Brucella Serology	Albumin	Albumin	
GGT	LP	Na	Na	
Bili	Brain MRI	Uric acid	Uric acid	
Albumin	PET CT Scan	LDH	LDH	
Na	CT (C/A/P)	Triglycerides	Triglycerides	
Uric acid	TTE/MUGA Scan	PT	PT	
LDH	HLA Typing	PTT	PTT	
Triglycerides	SCT consult	D-dimer	D-dimer	
Ferritin	Tissue Biopsy	Fibrinogen	Fibrinogen	
ANA Profile	Genetic studies	Ferritin	Ferritin	
Cytokine 12 panel	PPD	Cytokine 12		
soluble IL2R-alpha (sCD25)	HIV	Immunoglobulins		
sCD163		EBV/CMV PCR		
NK Function		Blood Cx		
T cell subsets		LP + CSF studies		
CD107 mobilization				
PRF1/Granzyme proteins				
Immunoglobulins				
LP + CSF studies				
Blood-cx				
BM + cx + PCR (EBV/CMV)				
Blood-PCR (EBV/CMV)				

Shah A, Daver N et al, BBA 2016

New Kids on the Block

Covid-19

CAR-T

HLH PROGNOSIS

Sandler et al, Frontiers in Immunology 2020

PROGNOSIS – HLH IN ADULTS

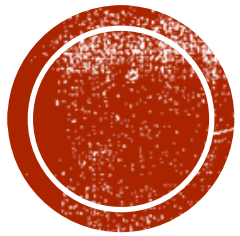
- Prognosis is worse in those with:
  - underlying malignancy
  - older age

- Review of 162 adults with HLH – poor prognostic factors
  - Lymphoma (especially T-cell)
  - Thrombocytopenia
  - Elevated AST/LDH
  - >50 yo

Adult M-HLH Outcomes at Major Centers

Study	Number of patients	Most frequent associated malignancy	6-month survival rate	Median survival (months)
Tamamyam et al.[26] (2016)	35	AML/MDS (n=13), T-lymphoma (n=10), DLBCL (n=6), HL (n=6), CLL (n=4), CML (n=2), Follicular lymphoma (n=2)	30%	2.0
Parikh et al.[23] (2014)	32	T-lymphoma (n=19), DLBCL (n=6), EBV-associated PTLD (n=3), HL (n=1), CMML (n=1), hemangioendothelioma (n=1), systemic histiocytosis (n=1)	Not reported	1.4
Otrock et al.[33] (2015)	21	B-neoplasms (n=10), T-neoplasms (n=6), HL (n=3), AML (n=1), MDS (n=1)	20%	1.1
Lehmberg et al.[25] (2015)	21	T-neoplasms (n=12), B-neoplasms (n=7)	67%	1.2
Machaczka et al.[10] (2011)	8	HL (n=2), MM (n=2), 1 each of B-CLL, PTCL, AILD, WM, ATL	38%	2.4
Shabbir et al.[24] (2011)	6	AML (n=2), T-cell lymphoma (n=2), post auto-SCT for MM (n=2)	Not reported	1.2 (entire adult HLH cohort, n=18)

Daver N, Kantarjian H et al Cancer 2017



CASE STUDIES

CASE STUDY #1

- 46 yo male from Florida diagnosed with multiple myeloma in 12/2015 status post multiple regimens of chemotherapy, autologous SCT 8/22/16, and haploidentical SCT 10/24/19. Complications: PE, BKV
- Brief admission at MDACC 11/21/19-11/24/19 due to severe HA and fever; PET showed new splenomegaly with avid lesions. Discharged with impression that HA was related to IVIG. CMV and EBV negative.
- PET 12/10/19 confirms relapsed MM
- Admission MDACC 12/31/19-1/12/20 for right facial pain; found to have right maxilla plasmacytoma. Received RT (1/3/20) x 8 and started C1 (1/4/20) of cyclophosphamide, carfilzomib, daratuzumab, dexamethasone and Fulphila.
- Admitted to Florida hospital 1/14/20-1/27/20; presented with NF (103), AMS and hypotension requiring low-dose pressor support in ICU. Placed on cefepime/vancomycin. EF noted to be 35%. Increased LFTs/TBil. Developed rash. RVP + adenovirus. Worsening AMS throughout. Due to concern for fungal infection, anidulafungin was started, but stopped due to rash.

CASE STUDY #1

- 1/27/20 transferred to MDACC from OSH.
  - ANC 0.3, Platelets 4
  - Tbil 21.7, ALKP 287, AST/ALT 131/67
  - Rash , AMS
  - ID consulted 1/28: mero/linezolid, LP, ACY for emperical HSV coverage, RVP
  - Ferritin level 43,247, IL 2 2223, Fibrinogen 722, Triglycerides 423, Adenovirus 4.7E+07 (11/4/10 neg), EBV neg, (12/19 < 400, 11/20 493)

  - Did not receive treatment for HLH due to rapid decline, worsening MOF
  - Expired within one month of hospitalization

CASE STUDY #2

- 20 yo from El Paso, TX oil field worker presented with CP, SOB, and pancytopenia found to have right mediastinal mass + mixed germ cell tumor. Transferred to MDACC for further management.
- ID consulted for NNF
- Exam: +HSM
- Labs: pancytopenia, Ferritin 9647, IL2R 35, triglycerides 156, BM: histoplasmosis, hemophagocyctic histiocytes
- Got etoposide x 2 doses with steroids, no improvement
- Patient expired after one month in hospital

CASE STUDY #3

- 61 y.o. female with EBV-related NHL diagnosed in 4/2019, s/p R-CHOP IT MTX on 5/13/19, evaluated at that time by ID for neutropenic fevers with unclear source. After further chemotherapy was undergoing evaluation for autologous SCT in 2/2019, at which time she was admitted for persistent fevers. Ferritin at that time noted to be newly elevated >1000. POD also noted so was started on RICE chemotherapy.
- Starting in 2/2020, patient had multiple admission for fevers, and ID were consulted multiple times for recurrent fevers, during this course her NHL progressed.
- 5/2020 serum EBV and CMV in bone marrow were elevated. BM pathology showed histiocytes with hemophagocytosis. +splenomegaly

Component	Latest Ref Rng & Units	5/4/2020	5/12/2020	5/14/2020	5/16/2020 2:21 AM	5/16/2020 2:44 AM	5/16/2020 3:56 PM	5/18/2020	5/19/2020 3:21 AM	5/19/2020 3:21 PM	5/19/2020 6:25 PM	5/20/2020
Trig	<=149 mg/dL	310 (H)					426 (H)					
Ferritin Lvl	13 - 150 ng/mL	7,503 (H)	5,829 (H)	5,960 (H)		13,339 (H)	15,930 (H)		>100,000 (H)			>100,000 (H)
Fibrinogen	214 - 503 mg/dL	249			308		244	95 (L)	36 (C)	88 (L)	68 (C)	88 (L)
IL 2 Recp(CD25) Sol-Mayo	175.3 - 858.2 pg/mL						80444.0 (H)					

- She was treated with tocilizumab and high dose steroids, developed multiorgan failure and passed away.

CASE STUDY #4

- 45 yo from Houston, TX with PMH, HBV (treated), DM, HTN diagnosed/treated for DLBCL in 20s, remained in remission until 2015, while incarcerated developed fevers, night sweats and weight loss. Presented to MDACC in 10/2015 for management of sepsis/PNA in the setting of DLBCL relapse.
- ID consulted for NF, reactivation of HBV (DNA level 920,000)
- Ferritin peaked at 31,973, IL2R 13,518, WBC 0.7, triglycerides 279, fibrinogen 182
- Ferritin rapidly decreased following treatment for lymphoma
- Last seen in ID clinic for HBV follow-up in 12/2019 and is doing well.

THANK YOU, AUDIENCE

ADDITIONAL REFERENCES

- Henter JI, Horne A, Aricó M, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 2007; 48:124.
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