

## SUPPLEMENTAL FIGURES/TABLES

Supplemental Table 1 – Specialized Laboratory & Biomarker Testing in HLH/MAS						
Test	In HLH/MAS	Biology	Criteria	Monitoring Frequency	Prognostic Utility*	Caveats
Bone Marrow Biopsy	↑	Identify HPCs, evaluate for malignancy	1,3	n/a	√ <sup>1</sup>	HPC visualization aided by CD163 IHC
NK-cell killing	↓	NK cell killing of cell line	1	n/a		abnl in illness, meds, NK cytopenia
Procalcitonin	↑	Adipokine		prn		
IL-6	↑	Pleiotropic inflammatory cytokine		R, prn		
IFNg	↑	Classic Type 1/Th1 cytokine		R, prn	√ <sup>2</sup>	
Neopterin	↑	Metabolite of GTP, induced by IFNg		R, prn		
CD163	↑	Macrophage activation		prn	√ <sup>3</sup>	
CD107a mobilization	↓	Functional test of degranulation		n/a		a.k.a. LAMP1
Flow cytometry	↓	Detect specific protein deficiency (e.g. Perforin, SAP, XIAP)		n/a		
HPC=hemophagocyte (macrophage that has engulfed other blood cells); IHC=immunohistochemistry; NK=Natural Killer; IL=Interleukin; IFNg=Interferon gamma						
*=Degree of abnormality and/or failure to improve correlated with worse outcomes						
1=HLH-04 <sup>4</sup> , 2=MAS-2016 <sup>5</sup> , 3=H-score <sup>6</sup>						
F=frequent (e.g. daily), I=Intermittent (e.g. weekly), R=Rarely (e.g. monthly), PRN=as needed, n/a=not applicable						

Supplemental Table 2: Comparison of HLH/MAS Criteria			
Test	HLH-04 <sup>4a</sup>	MAS-2016 <sup>5</sup>	H-Score <sup>6e</sup>
Fever	✓	✓ <sup>d</sup>	<38.4 / 38.4-39.4 / >39.4
Ferritin (ng/mL)	>500	>684 <sup>d</sup>	<2000 / 2000-6000 / >6000
Splenomegaly	✓		Y/N
Hepatomegaly			Y/N
Neutrophils (cells/uL)	<1000 <sup>b</sup>		Leukocytes <1000
Hemoglobin (g/dL)	<9 <sup>b</sup>		<9.2
Platelet Count (10 <sup>9</sup> /L)	<100 <sup>b</sup>	<182	<110
AST (U/L)		>48	>30
Triglycerides (mg/dL)	>265 <sup>c</sup>	>156	<133 / 133-354 / >354 <sup>f</sup>
Fibrinogen (mg/dL)	<150 <sup>c</sup>	<361	<250
Hemophagocytosis	✓		Y/N
Low/absent NK-cell killing	✓		
Soluble IL-2Ra (CD25) U/L	>2400		
Immunosuppression			Y/N

Developed primarily in	children with primary and secondary HLH	sJIA-MAS	adults with cancer or infection
<sup>a</sup> 5 of 8  <sup>b</sup> as part of cytopenias affecting 2 or more lineages  <sup>c</sup> part of same criterion  <sup>d</sup> Required, plus any 2 of 4 other parameters  <sup>e</sup> point system, see <a href="http://saintantoine.aphp.fr/score/">http://saintantoine.aphp.fr/score/</a>  <sup>f</sup> converted from mmol/L  AST=aspartate aminotransferase; sJIA=systemic juvenile idiopathic arthritis			

## METHODOLOGY SUPPLEMENT

### Part I. PICO questions

### Part II. Systematic Literature Review search terms and filtering strategy

#### Part I. PICO Questions

##### TERMINOLOGY

**PICO-1a:** For patients in the early, undefined stages of possible HLH/MAS, what terminology compared to the term HLH/MAS best describes these patients?

- P: For patients in the early, undefined stages of possible HLH/MAS,  
I: Selecting terminology  
C: Compared to the term HLH/MAS  
O: Patient description

- a. HLH/MAS
- b. HLH/MAS Syndrome
- c. Hyperinflammation
- d. Hyperinflammatory Syndrome
- e. HLH/MAS Spectrum Disorder
- f. Hemophagocytic Syndrome
- g. HLH/MAS-Like Syndrome
- h. Inflammatory Immune Dysregulation
- i. Hyperinflammatory Immune Dysregulation
- j. Hemophagocytic Lymphohistiocytosis
- k. Hyperinflammatory Lymphohistiocytosis
- l. Cytokine Storm Syndrome
- m. Hyperferritinemic Inflammation
- n. Systemic Inflammation
- o. Hyperferritinemic Systemic Inflammation
- p. T Cell Activation Disorder
- q. Hypercytokinemic Inflammatory Syndrome

**PICO-1b:** For patients with proven HLH/MAS, what terminology compared to the term HLH/MAS best describes these patients?

P: For patients with proven diagnosis of HLH/MAS,

I: Selecting terminology

C: Compared to the term HLH/MAS

O: Patient description

- a. HLH/MAS
- b. HLH/MAS Syndrome
- c. Hyperinflammation
- d. Hyperinflammatory Syndrome
- e. HLH/MAS Spectrum Disorder
- f. Hemophagocytic Syndrome
- g. HLH/MAS-Like Syndrome
- h. Inflammatory Immune Dysregulation
- i. Hyperinflammatory Immune Dysregulation
- j. Hemophagocytic Lymphohistiocytosis
- k. Hyperinflammatory Lymphohistiocytosis
- l. Cytokine Storm Syndrome
- m. Hyperferritinemic Inflammation
- n. Systemic Inflammation
- o. Hyperferritinemic Systemic Inflammation
- p. T Cell Activation Disorder
- q. Hypercytokinemic Inflammatory Syndrome

**PICO-1c:** Which patients are suitable candidates for term macrophage activation syndrome or be terminology compared to the term HLH to indicate the pathophysiology?

P: Patients with proven HLH/MAS

I: Suitable for term “macrophage activation syndrome”,

C: Compared to HLH

O: Suitable terminology

- a. Patients with rheumatologic disorders
- b. Patients with autoinflammatory disorders

- c. Patients with persistent and significant serum elevation of IL-18 levels.

**PICO-1d:** In patients with known genetic predisposition to HLH/MAS, what terminology best describes the inborn errors of immunity such as pathogenic variants of PRF1, UNC13D, STX11, STXBP2, RAB27A, LYST, SH2D1A, XIAP/BIRC4, CD27, and others as a predisposing factor for the development of HLH/MAS?

P: Patients with known genetic predisposition to HLH/MAS,

I: Describe the inborn errors of immunity

C: Compared to the term HLH/MAS

O: Best Terminology

- a. Primary HLH
- b. Genetic HLH
- c. Primary HLH Disease
- d. Genetic HLH Disease
- e. Primary HLH Disorder
- f. Genetic HLH Disorder

#### INITIAL SUSPICION

**PICO-2:** In acutely ill patients, what routine clinical/laboratory characteristics should raise concern for possible HLH/MAS compared to no HLH/MAS and prompt further work up?

P: Acutely ill patients

I: Clinical/laboratory characteristics that raise concern for possible HLH/MAS

C: Patients without HLH/MAS

O: Identify tests that can prompt further work up for HLH/MAS?

- a. Persistent fever
- b. Cytopenias in at least two lineage
- c. Inappropriately low WBC and/or platelet counts relative to the degree of inflammation
- d. Hepatitis or liver failure
- e. Coagulopathy
- f. Encephalopathy or seizures of unknown cause
- g. Splenomegaly
- h. Hepatomegaly
- i. Elevated CRP
- j. Elevated ESR

- k. Elevated Ferritin
- l. Known immunologic disorder
- m. Known infection or malignancy

#### FERRITIN

**PICO-3a:** In patients with suspected HLH/MAS, can we use elevation of ferritin compared to non-elevation to identify patients more likely to have a syndrome of HLH/MAS?

- P: Patients with suspected HLH/MAS
- I: Ferritin measurement
- C: Patients without HLH/MAS
- O: Screening of HLH/MAS

**PICO-3b:** In patients with suspected HLH/MAS, what ferritin level elevation compared to values below that threshold strengthens the likelihood of a syndrome of HLH/MAS?

- P: Patients with suspected HLH/MAS
  - I: Minimum ferritin level
  - C: Other causes of elevated ferritin level.
  - O: Higher likelihood of HLH/MAS?
- 
- a. Ferritin >500
  - b. Ferritin >684
  - c. Ferritin >1,000
  - d. Ferritin >2,000
  - e. Ferritin >10,000
  - f. No firm diagnostic threshold can be used for all patients.
  - g. Other [free text]

**PICO3c:** In patients suspected of HLH/MAS, is an elevated ferritin alone compared to grouped with other HLH related biomarkers such as soluble IL-2R a sufficient and reasonable screening test to trigger HLH work-up (and conversely, does a normal ferritin mean rule out the need for HLH/MAS work up).

- P: Patients suspected of HLH/MAS
- I: Measurement of ferritin
- C: Compared to ferritin grouped with other HLH related biomarkers such as soluble IL-2R

O: Serve as sufficient and reasonable screening test for HLH/MAS

**PICO-4:** In patients with suspected HLH/MAS and an elevated ferritin, which laboratory investigations are essential for general clinicians to obtain during the initial evaluation compared to specialty labs or invasive evaluations that are informative but not essential or not readily available to further strengthen the likelihood of a diagnosis of a syndrome of HLH/MAS.

P: Patients with suspected HLH/MAS and elevated ferritin

I: Essential laboratory investigations

C: Compared to specialty labs or invasive evaluations that are informative but not essential or not readily available

O: To further strengthen the likelihood of a diagnosis of a syndrome of HLH/MAS?

- a. CBC + Differential
- b. Liver Panel (AST, ALT, Bilirubin, GGT)
- c. ESR
- d. CRP
- e. Soluble IL-2R
- f. Triglycerides
- g. Fibrinogen
- h. LDH
- i. Ddimer
- j. Bone marrow aspirate and biopsy
- k. NK cell function (Cr51 release assay)
- l. T cell HLA-DR expression
- m. Soluble CD163
- n. Neopterin
- o. CXCL9
- p. IL-18
- q. Perforin
- r. CD107a
- s. SAP
- t. XIAP

#### **ETIOLOGIC WORK-UP**

**PICO-5:** In patients with presumed HLH/MAS, which triggering factor(s), should be immediately investigated compared to standard assessments (i.e. vital signs, standard tests) to establish contributors to the development of a syndrome of HLH/MAS?



P: In patients with presumed HLH/MAS

I: Immediate investigation of underlying condition(s) and/or triggering factor(s), stratified by clinical and geographic indications

C: Standard assessment (i.e. vital signs, standard tests)

O: to establish factors contributing to the development of a syndrome of HLH/MAS?

- a. Blood Culture
- b. Urine Culture (if symptomatic or young child)
- c. EBV PCR
- d. CMV PCR
- e. HHV6 Plasma PCR
- f. Adenovirus PCR
- g. HSV PCR
- h. HIV testing
- i. SARS-CoV-2 PCR during pandemic
- j. Influenza PCR if symptomatic and appropriate season
- k. Histoplasma urine antigen if appropriate geographic location or travel history
- l. Leishmania PCR testing if appropriate geographic location or travel history
- m. Tick-borne illness testing if appropriate geographic location/season
- n. Peripheral smear and bone marrow aspirate and biopsy to evaluate for lymphoma and leukemia if cytopenias are present
- o. Imaging studies of the brain, neck, chest, abdomen, and pelvis to evaluate for infections and malignancies
- p. Appropriate investigations of any imaging abnormalities that are suspicious for malignancy or infection
- q. ANA
- r. Lymphocyte subsets
- s. Neutrophil oxidative burst
- t. IgG
- u. Perforin protein expression testing
- v. CD107a testing
- w. Urine organic acids
- x. Plasma amino acids
- y. SAP protein expression testing (male patients)
- z. XIAP protein expression testing (male patients)
- aa. IL-18 if not previously done
- bb. CXCL9 if not previously done
- cc. Interferon alpha and/or beta

## **GENETIC TESTING**

**PICO-6a:** In patients with presumed HLH/MAS, which patient or clinical features should prompt testing for an underlying genetic cause of HLH/MAS predisposition?

- P: In patients with presumed HLH/MAS,
- I: Patient characteristics or clinical features
- C: Absence of patient or disease features
- O: Support genetic testing for an underlying genetic cause of HLH/MAS
- a. Severe disease
  - b. Recurrent disease (history of 2 or more episodes)
  - c. Refractory disease
  - d. Suggestive family history
  - e. Albinism
  - f. History of recurrent infections
  - g. History of progressive or persistent neurologic dysfunction, developmental delay, or hearing loss
  - h. History of previous inflammatory problems such as inflammatory bowel disease

**PICO-6b:** In patients with presumed HLH/MAS, patients in which age category may benefit from genetic testing due to genetic disorder associated with a predisposition to HLH/MAS compared to not likely to have a genetic disorders and suggest that genetic testing should be pursued?

- P: In patients with presumed HLH/MAS,
- I: Age category likely to have a genetic disorder as a predisposition to HLH/MAS
- C: Compared to patients without genetic predisposition to HLH/MAS
- O: Get genetic testing
- a. Infants
  - b. Children
  - c. Adolescents
  - d. Young Adults
  - e. Adults

**PICO-6c:** In patients with presumed HLH/MAS requiring genetic testing, which approach to genetic testing is most appropriate to establish a potential underlying genetic disorder?

- P: In patients with presumed HLH/MAS requiring genetic testing
- I: Most appropriate genetic testing
- C: Lower yield genetic testing
- O: To establish a potential underlying genetic disorder

- a. NGS Panel to evaluate genes that are considered as causes of genetic HLH diseases at the time of testing
- b. NGS Panel to evaluate genes that cause inborn errors of immunity (including primary immune deficiencies, primary immune regulatory diseases, and autoinflammatory diseases) other than those considered as causes of genetic HLH diseases at the time of testing
- c. NGS Panel to evaluate genes that cause inborn errors of metabolism
- d. Whole exome or whole genome sequencing

**PICO-6d:** In patients with suspected HLH/MAS, should the following clinical features or medical center capability/access to NGS testing versus absence of these features or capabilities lead to genetic testing for limited single gene or few gene testing in place of NGS panel testing?

P: In patients with suspected HLH/MAS,

I: should the following clinical features or medical center capability/access to NGS testing

C: versus absence of these features or capabilities

O: lead to genetic testing for limited single gene or few gene testing *in place of NGS panel testing?*

Pigment abnormality

Inflammatory Bowel Disease

Presence of Lymphoma

Family history of a specific genetic disorder

Lack of access to NGS Testing Panels or Whole Exome or Whole Genome testing

#### **DISEASE PROGNOSIS/SEVERITY**

**PICO-7a:** In patients with a clinical diagnosis of the syndrome of HLH/MAS, the presence of which clinical manifestations versus their absence suggest poor prognosis?

P: Patients with suspected HLH/MAS

I: Clinical features at presentation

C: Absence of these features

O: Indicative of poor prognosis (higher mortality, increased length of admission, long term sequelae, longer ICU stay)

- a. Underlying Active Lymphoma
- b. Active Malignancy other than lymphoma
- c. CNS involvement
- d. Need for ICU admission at the time of presentation

- e. Renal failure at presentation
- f. Underlying rheumatic disease other than sJIA and Still's (Lupus, Dermatomyositis, and Vasculitis)
- g. Prior Immune suppressive medication use (malignancy, transplant, autoimmune diseases)
- h. Liver Failure
- i. Multiple Organ Dysfunction (more than 1 organ failure)
- j. Presence of EBV infection
- k. Other infections

**PICO-7b:** In patients with a clinical diagnosis of a syndrome of HLH/MAS, the presence of which laboratory biomarker observations may indicate worsening disease?

- P: Patients with a clinical diagnosis of HLH/MAS
- I: Laboratory or biomarker abnormality
- C: Normal or expected value
- O: Indicative of disease worsening
- a. High or rising CRP
  - b. New or worsening DIC markers (d-dimer, PT/INR, Fibrinogen,...)
  - c. High or rising LDH
  - d. High or rising liver enzymes (AST, ALT) or bilirubin
  - e. High or rising ferritin
  - f. Low or dropping platelet count
  - g. Low or dropping WBC
  - h. Low or dropping neutrophil count
  - i. High or rising IL-18 (when/where available)
  - j. High or rising soluble IL-2R (when/where available)
  - k. High or rising CXCL9 (when/where available)

### **CNS DISEASE**

**PICO-9a:** In patients with probable HLH/MAS, which of the following factors suggest that patients should be screened for CNS involvement, versus not, to establish the presence or absence of CNS disease?

- P: Patients with probable HLH/MAS
- I: Suggestive findings of CNS involvement
- C: No clinical CNS features
- O: Presence of CNS disease

- a. Age 2-5 years
- b. Age 6-10 years
- c. Age 11-18 years
- d. Adults
- e. Seizures
- f. Encephalopathy/Altered Mental Status/Irritability
- g. Meningismus
- h. Headaches
- i. Vision Changes
- j. Motor Defects
- k. Known Genetic HLH Disease (PRF1, UNC13D, etc)

**PICO-9b:** In early HLH/MAS patients who are screened for CNS involvement, screening with the following tests, versus not, should be performed.

- P: Patients with early HLH/MAS screened for CNS involvement
- I: Which tests
- C: No testing
- O: CNS disease diagnosis
- a. Brain MRI
  - b. Spine MRI
  - c. Lumbar puncture for cell count, differential, glucose, ...
  - d. Lumbar puncture for pathologic review
  - e. EEG

### EARLY TREATMENT

**PICO-10a:** In patients with early suspected or probable HLH/MAS syndrome, what clinical/laboratory features, versus their absence, indicate the need for treatment of HLH/MAS syndrome despite ongoing diagnostic workup?

- P: Patients with early, suspected, or probable HLH/MAS
- I: Clinical/laboratory features
- C: No specific features
- O: Need for early treatment
- a. Any organ failure (respiratory, cardiac, CNS, renal, liver)
  - b. Rapidly or persistently worsening liver function

- c. Rapidly or persistently worsening coagulopathy
- d. Rapidly or persistently worsening CNS disease
- e. Rapidly or persistently worsening cytopenias
- f. Need for ICU admission
- g. Rapidly or persistently rising ferritin
- h. Rapidly or persistently rising CRP
- i. Rapidly or persistently rising soluble IL-2R
- j. Underlying known rheumatic disease
- k. Underlying known malignancy
- l. Underlying known genetic HLH disorder
- m. Underlying known inborn error of immunity that may contribute t...
- n. Underlying known metabolic disease

**PICO-10b:** In patients with early suspected or probable HLH/MAS syndrome, which evaluations should be completed prior to treatment with glucocorticoids, chemotherapy, or lymphodepleting therapies, versus not completed, to avoid hindering diagnostics for

malignancy?

- P: Patients with early, suspected, or probable HLH/MAS
- I: Evaluations completed before start of specific treatments
- C: No specific pre-treatment testing
- O: Avoid hindering treatment.

- a. If cytopenias are present, peripheral smear and bone marrow aspirate and biopsy
- b. Imaging studies of the brain, neck, chest, abdomen, and pelvis to evaluate for infection and malignancy
- c. Biopsy of any imaging abnormality suspected to be malignant

**PICO-10c:** In patients with early suspected or probable HLH/MAS syndromes undergoing evaluations including infectious and malignancy evaluations, what therapeutics are appropriate to give (if available), versus not appropriate, to improve patient status with the least likelihood of causing harm or hindering diagnostics?

- P: Patients with early, suspected, or probable HLH/MAS undergoing diagnostic work-up
- I: Appropriate treatments
- C: therapeutics that increase likelihood of harm of hinder diagnosis
- O: improve patient status

- a. Dexamethasone
- b. Methylprednisolone or prednisone
- c. IVIG
- d. Anakinra
- e. Etoposide
- f. Ruxolitinib
- g. Emapalumab
- h. Tocilizumab
- i. ATG
- j. Alemtuzumab
- k. Cyclosporine
- l. Tacrolimus
- m. Plasmapheresis

**PICO-10d:** In patients treated for suspected or probable syndrome of HLH/MAS, which of the following tests should be included in regular monitoring of disease activity, compared to symptom-driven evaluation only, to monitor treatment response and flares?

P: Patients with early, suspected, or probable HLH/MAS

I: Regular testing

C: Symptom-driven testing

O: Monitor treatment response and flares

- a. CBC + differential
- b. Liver Panel (ALT, AST, Bilirubin, GGT)
- c. Ferritin
- d. ESR
- e. CRP
- f. Soluble IL-2R
- g. Triglycerides
- h. Fibrinogen
- i. LDH
- j. Bone marrow aspirate and biopsy in the setting of cyto...
- k. NK cell function (Cr-51 release assay)
- l. T cell HLA-DR expression
- m. Soluble CD163
- n. Neopterin
- o. CXCL9
- p. IL-18
- q. Serial physical assessment of hepatosplenomegaly
- r. PT/INR

- s. Monitoring of need for invasive support measures (int...
- t. Monitoring of need for blood products (increase or de...
- u. Temperature monitoring

### **MULTIDISCIPLINARY APPROACH**

**PICO-11a:** For patients with suspected or probable HLH/MAS syndrome, should the evaluation and care be led by a multidisciplinary HLH/MAS expert team, compared to individual non-specialist physicians, to optimize diagnostics and care?

- P: Patients with early, suspected, or probable HLH/MAS
- I: Evaluation and Care by Multi-disciplinary Expert Team
- C: Individual specialist or nonspecialist.
- O: Optimize diagnostics and care.

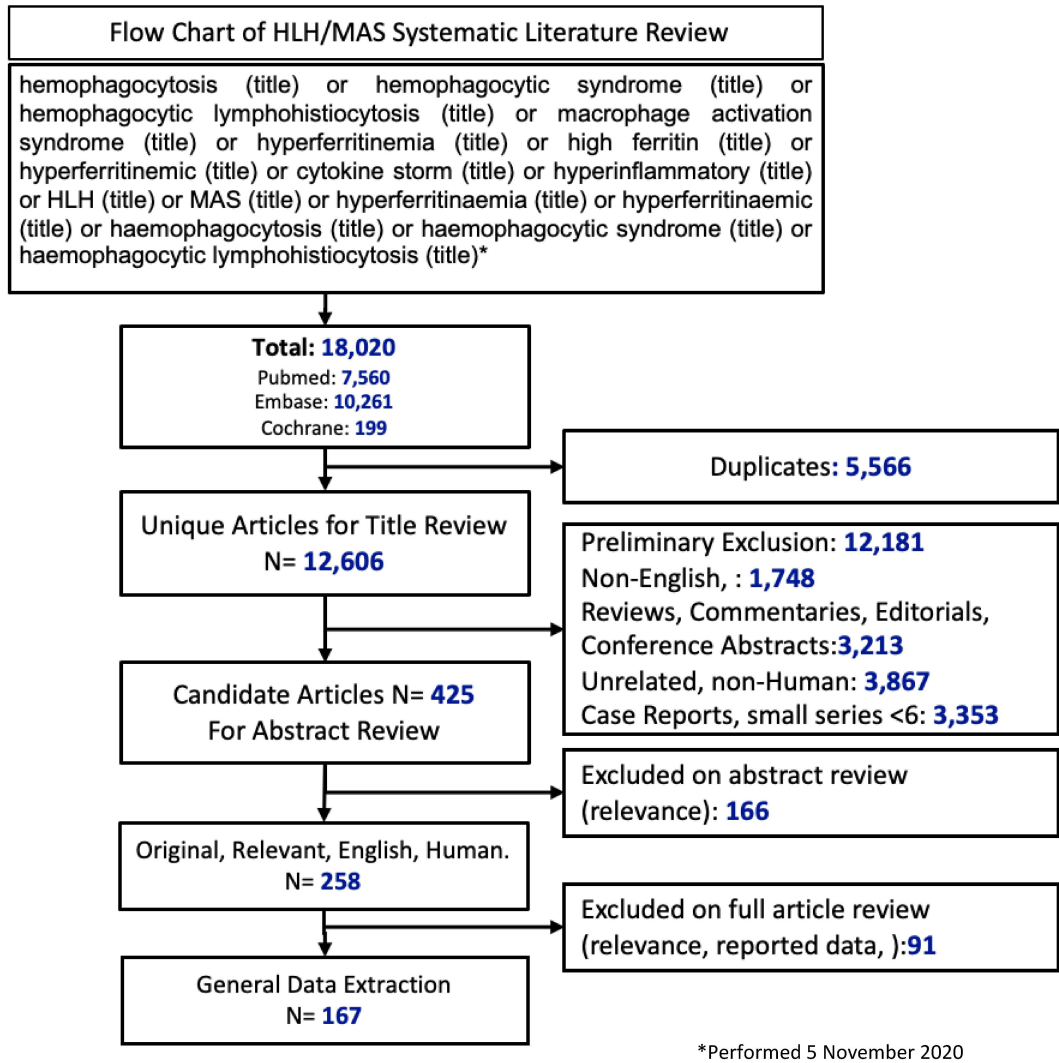
**PICO-11b:** In patients with suspected or probable HLH/MAS syndrome, does a multi-disciplinary HLH/MAS team that routinely includes the following members, versus their absence, best fulfill the needs of patients with HLH/MAS?

- P: Patients with early, suspected, or probable HLH/MAS
- I: Predefined specialists/experts in the multi-disciplinary team
- C: non-specific group of specialists/experts
- O: Best address the clinical care needs of the patients.

- a. Hematologist/oncologist
- b. Immunologist
- c. Rheumatologist
- d. Infectious disease physician
- e. Neurologist
- f. Intensivist
- g. Geneticist
- h. Genetic counselor
- i. Social Worker
- j. Nurse care manager or mid-level nurse practitioner
- k. Pharmacist



Part II.



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