Ferritine glycosylée et autres marqueurs biologiques des syndromes hémophagocytaires

Debaugnies France

Laboratoire d'Immunologie IrisLab, Bruxelles

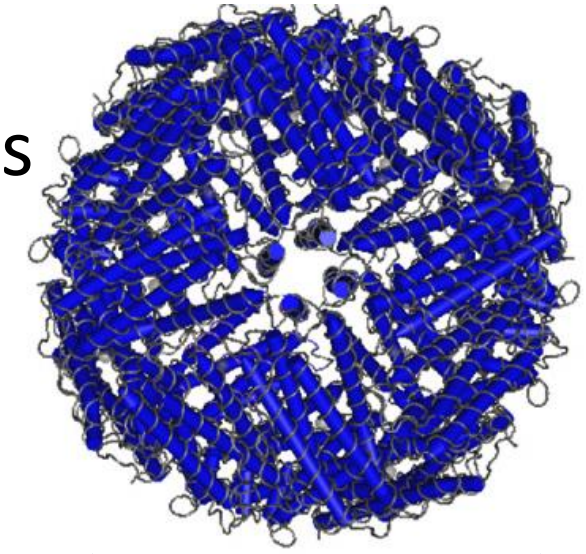
23/9/15

iris-Lab

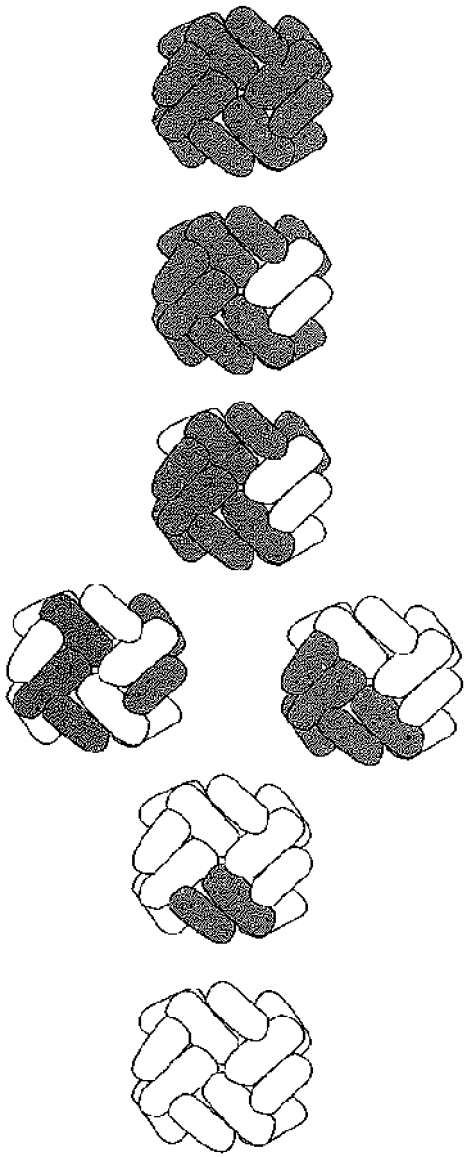
Ferritine

- Glycoprotéine de 440 à 800 kDa
- Principalement intracellulaire
- peut contenir jusque 4500 atomes de fer (Fe^{+++})

- Hépatocytes et macrophages
  = compartiment
  de stockage du fer



Ferritine



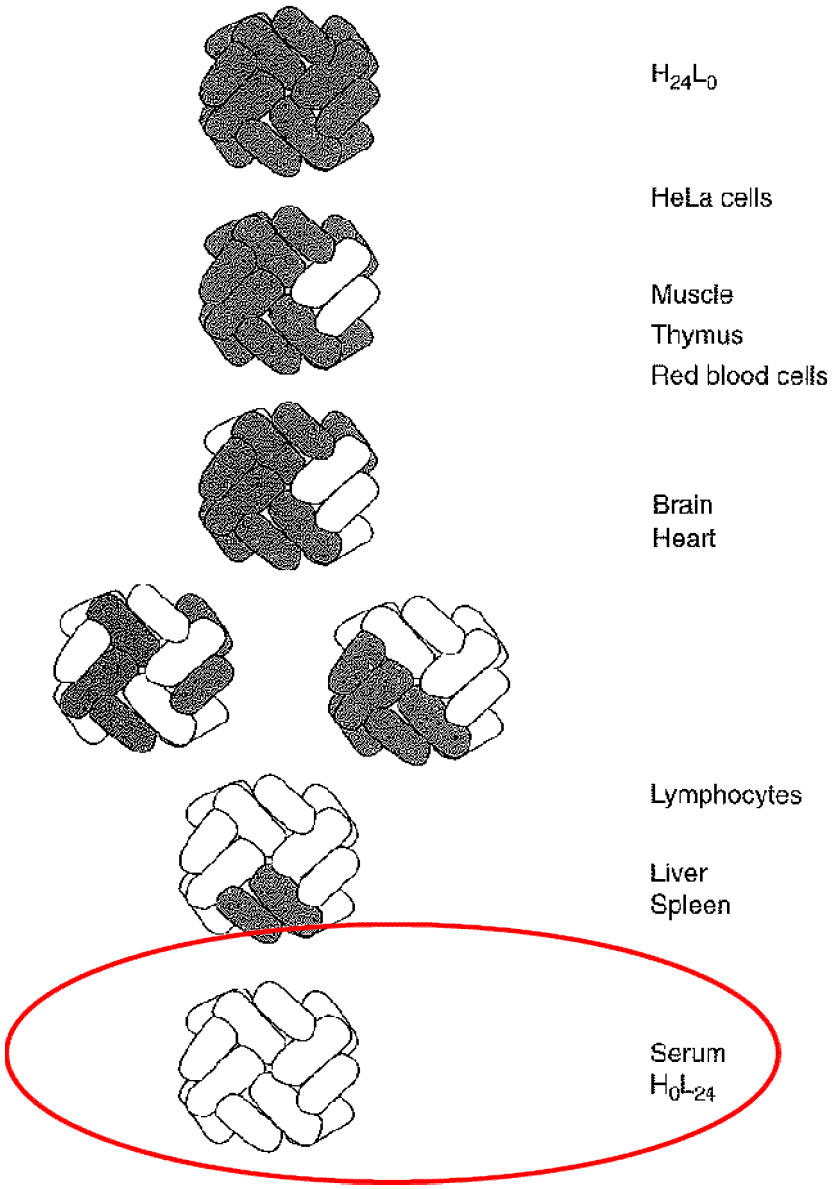
24 sous unités L (19kDa)
H (21 kDa)

Sous-unités L:
stockage à long terme du fer
→ Foie, cellules du système réticulo-endothélial

Sous-unités H:
Turn-over rapide du fer
→ GR, myocytes

Biochim Biophys Acta 1996;1275:161-203

Ferritine sérique

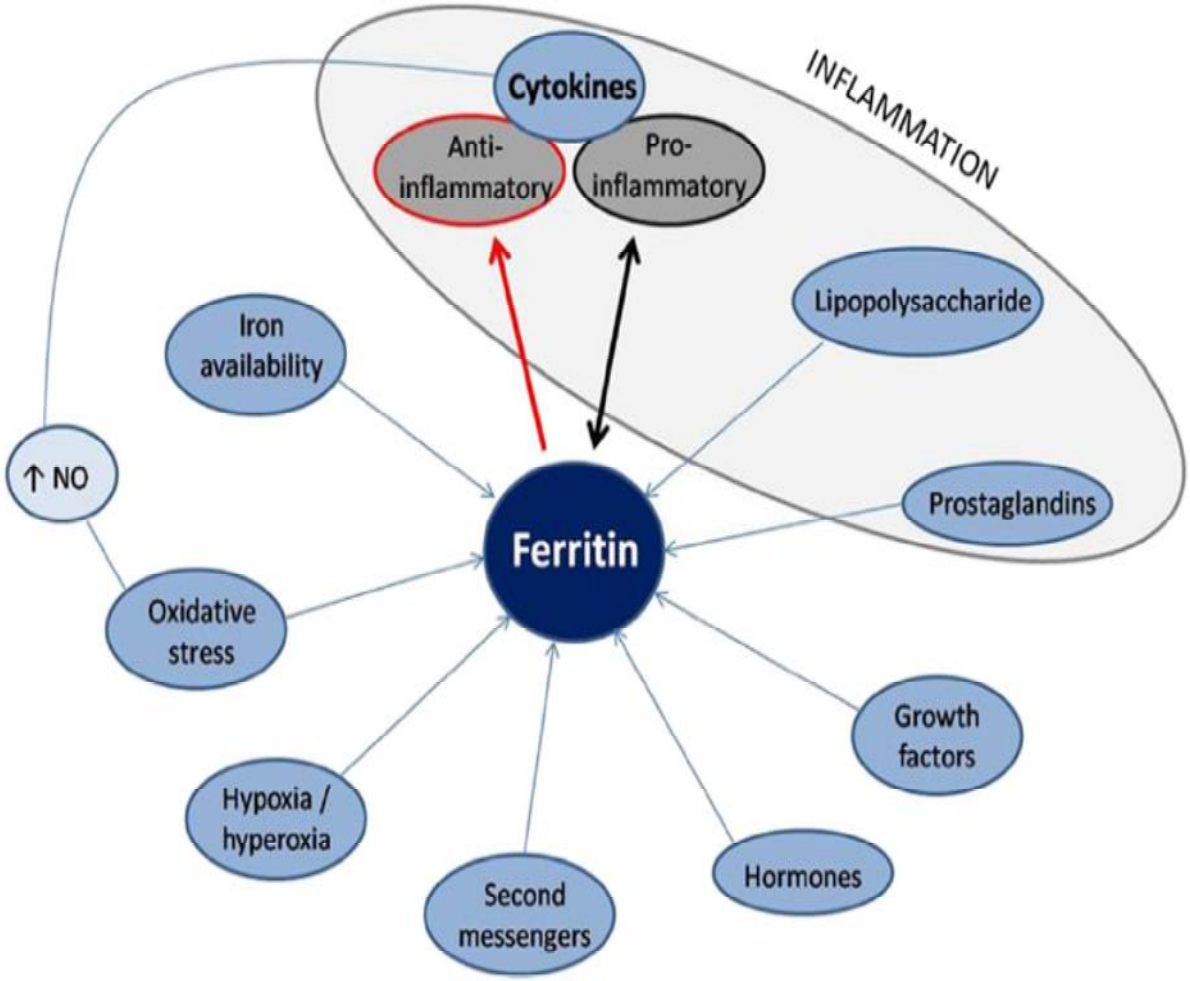


Biochim Biophys Acta 1996;1275:161-203

- **30-350 µg/L**
- Apoferritine pauvre en fer formée de sous unités de type L en partie glycosylée
- Sujet sain : **50-80%** ferritine sérique glycosylée

(basé sur sa capacité à lier la concanavalline)

Contrôle de la synthèse de la ferritine



BMC Medicine 2013;11:185

Ferritine sérique

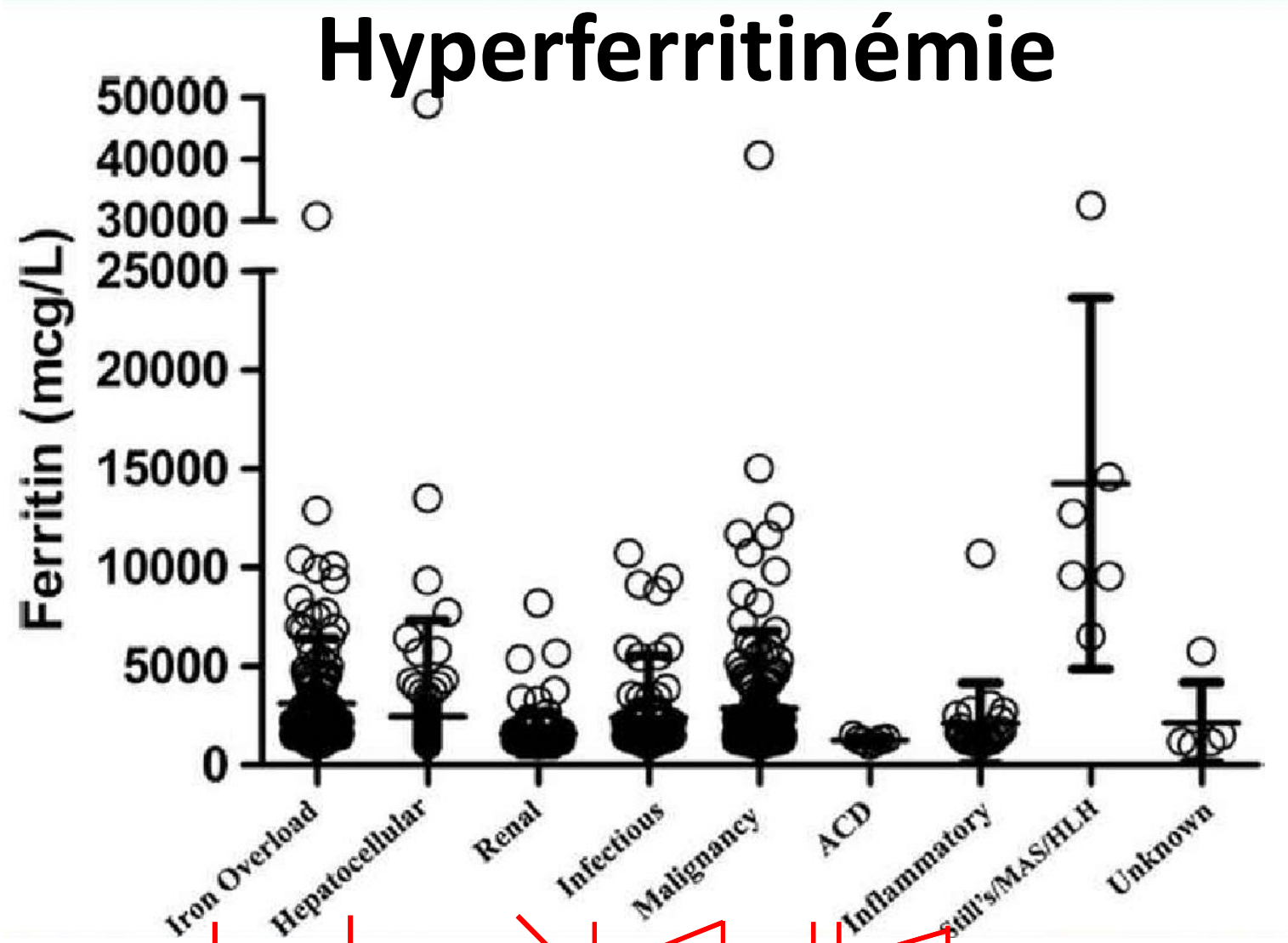
Origine: processus de sécrétion actif et régulé + cellules lysées

Origine cellulaire?

Sécrétion de ferritine de type L sous forme N-glycosylée à partir des **hépatocytes** par la voie classique (RE-Golgi) *Blood 2004, 103 (6) 2369-2376*

Sécrétion à partir des **macrophages** par des lysosomes sécrétoires et glyquée secondairement. *Blood 2010, 116 (9) 1574-1584*

Hyperferritinémie



J Clin Rheumatol 2013;19:324

- A quoi correspondent ces « syndromes d'hyperferritinémie »?
- Quelle est la place du dosage de la ferritine dans le diagnostic du syndrome hémophagocytaire?
- Et la ferritine glycosylée?

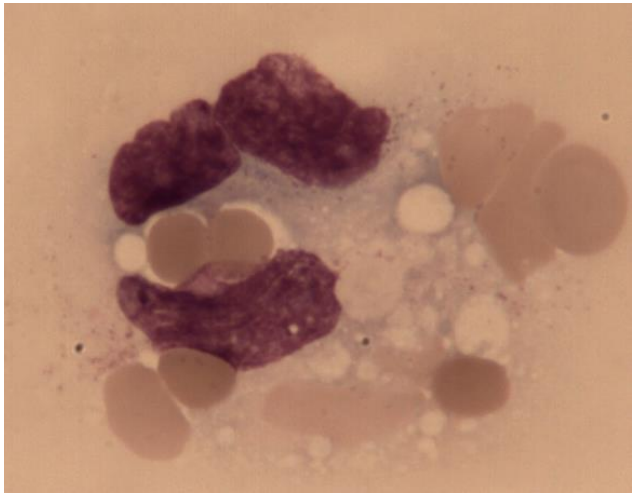
Syndrome hémophagocytaire

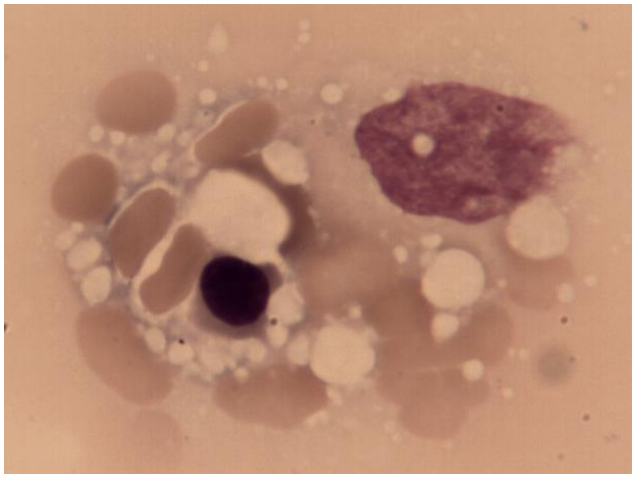
Définition:
Affection sévère résultant d'une stimulation anarchique et non contrôlée du système immunitaire:

des cellules cytotoxiques et des macrophages activés
avec **hémophagocytose**
et production+++ de cytokines proinflammatoires

aboutissant à une infiltration et une
destruction de multiples organes

=HYPER-INFLAMMATION SYSTEMIQUE





Syndrome hémophagocytaire

hemophagocytic syndrome

Å Hémophagocytose lymphohistiocytaire **(HLH)**

hemophagocytic lymphohistiocytosis

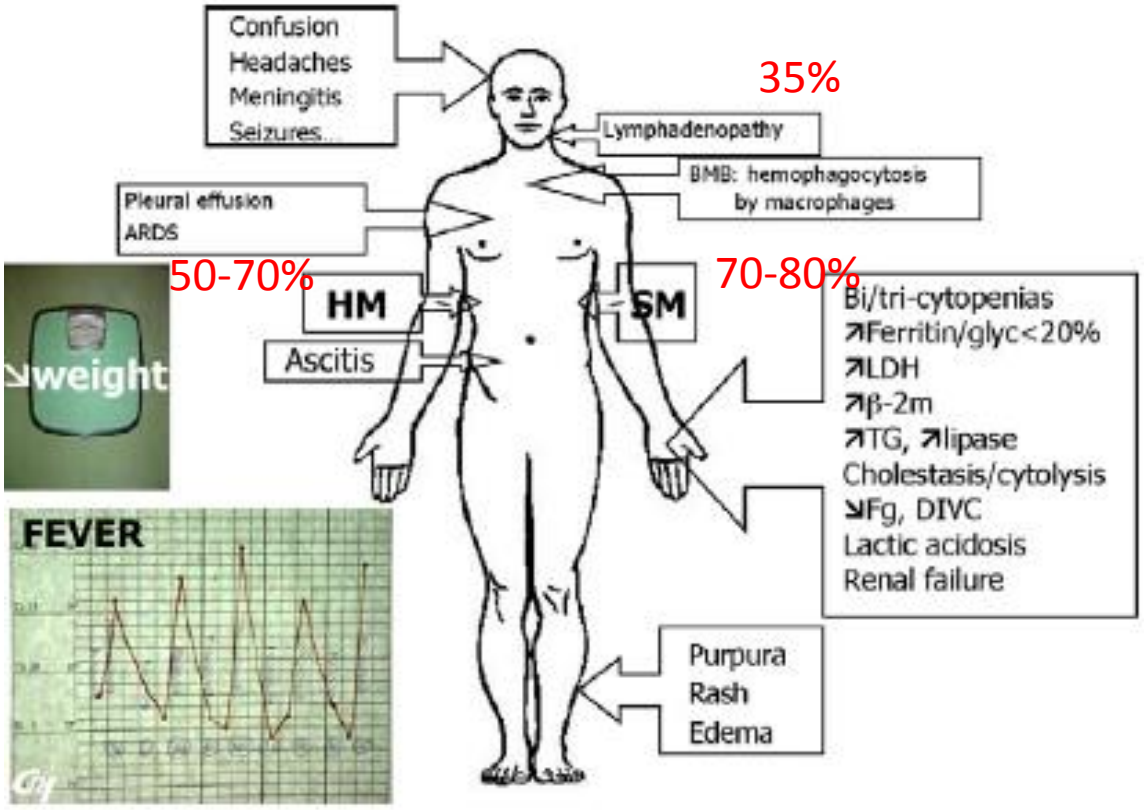
Å Syndrome d'activation lymphohistiocytaire

Å Syndrome d'activation (lympho-) macrophagique *macrophage activation syndrome*

→ 2air à une pathologie autoimmune ou autoinflammatoire

Association de signes cliniques et biologiques **non spécifiques**

signes cliniques

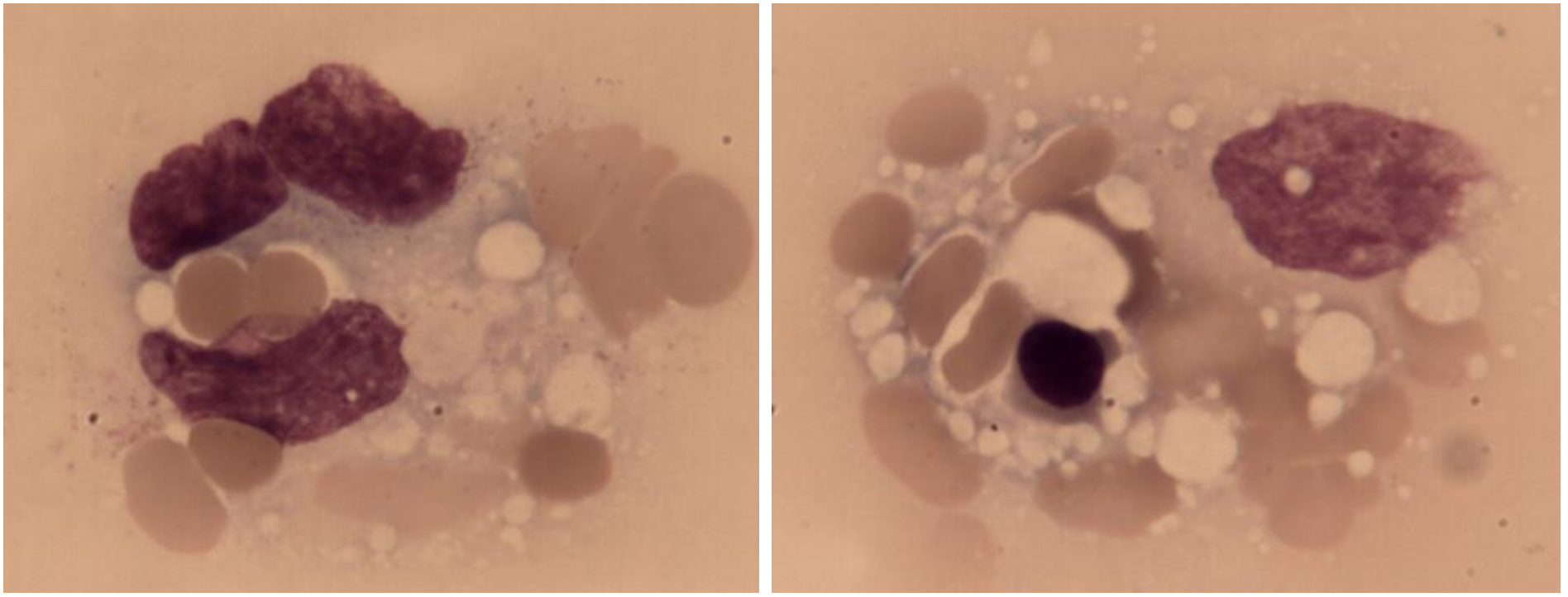


Laroche. Joint Bone Spine 2012

Paramètres biologiques

	Adultes				Enfants	
	n = 775 Ramos-Casals et al. Lancet 2014	**n = 103** Li et al. Medicine 2014	**n = 73** Otrock et al AJH 2014	**n = 20** CHU Brugmann, STP et Bordet	**n=38 FHL** Henter et al. Pediatr Blood Cancer 2015	**n = 16** Huderf
Bi ou pancytopénie	NR	98%	85%	95%	100%	81%
Hb <9g/l	67%	59%	NR	100%	NR	81%
PLT < $100x10^9$/L	78%	86%	NR	95%	NR	88%
PMN <1 $x10^9$/L	42%	74%	NR	75%	NR	69%
Triglycerides ≥ 265 mg/dl	42%	89%	71%	63%	85%	47%
Fibrinogen ≤ 1.5 g/L	48%	61%	38%	60%	79%	38%
Ferritin ≥ 500 ng/ml	90%	98%	100%	100%	90%	100%
AST≥30 UI/L	57%	84%	NR	100%	79%	94%
Hemophagocytosis	85%	NR	77%	70%	67%	69%

Les images d'hémophagocytose ne sont ni spécifiques ni sensibles pour le diagnostic d'HLH.



Sensibilité:83% Spécificité: 60% *Ann Clin Lab Sci 2012;42:21-25*

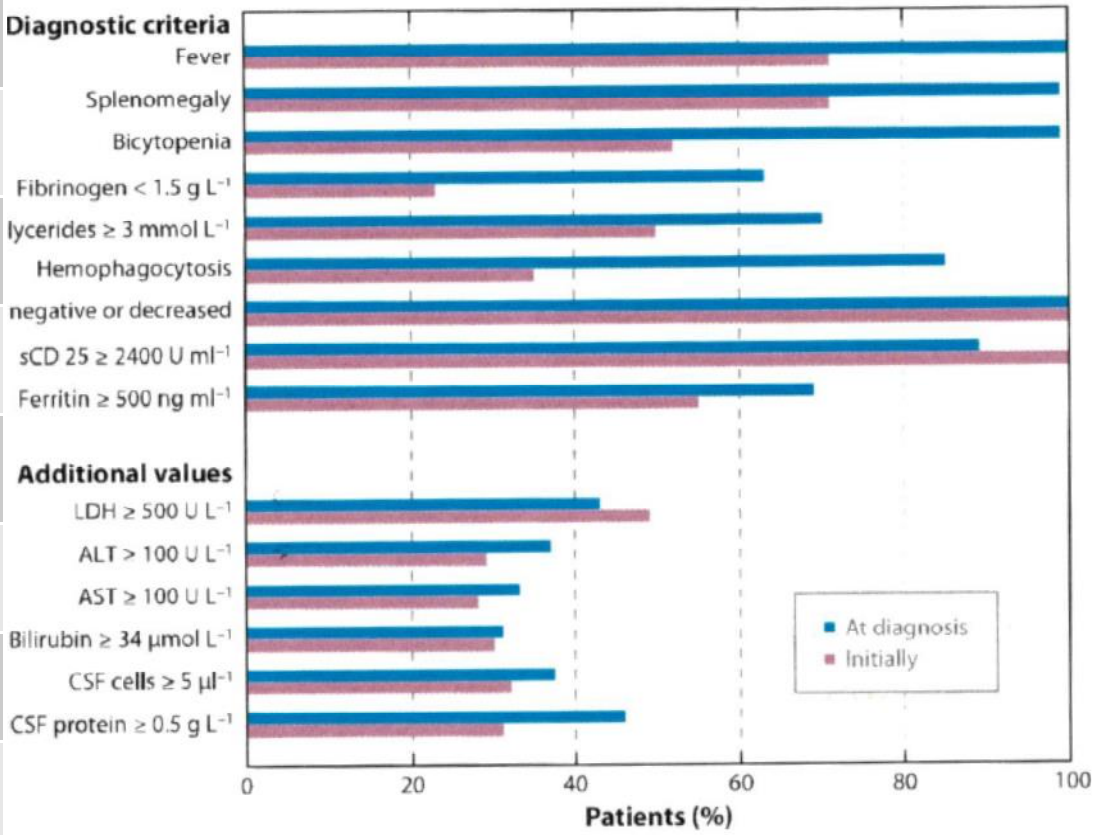
Comment diagnostiquer un syndrome hemophagocytaire?

Association de signes cliniques et biologiques **non spécifiques**

Seul l'intensité des anomalies cliniques et biologique et l'évolution des symptômes vont devenir caractéristiques au cours du développement de la pathologie

L'évolution des paramètres au cours du temps est un élément essentiel

	ADULTES n = 20 CHU Brugmann, STP et Bordet		**ENFANTS n = 16** Huderf	
Bi ou pancytopénie	75%	95%	69%	81%
Hb <9g/l	65%	100%	56%	81%
PLT < $100x10^9$/L	90%	95%	81%	88%
PMN <1 $x10^9$/L	50%	75%	44%	69%
Triglycerides ≥ 265 mg/dl	47%	63%	36%	47%
Fibrinogen ≤ 1.5 g/L	41%	60%	7%	38%
Ferritin ≥ 500 ng/ml	100%	100%	100%	100%
AST ≥30 UI/L	95%	100%	81%	94%



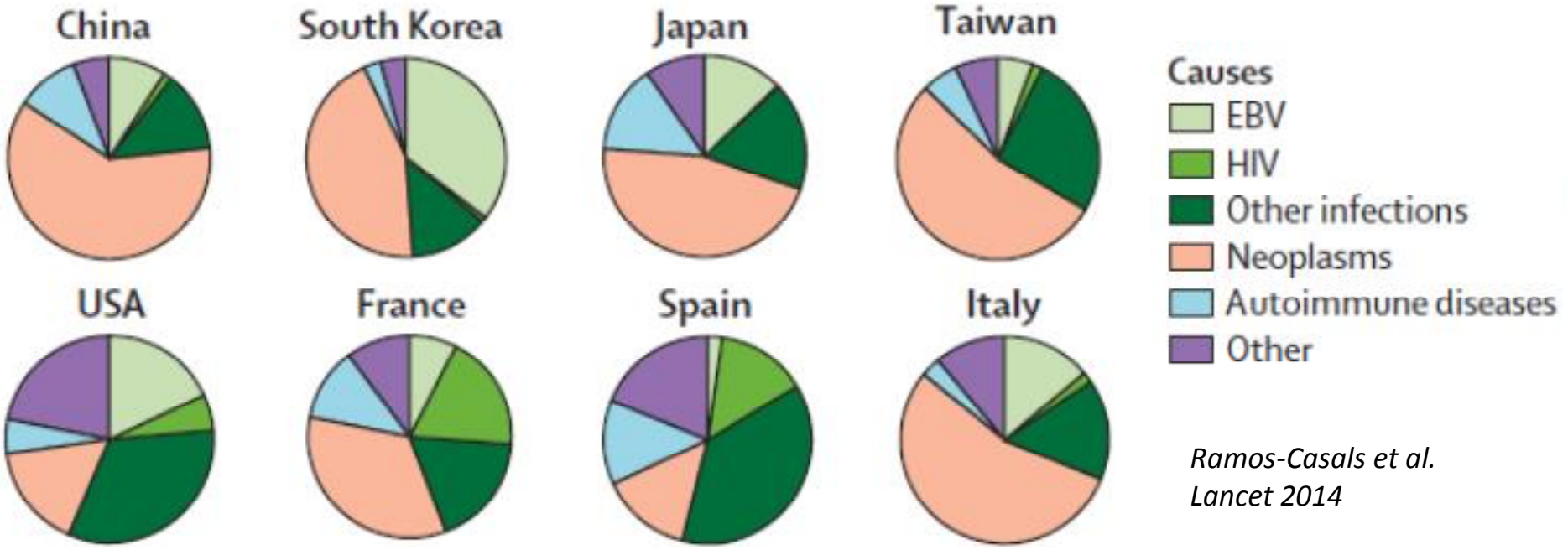
Janka GE. Annu Rev Med 2012

Quelles en sont les causes?

		Genetic defects
Familial HLH	Primaire	*PRF1* (perforin) – FHL2
		UNC13D (MUNC13-4) – FHL3
		STX11 (syntaxin 11) – FHL4
		STXBP2 (MUNC18-2) – FHL5
Albinism syndromes		*RAB27A* – Griscelli syndrome 2
		LYST – Chediak-Higashi syndrome
		AP3B1 – Hermansky-Pudlak syndrome type 2
Others		*SH2D1A* (SAP) - X-linked lymphoproliferative disease (XLP) 1
		XIAP (BIRC4) – X-linked lymphoproliferative disease 2
		ITK (IL2-inducible T-cell kinase)
		CD27
Conditions associated with acquired HLH	Secondaire	Infections
		Malignancies
		Autoinflammatory disease
		Autoimmune disease
		Immunosuppression
		Inborn errors of metabolism

Lehmberg et al. British Journal of Haematology 2013

ADULTES



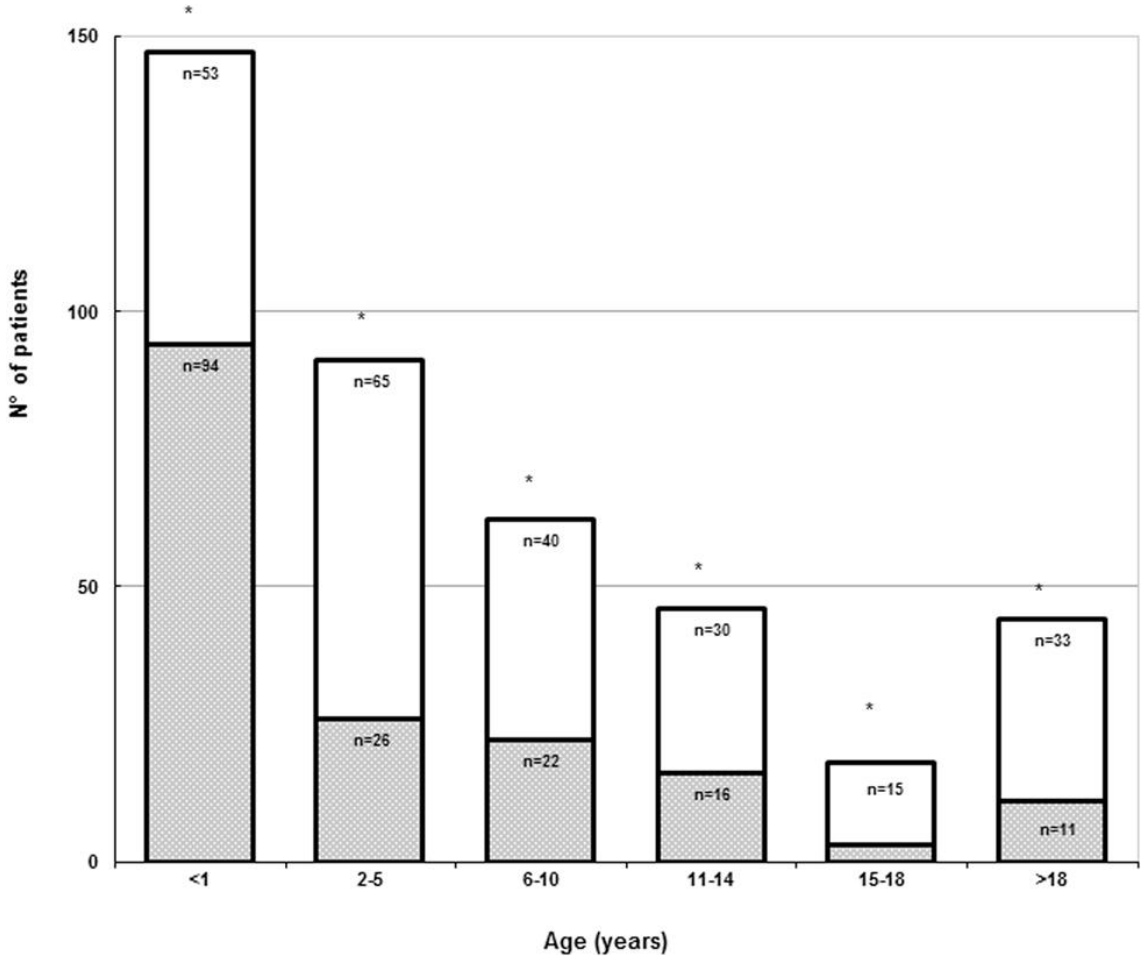
Ramos-Casals et al.
Lancet 2014

En Belgique:

ADULTES: 30% **Hémopathie maligne** + 15% (Hémopathie maligne+ Infections)
35% Infections
10% maladies auto-immunes ou auto-inflammatoires + Infections
5% Autres/inconnu

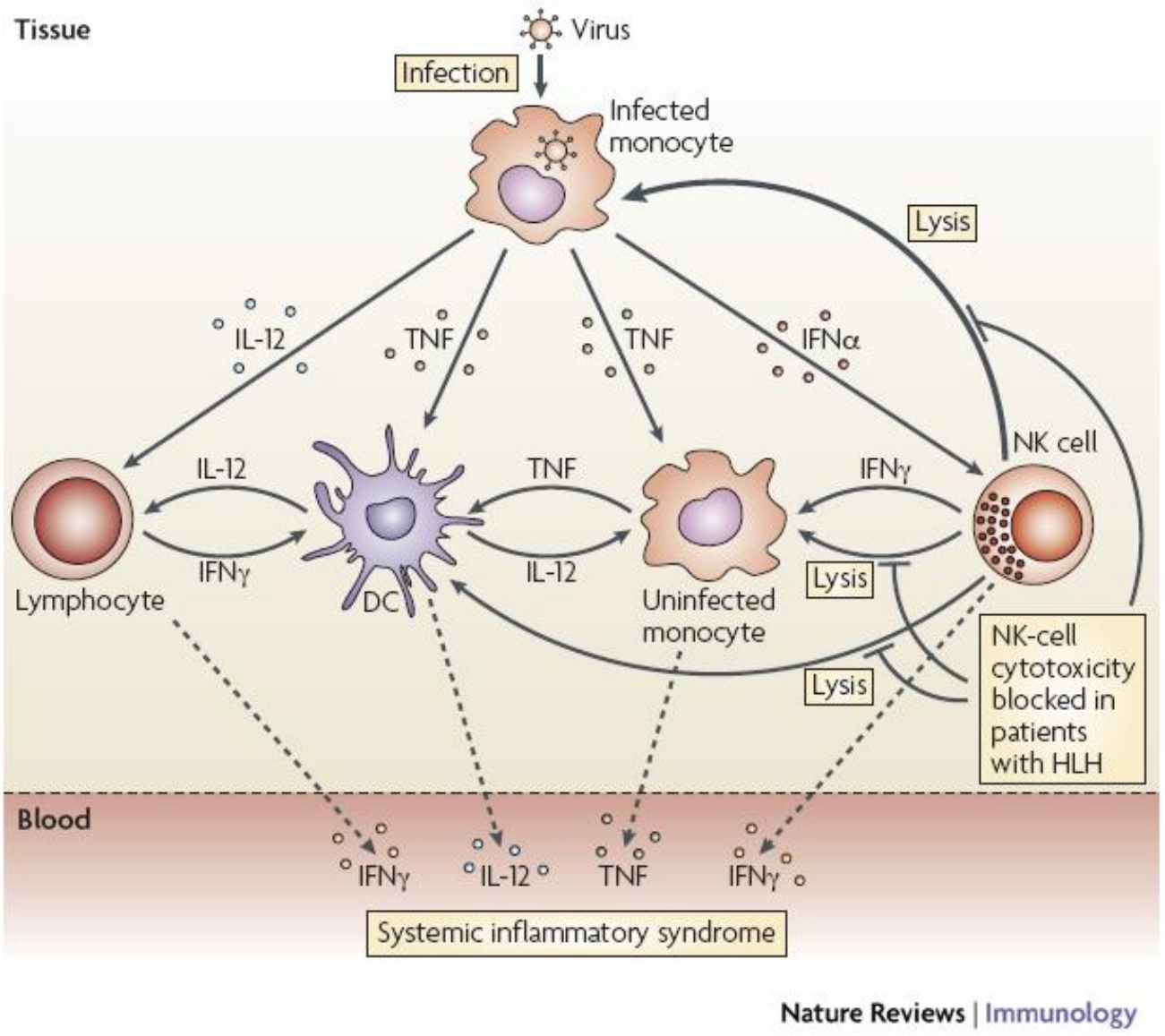
ENFANTS: 50% Infection
25% Maladies auto-immunes ou auto-inflammatoires
13% Hémopathie maligne+ 6% (Hémopathie maligne+ Infections)
6% FHL

Environ 2/3 des patients diagnostiqués avant un an sont des cas de FHL.



Distribution of patients with FHL (biallelic mutations; *gray bars*) or sporadic HLH (no biallelic mutations; *white bars*), according to age at diagnosis. The number of patients for each age group is indicated within each bar. *$P < .001$, χ^2 test.

Journal of Allergy and Clinical Immunology DOI: (10.1016/j.jaci.2015.06.048)



Orange. Nature Reviews Immunology 2008

Critères de diagnostic pour le syndrome d'hémophagocytose

Clinique

Fièvre (>7 jours, température ≥38.5°C)

Splénomégalie (>3cm au dessous du rebord costal)

Biologique

Cytopénie de plus de 2 lignées (Hémoglobine < 9g/dL, plaquettes $<100.10^3/\mu L$, neutrophiles $<1.10^3/\mu L$)

Hypertriglycéridémie (265 mg/dL) et/ou diminution du fibrinogène (<150mg/dL)

Hyperferritinémie (>500ng/mL)

Activité des cellules NK faible ou nulle

Elévation du récepteur soluble de l'IL-2 >2400 unités/mL)

Histopathologique

Hémophagocytose sans signe de malignité

Henter et al. Crit Rev Oncol Hematol 2004

Hscore

Validés pour les formes 2 aires chez l'adulte

Hscore	
Parameter	**No. of points**
Fever (°C)	0 (<38.4), 33 (38.4ï 39.4), or 49 (>39.4)
Organomegaly	0 (no), 23 (hepatomegaly or splenomegaly), or 38 (hepatomegaly and splenomegaly)
Cytopenia**	0 (1 lineage), 24 (2 lineages), or 34 (3 lineages)
Ferritin (ng/ml)	0 (<2000), 35 (2000ï 6000), or 50 (>6,000)
Triglycerides (mmol/l)	0 (<1.5), 44 (1.5ï 4), or 64 (>4)
Fibrinogen (g/l)	0 (>2.5) or 30 (Ò2.5)
Hemophagocytosis in bone marrow	0 (no) or 35 (yes)
Aspartate aminotransferase (UI/l)	0 (<30) or 19 (Ó30)
Known underlying immunosuppression	0 (no) or 18 (yes)

Cutoff à 169: sensibilité 93% et spécificité de 86%

http://saintantoine.aphp.fr/score/

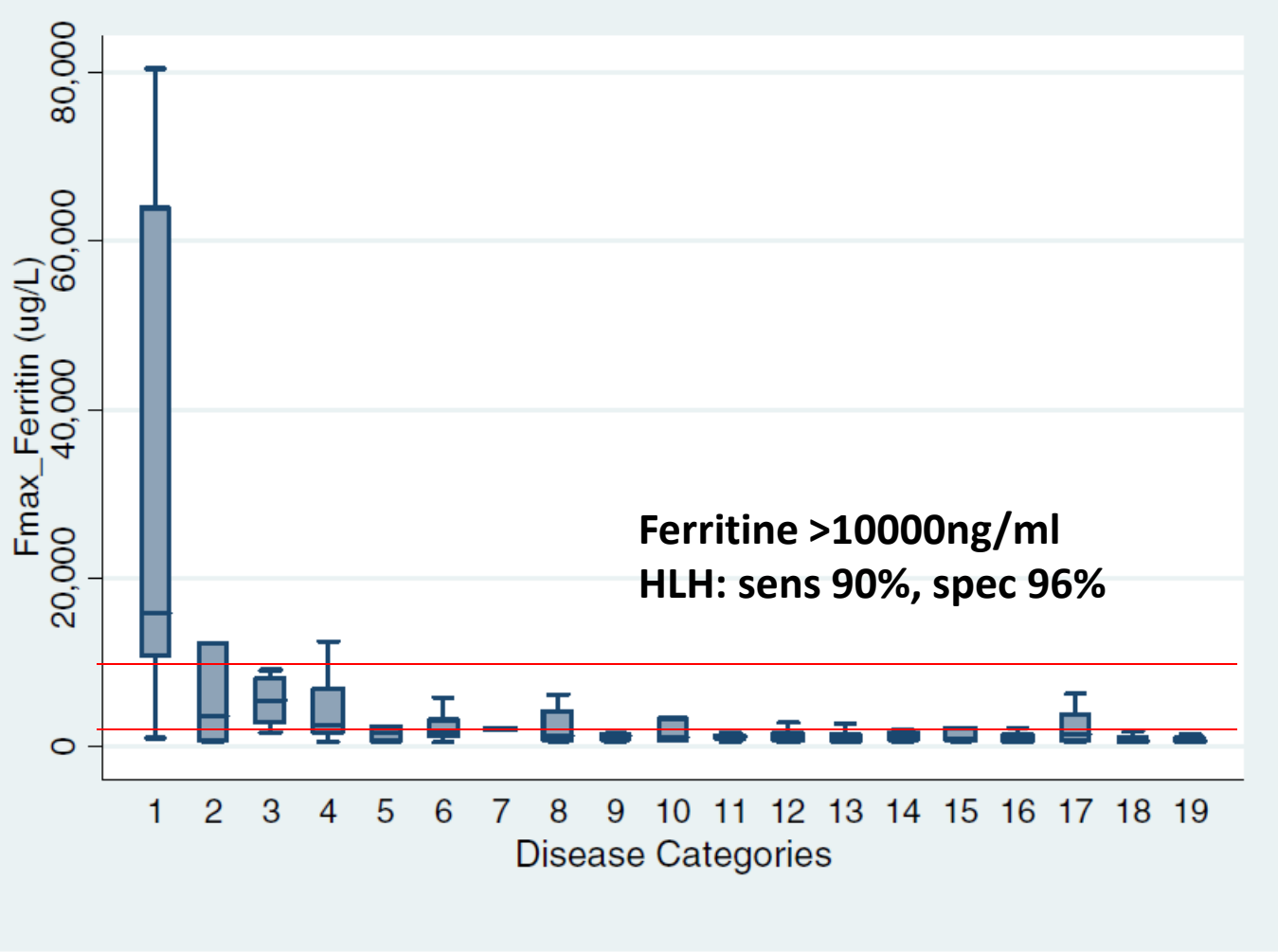
Score

Known underlying immunodepression	----Select ----
Maximal Temperature (C)	----Select ----
Hepatomegaly	----Select ----
Splenomegaly	----Select ----
Lower Hemoglobin level	----Select ----
Lower Leucocytes count	----Select ----
Lower Platelets count	----Select ----
Higher Ferritin level (ng/ml)	----Select ----
Higher Triglyceride level (mmol/l)	----Select ----
Lower Fibrinogen level (g/l)	----Select ----
Higher SGOT/ASAT level (UI/L)	----Select ----
Hemophagocytosis features on bone marrow aspirate	----Select ----
	Calculate
HScore	**0**
Probability of having HS (%)	**0**

Score issued from the manuscript « Development and validation of a score for the diagnosis of reactive hemophagocytic syndrome (HScore) » by L Fardet, L Galicier, O Lambotte et col. Arthritis & Rheumatology. 2014

- A quoi correspondent ces « syndromes d'hyperferritinémie »?
- Quelle est la place du dosage de la ferritine dans le diagnostic du syndrome hémophagocytaire?
- Et la ferritine glycosylée?

Dans la population pédiatriques des taux élevés de ferritine sont relativement spécifiques pour le HLH.



Disease Categories
1-HLH
2-Unknown
3-Shock
4-Stem cell transplant
5-Heart disease
6-Chronic transfusion
7-Immunodeficiency
8-Liver disease
9-Cystic fibrosis
10- Bone marrow failure
11-Prematurity
12-Malignancy
13-Viral infection
14-Bacterial infection
15-Solid organ transplant
16-Trauma
17-Autoimmune disease
18-Hemoglobinopathy
19-Renal disease

Population pédiatrique 2003-2005 Texas Children's Hospital, ferritine >500 ng/mL
HLH n=10 , non-HLH n=320

Pediatr Blood Cancer 2008; 50:1227-35

n= 627 adultes
Ferritine >1000 ng/mL

Table 2. Prevalence of Each Category by Age

	n (%)
<50 y old	
Iron overload	68 (30)
Hepatocellular	41 (18)
Infectious	38 (16.7)
Renal failure/insufficiency	34 (14.9)
Malignancy	33 (14.5)
Inflammatory	10 (4.4)
Anemia of chronic disease	2 (0.9)
Unknown	2 (0.9)

≥50 y old	
Malignancy	120 (30.1)
Iron overload	68 (17)
Hepatocellular	66 (16.5)
Infectious	62 (15.5)
Renal failure/insufficiency	58 (14.5)
Inflammatory	17 (4.3)
Anemia of chronic disease	5 (1.3)
Unknown	3 (0.8)

J Clin Rheumatol 2013;19:324

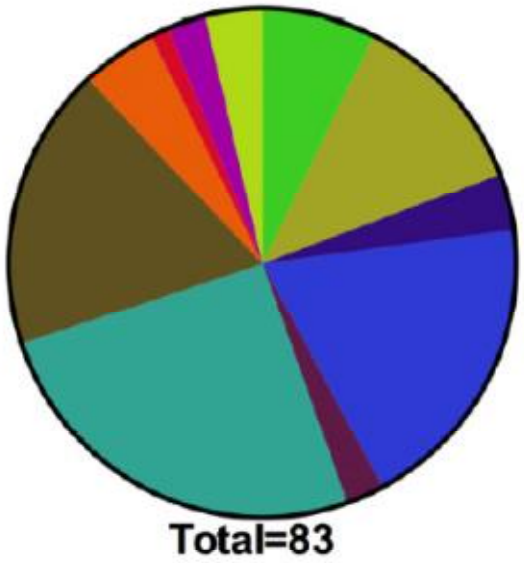


Figure 1 Cause of ferritin elevation >3000 µg/L category. (▬), Haemophagocytic lymphohistiocytosis 7% (*n* = 6); (▬), infection 12% (*n* = 10); (▬), inflammation 4% (*n* = 3); (▬), liver disease 19% (*n* = 16); (▬), haemochromatosis 3% (*n* = 2); (▬), transfusions 25% (*n* = 21); (▬), mixed 18% (*n* = 15); (▬), unknown 5% (*n* = 4); (▬), adult onset Still disease 1% (*n* = 1); (▬), haematologic malignancy 2% (*n* = 2); (▬), solid organ malignancy 4% (*n* = 3).

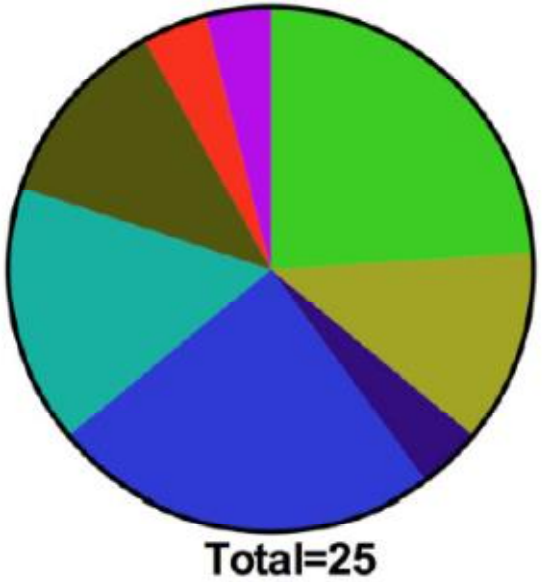
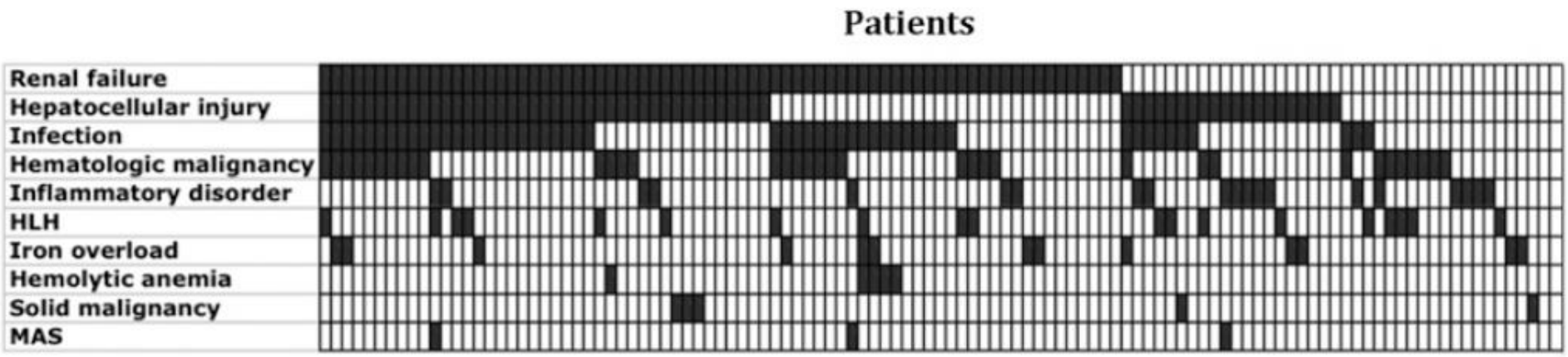


Figure 2 Cause of ferritin elevation greater than 10 000 µg/L. (▬), Haemophagocytic lymphohistiocytosis 24% (*n* = 6); (▬), infection 12% (*n* = 3); (▬), inflammation 4% (*n* = 1); (▬), liver disease 24% (*n* = 6); (▬), transfusions 16% (*n* = 4); (▬), mixed 12% (*n* = 3); (▬), adult onset Still disease 4% (*n* = 1); (▬), haematologic malignancy 4% (*n* = 1).

Internal medicine Journal 2015. doi 10.111/img.12768

Ferritine >50000 ng/mL
N=111



Key Points

- Highly elevated ferritin is not specific for hemophagocytic lymphohistiocytosis in adults.
- Marked hyperferritinemia in adults most often occurs in the setting of renal failure, hepatocellular injury, infection, or malignancy.

65% insuffisance rénale
54% atteinte hépatique
46% infection
32% Hémopathie maligne
17% HLH
3% MAS
12% Surcharge en fer

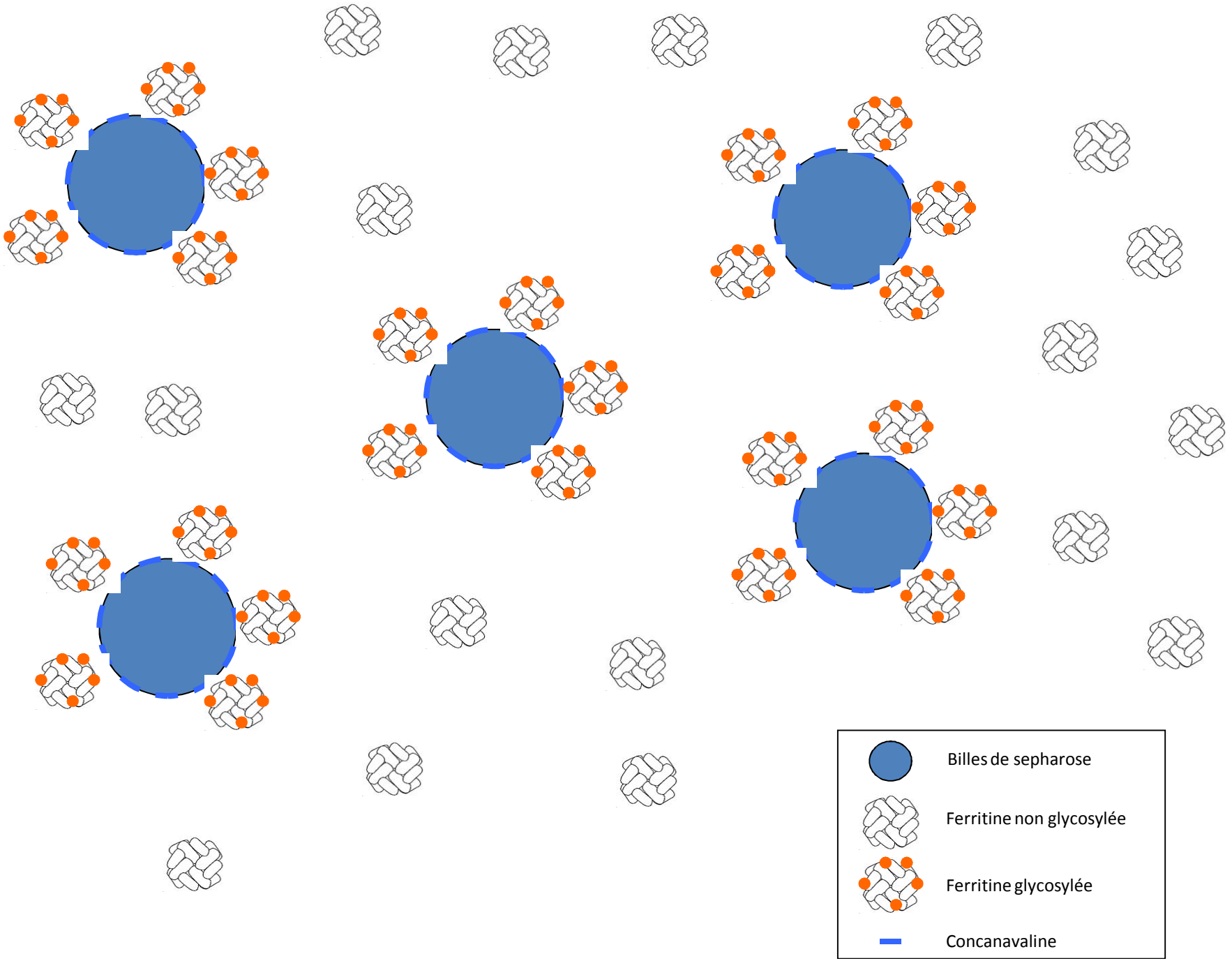
Blood 2015;125:1548-1552

- A quoi correspondent ces « syndromes d'hyperferritinémie »?
- Quelle est la place du dosage de la ferritine dans le diagnostic du syndrome hémophagocytaire?
- Et la ferritine glycosylée?

Détermination du % de ferritine glycosylée

- Le dosage de la ferritine totale
- Le dosage de la ferritine non glycosylée après adsorption de la ferritine glycosylée sur une lectine couplée à un gel et centrifugation

$$\text{Pourcentage de ferritine glycosylée} = \frac{(\text{concentration totale de ferritine} - \text{concentration ferritine non glycosylée}) \times 100}{\text{concentration totale de ferritine}}$$


Billes de sepharose
Ferritine non glycosylée
Ferritine glycosylée
Concanavaline

Ferritine sérique: % de glycosylation

- Sujet sain : **50-80%** ferritine sérique glycosylée
- Pourcentage diminué (**entre 20 et 50%**)**:** syndrome inflammatoire, néoplasie, infection
- Pourcentage effondré (**<20%**): maladie de Still de l'adulte, HLH, nécrose hépatique massive
- Hyperglycosylation (**>90%**): formes génétiques bénignes d'hyperferritinémie

Meilleur marqueur que le dosage total de ferritine sérique en cas de suspicion de syndrome d'HLH?

% Ferritine glycosylée: HLH

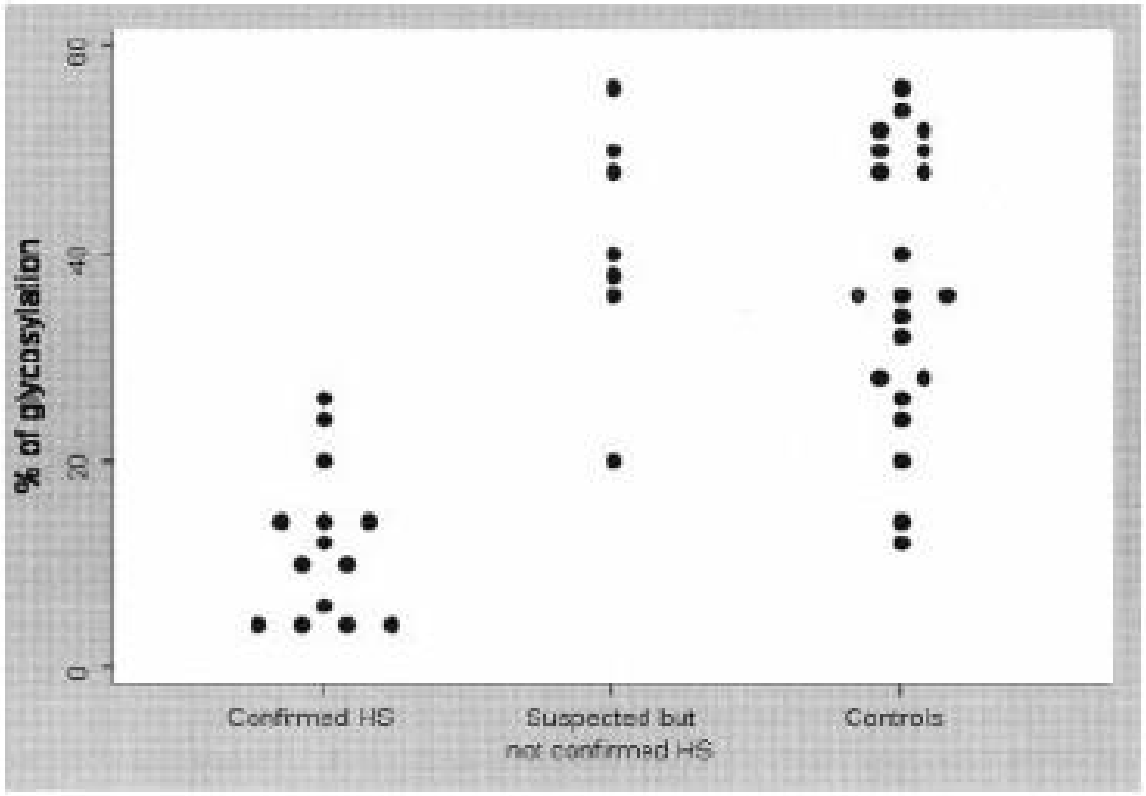


Figure 1. Percentage of glycosylated ferritin in 14 patients with confirmed hemophagocytic syndrome (HS), 7 patients with suspected but unconfirmed hemophagocytic syndrome, and 21 controls.

Fardet et al. Arthritis and Rheumatism 2008

Table 2. Characteristics of the 42 enrolled patients*

	Study group		
	Hemophagocytic syndrome confirmed (n = 14)	Hemophagocytic syndrome not confirmed (n = 7)	Control group (n = 21)
Ferritin, μg/liter	3,344 (2,074–7,334)	555 (464–1,420)	451 (126–929)
Glycosylated ferritin, %	10 (3–14)	40 (36–47)	36 (26–49)

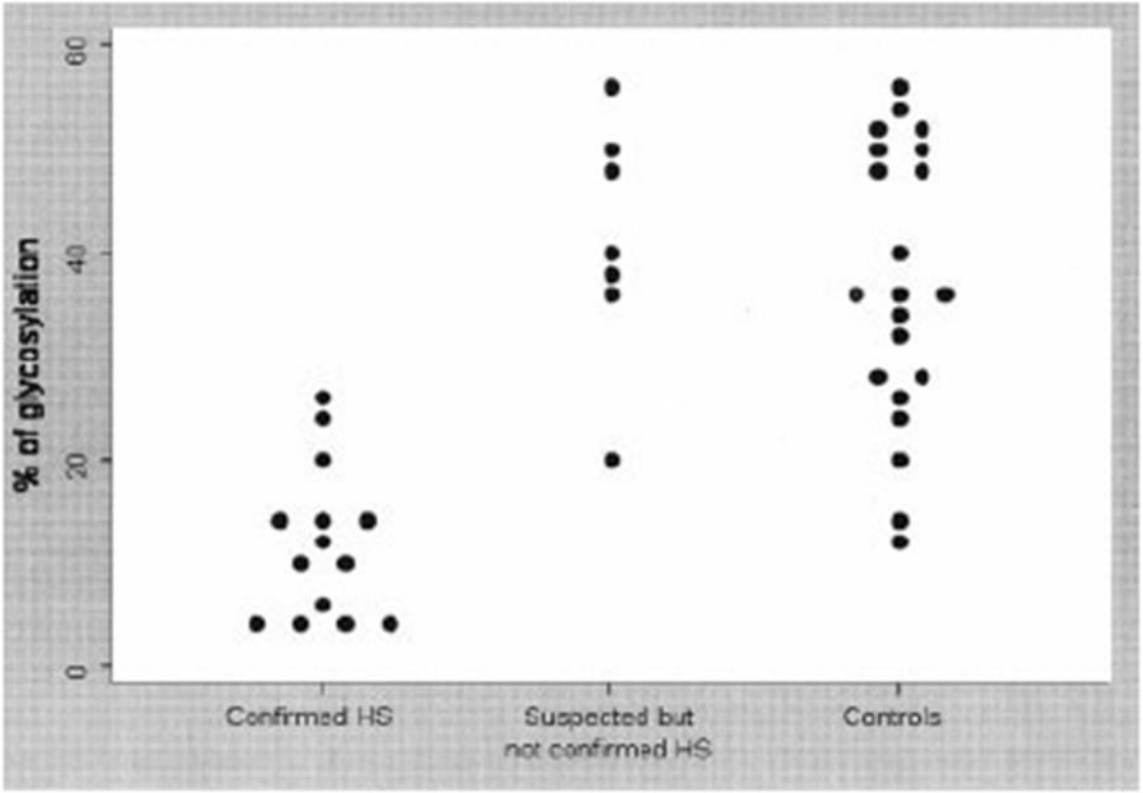
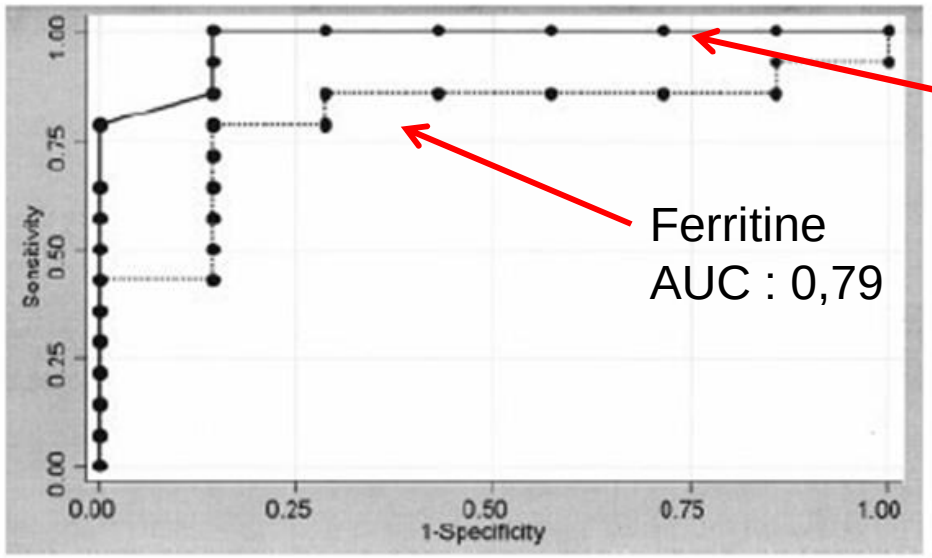


Figure 1. Percentage of glycosylated ferritin in 14 patients with confirmed hemophagocytic syndrome (HS), 7 patients with suspected but unconfirmed hemophagocytic syndrome, and 21 controls.



Ferritine glycosylée
AUC:0,97
Cutoff optimal: 25%

Ferritine
AUC : 0,79

Figure 2. Area under the receiver operating characteristic curves for patients with confirmed or suspected hemophagocytic syndrome. Solid lines represent glycosylated ferritin. Dotted lines represent total serum ferritin.

Table 3. Predictive usefulness of glycosylated ferritin for the diagnosis of hemophagocytic syndrome*

	Patients with confirmed hemophagocytic syndrome (n = 14) vs. patients with suspected but unconfirmed hemophagocytic syndrome (n = 7)		Patients with confirmed hemophagocytic syndrome (n = 14) vs. controls (n = 21)	
	Apparent performance	Cross-validation performance	Apparent performance	Cross-validation performance
Optimal cutoff, %†	26	25	26	25
Sensitivity	1.00 (0.77–1.00)	1.00 (0.77–1.00)	1.00 (0.77–1.00)	0.86 (0.57–0.98)
Specificity	0.86 (0.42–1.00)	0.71 (0.29–0.96)	0.76 (0.53–0.92)	0.76 (0.53–0.92)
Positive predictive value	0.93 (0.68–1.00)	0.88 (0.62–0.98)	0.74 (0.49–0.91)	0.71 (0.44–0.90)
Negative predictive value	1.00 (0.54–1.00)	1.00 (0.48–1.00)	1.00 (0.80–1.00)	0.89 (0.65–0.89)

* Values in parentheses are 95% confidence intervals.

Fardet et al. Arthritis and Rheumatism 2008

% Ferritine glycosylée: HLH

Early diagnostic value of low percentage of glycosylated ferritin in secondary hemophagocytic lymphohistiocytosis

	Scondary HLH confirmed group	HLH unconfirmed group	Control group
n	22	7	25
Age (years)	41.6 ± 12.4	34.9 ± 13.4	33.9 ± 9.1
Underlying disease	Non-Hodgkin's lymphoma ($n = 11$); viral infection ($n = 4$); bacterial infection ($n = 6$); unknown etiology for illness ($n = 1$)	Unknown etiology for illness ($n = 7$)	No

Wang et al. Int J Hematol 2009

Table 2 Total serum ferritin levels and the percentage of glycosylated ferritin in HLH confirmed, HLH unconfirmed, and control group

	Secondary HLH confirmed group	HLH unconfirmed group	Control group
Total serum ferritin (μg/L)	2897.6 ± 1837.2*,▲	653.1 ± 249.1	414.6 ± 212.6
Percentage of glycosylated ferritin (%)	20.5 ± 10.1*,▲	48.7 ± 12.1	53.6 ± 13.3

Values are expressed as mean ± SD

* $P < 0.01$, secondary HLH confirmed group versus HLH unconfirmed group; ▲ $P < 0.01$, secondary HLH confirmed group versus control group

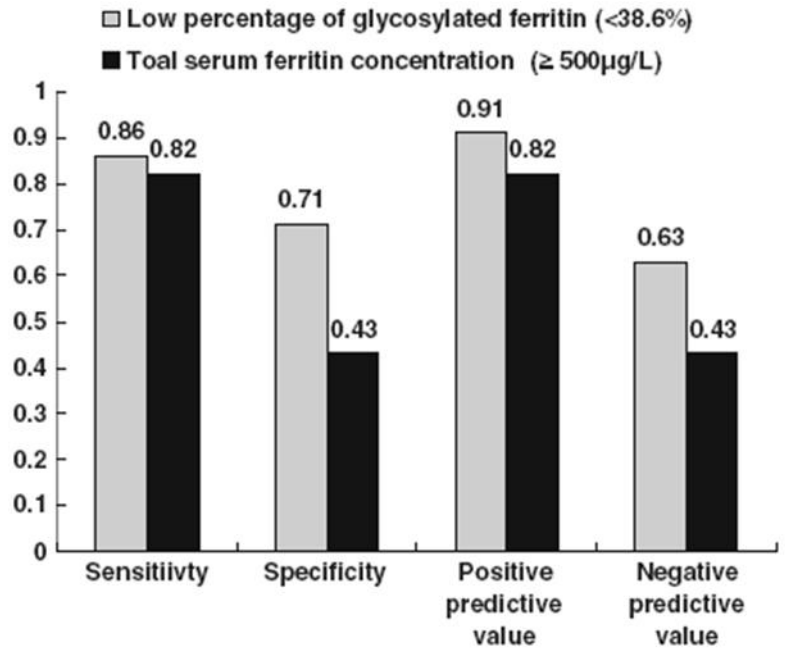


Fig. 1 Statistical parameters (sensitivity, specificity, positive and negative predictive values) derived from 2 × 2 tables for ability of low percentage of glycosylated ferritin and total serum ferritin levels to diagnosis of HLH

Normal values : 38,6-84,7%

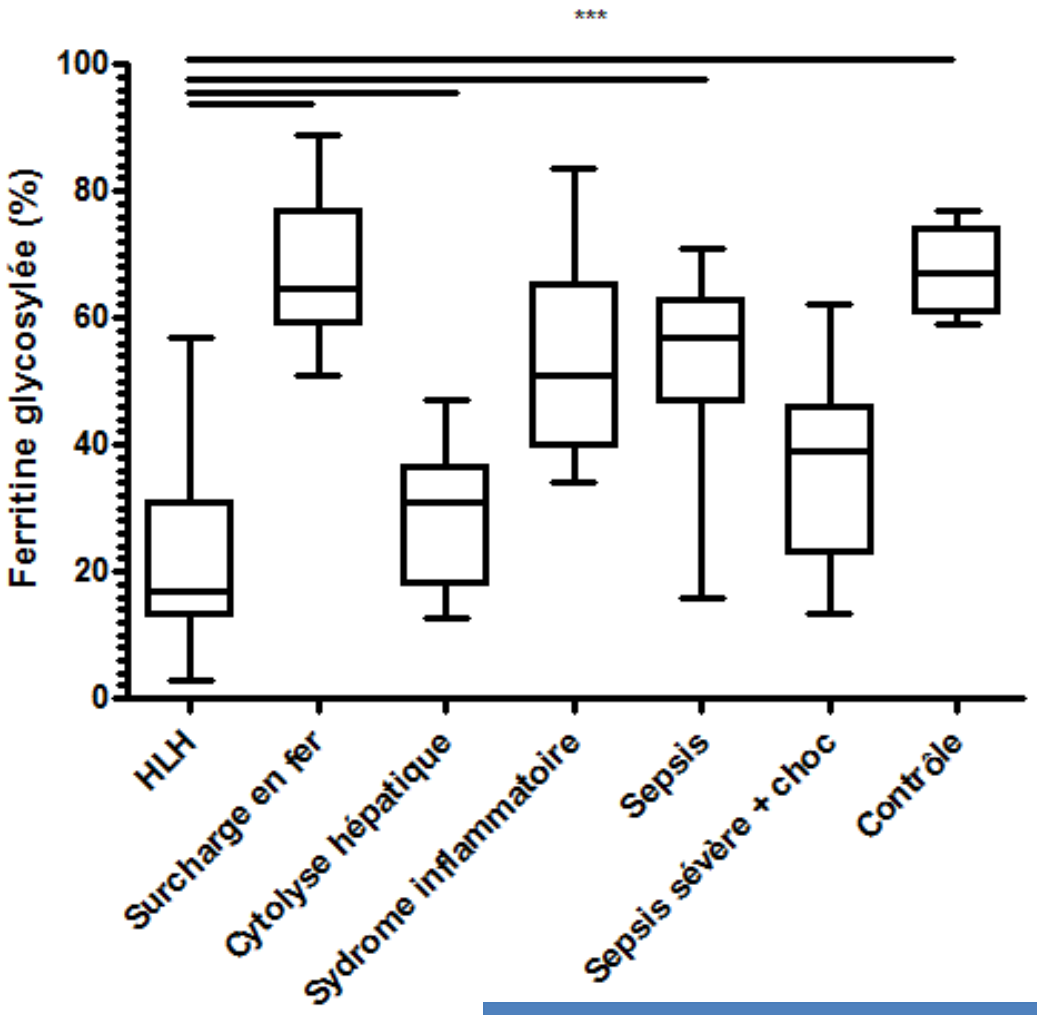
Wang et al. Int J Hematol 2009

Population étudiée

HLH n=27

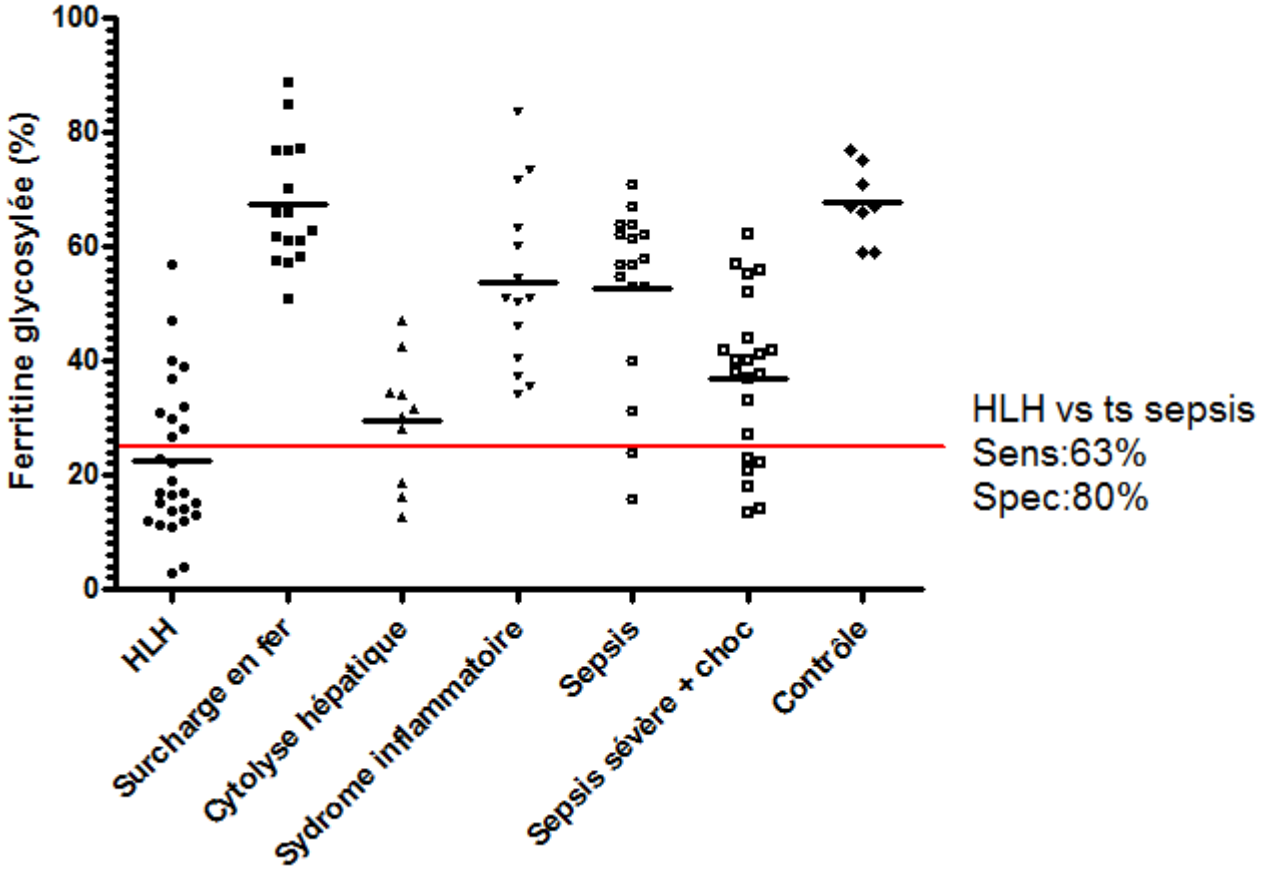
Groupe contrôle:

- **Surcharge en fer** (hémochromatose ou transfusion chronique) n=16
- **Cytolyse hépatique** n=10
- **Syndrome inflammatoire** (maladie AI, lymphome ou infection) n=14
- **Sepsis** n= 17
- **Sepsis sévère + choc** n= 22
- **Contrôle « sain »** n=8



	HLH	Surcharge en Fer	Cytolyse hépatique	Syndrome inflam.	Sepsis	Sepsis sev + choc	Contrôle
	n=27	n=16	n=10	n=14	n=17	n=22	n=8
Ferritine glycosylée	17	65	31	51	57	39	67
(%)	(13-31)	(59-77)	(18-36)	(40-65)	(46-63)	(23-46)	(61-74)

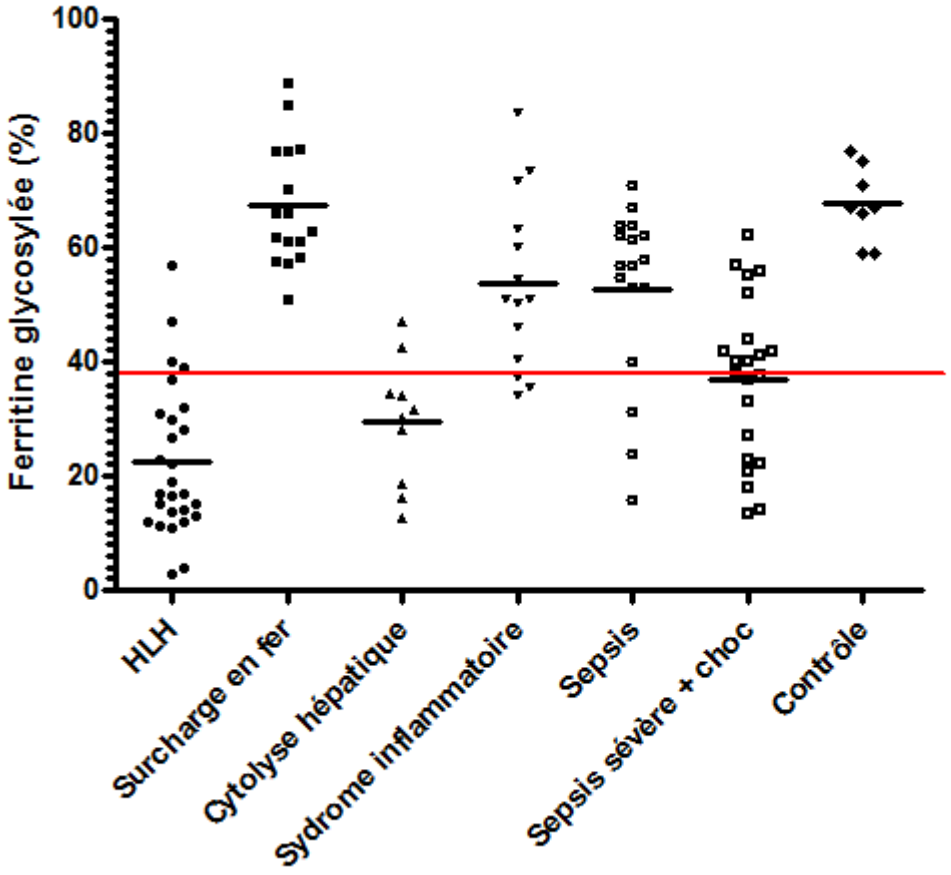
CUT-OFF:25%



Sens 100% et Spec 71%

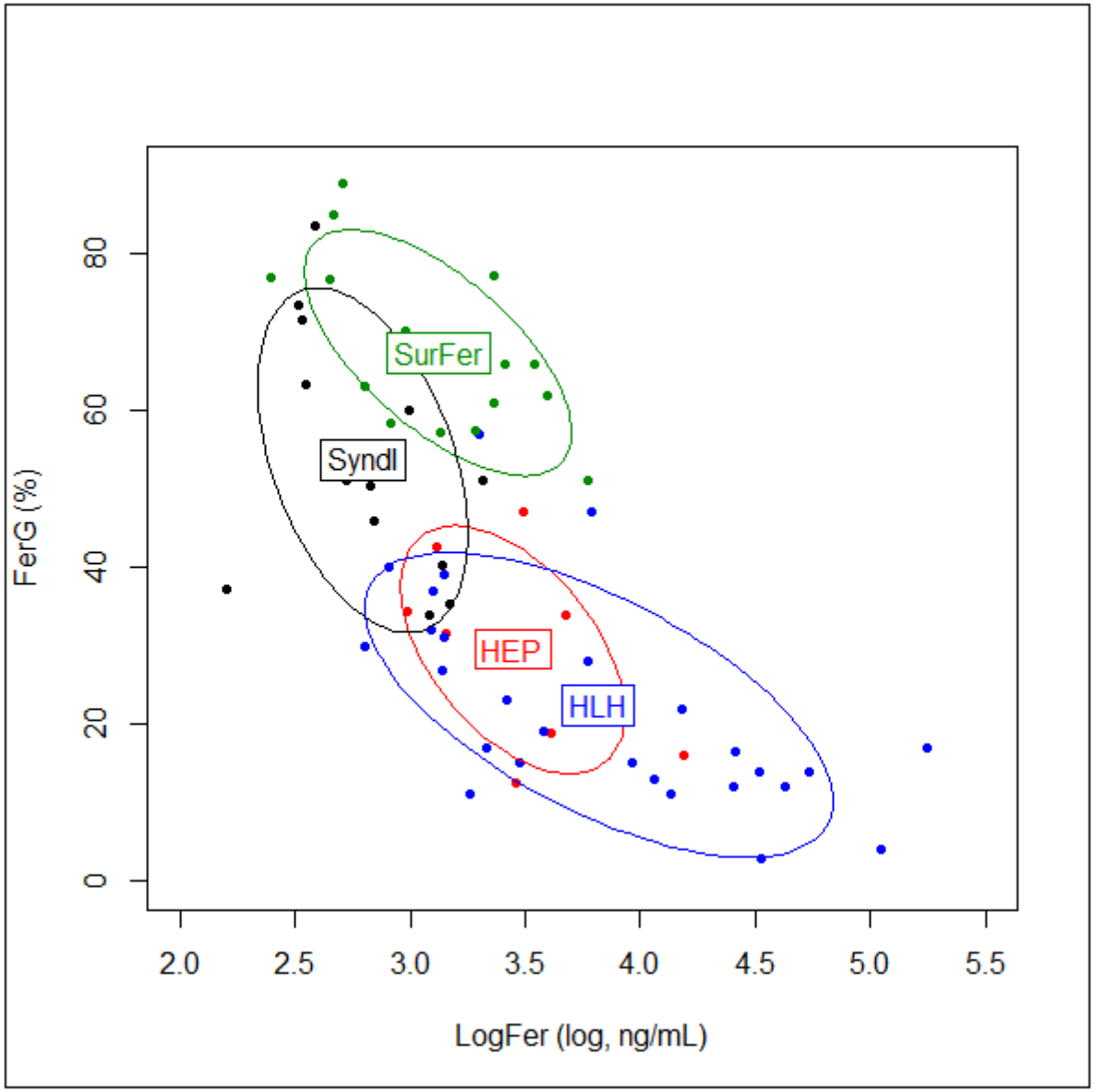
Fardet et al. Arthritis and Rheumatism 2008

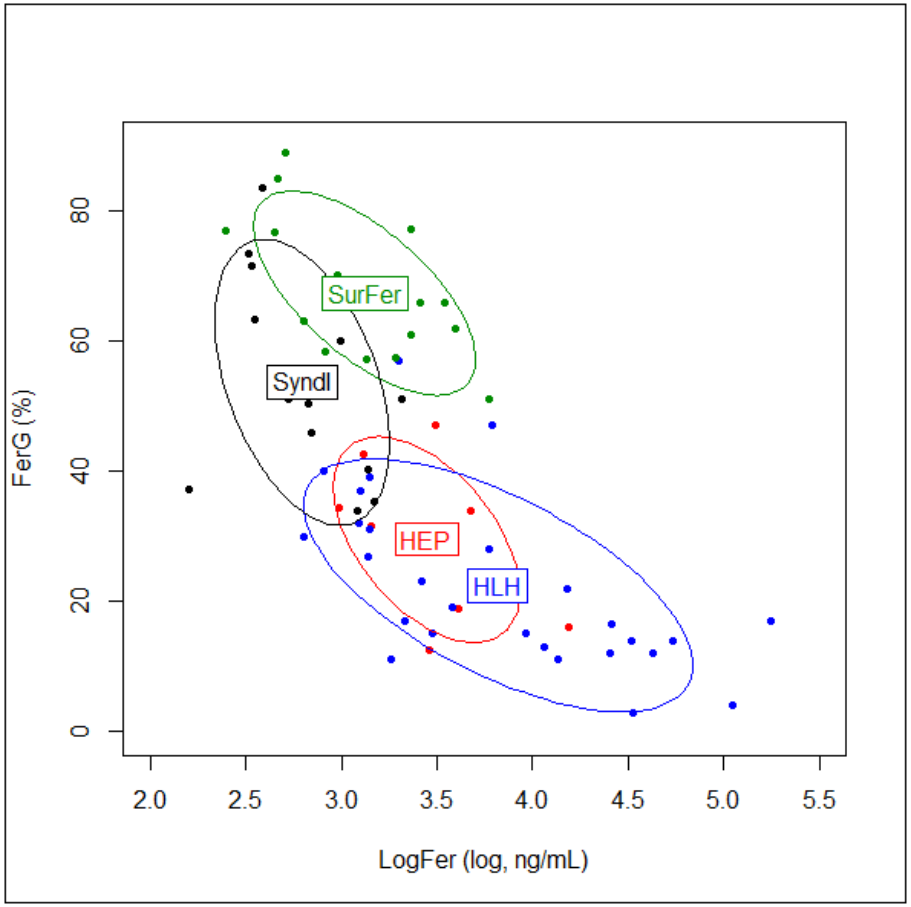
CUT-OFF:38,6%

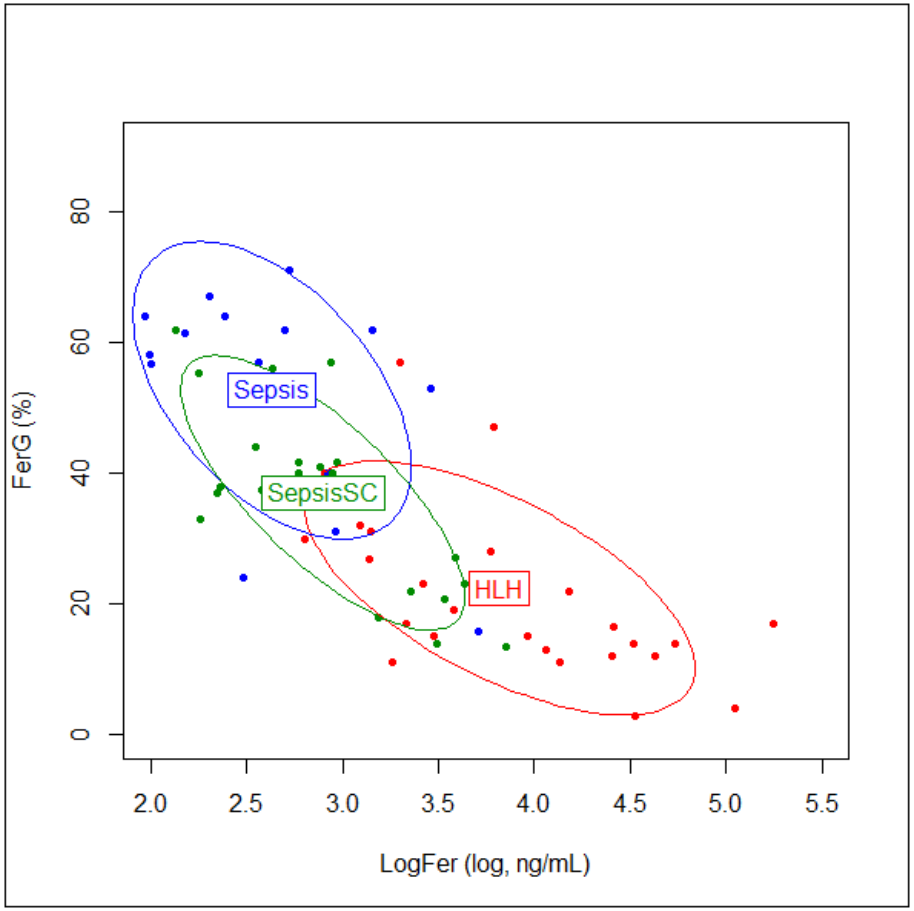


HLH vs ts sepsis
Sens:85%
Spec:64%

Sens 86% et Spec 71%
Wang et al. Int J Hematol 2009


SurFer
Syndl
HEP
HLH
FerG (%)
0
20
40
60
80
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
LogFer (log, ng/mL)


FerG (%)
0
20
40
60
80
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
LogFer (log, ng/mL)
SurFer
Syndl
HEP
HLH


FerG (%)
0
20
40
60
80
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
LogFer (log, ng/mL)
Sepsis
SepsisSC
HLH

Secondary hemophagocytic lymphohistiocytosis and severe sepsis/ systemic inflammatory response syndrome/multiorgan dysfunction syndrome/macrophage activation syndrome share common intermediate phenotypes on a spectrum of inflammation

Leticia Castillo, MD; Joseph Carcillo, MD

Pediatr Crit Care Med 2009 Vol. 10, No. 3

The Hyperferritinemic Syndrome: macrophage activation syndrome, Still's disease, septic shock and catastrophic antiphospholipid syndrome

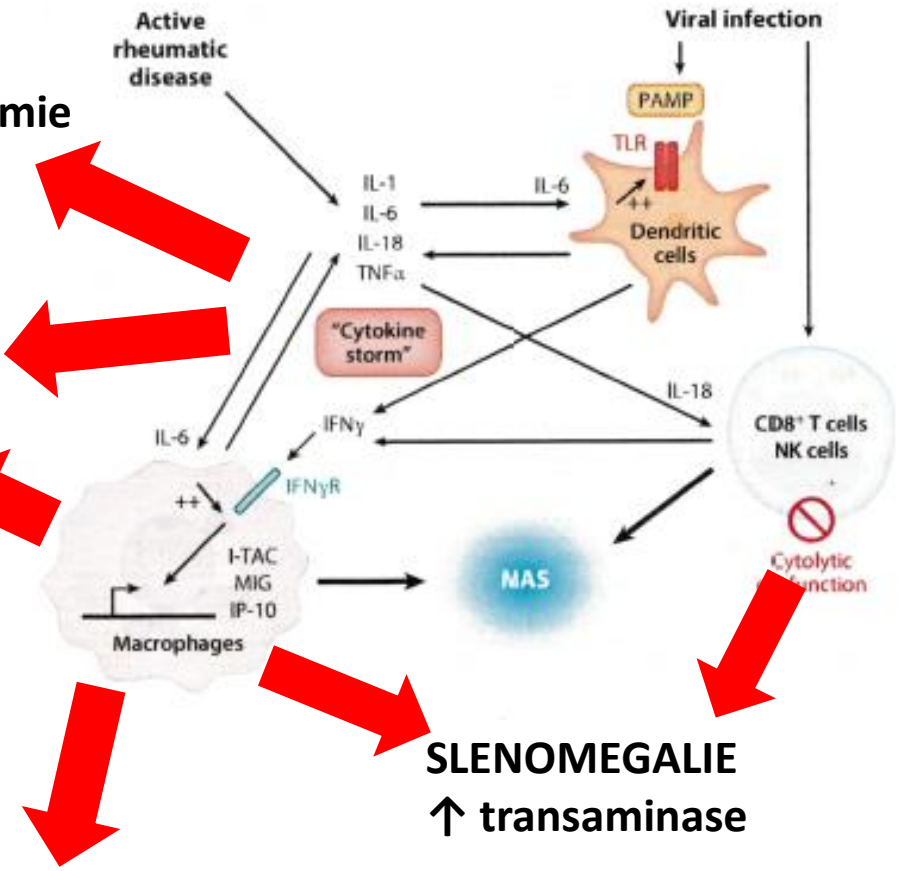
Cristina Rosário[1], Gisele Zandman-Goddard[2,3], Esther G Meyron-Holtz[4], David P D'Cruz[5] and Yehuda Shoenfeld[1,2*]

BMC Medicine 2013,11:185

FIEVRE

Hypertriglycéridémie
(inhibition de la lipoprotéine lipase)

Cytopénie

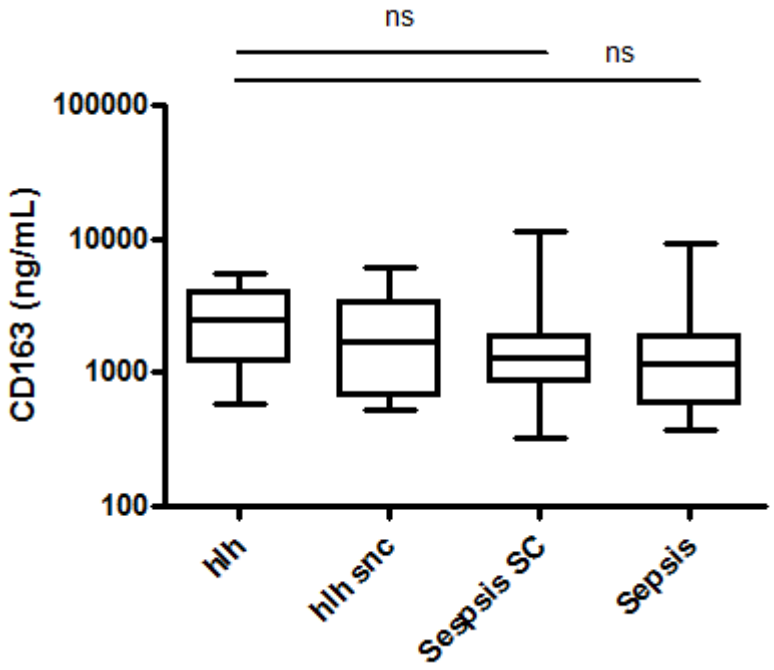


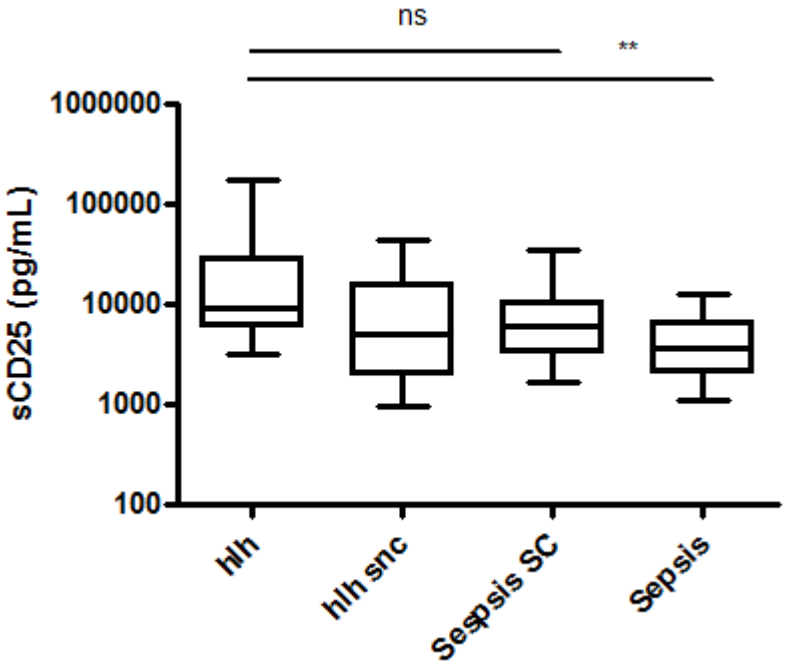
Soluble CD25 ↑

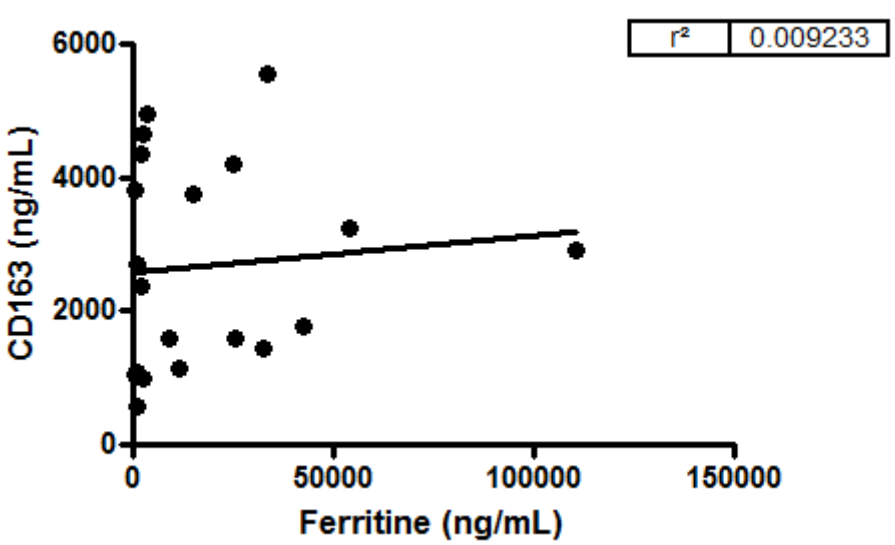
SLENOMEGALIE
↑ transaminase

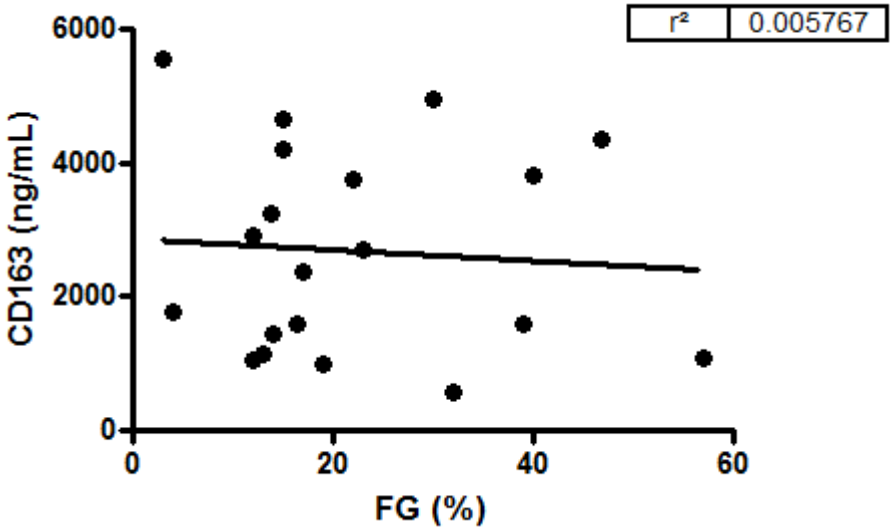
Hyperferritinémie (macrophage activation GDF15?)
Hypofibrinogénémie (plasminogen-activator)
Hémophagocytose
CD163 ↑

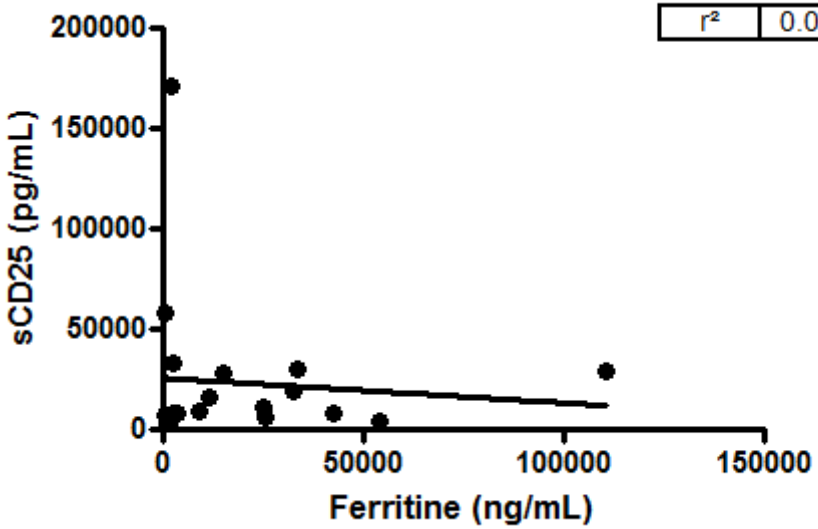
Schulert. Annu. Rev. Med. 2015

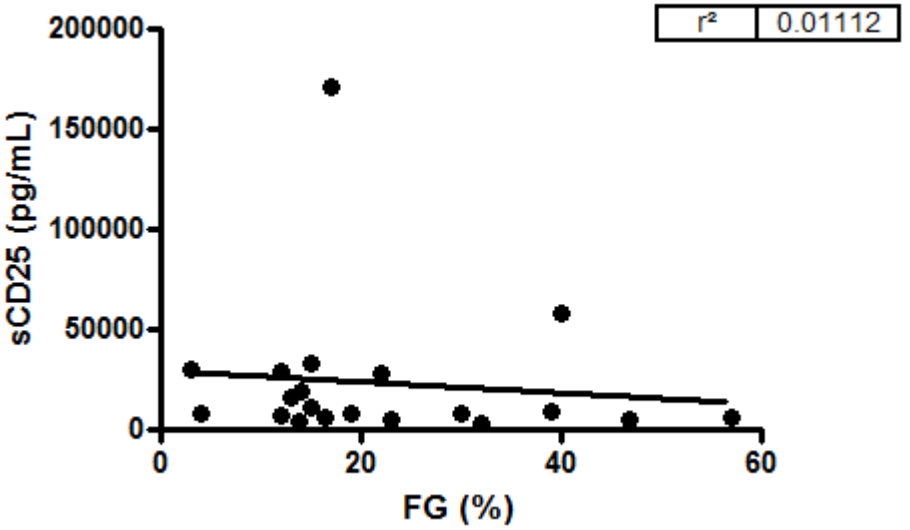

ns
ns
100000
10000
1000
100
CD163 (ng/mL)
hlh
hlh snc
Sespsis SC
Sepsis


ns
**
1000000
100000
10000
1000
100
sCD25 (pg/mL)
hlh
hlh snc
Sespsis SC
Sepsis


r²
0.009233
CD163 (ng/mL)
6000
4000
2000
0
0
50000
100000
150000
Ferritine (ng/mL)


r²
0.005767
CD163 (ng/mL)
6000
4000
2000
0
0
20
40
60
FG (%)

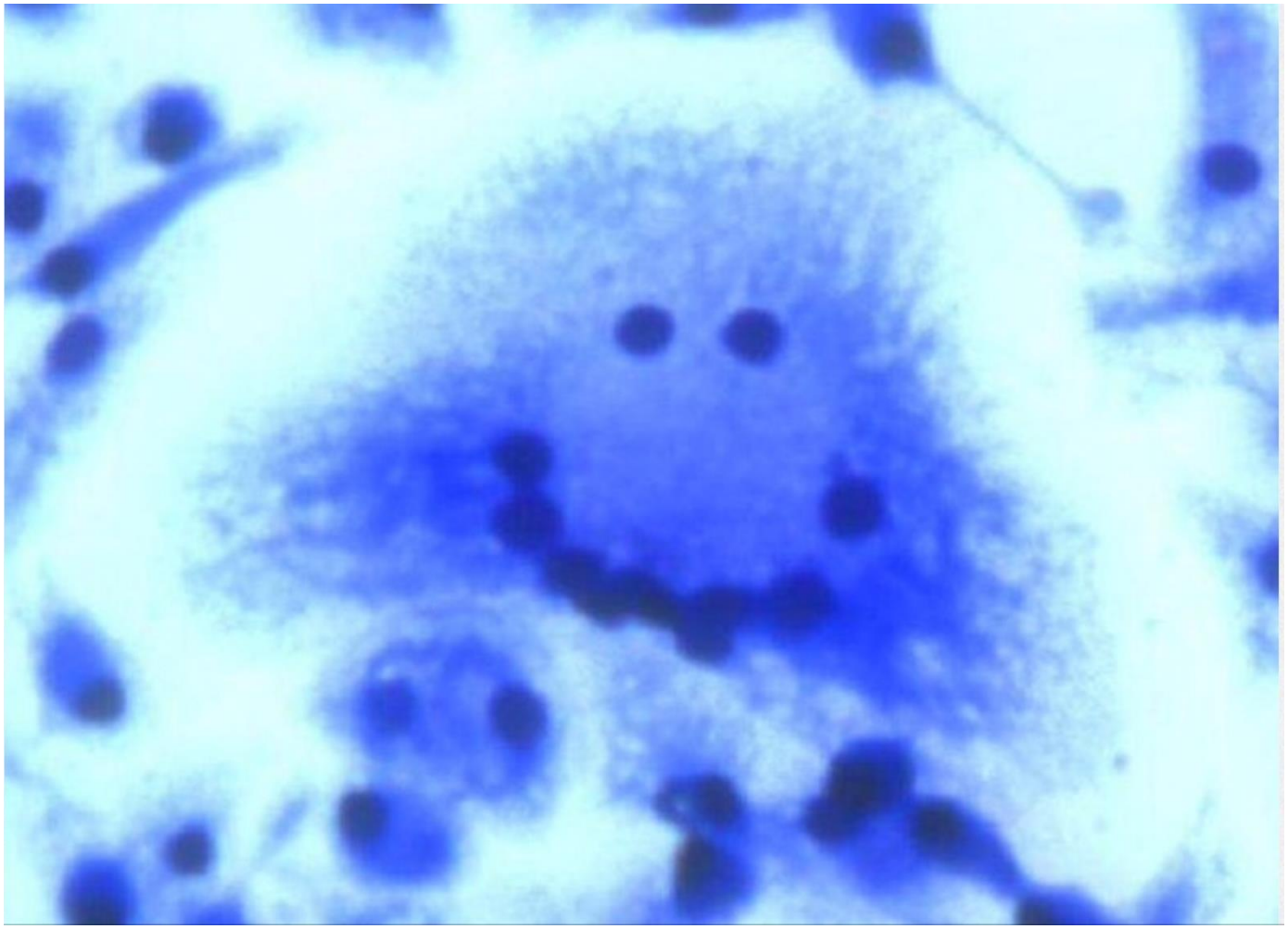

r²
0.008014
sCD25 (pg/mL)
200000
150000
100000
50000
0
0
50000
100000
150000
Ferritine (ng/mL)


r²
0.01112
sCD25 (pg/mL)
200000
150000
100000
50000
0
0
20
40
60
FG (%)

Perspectives

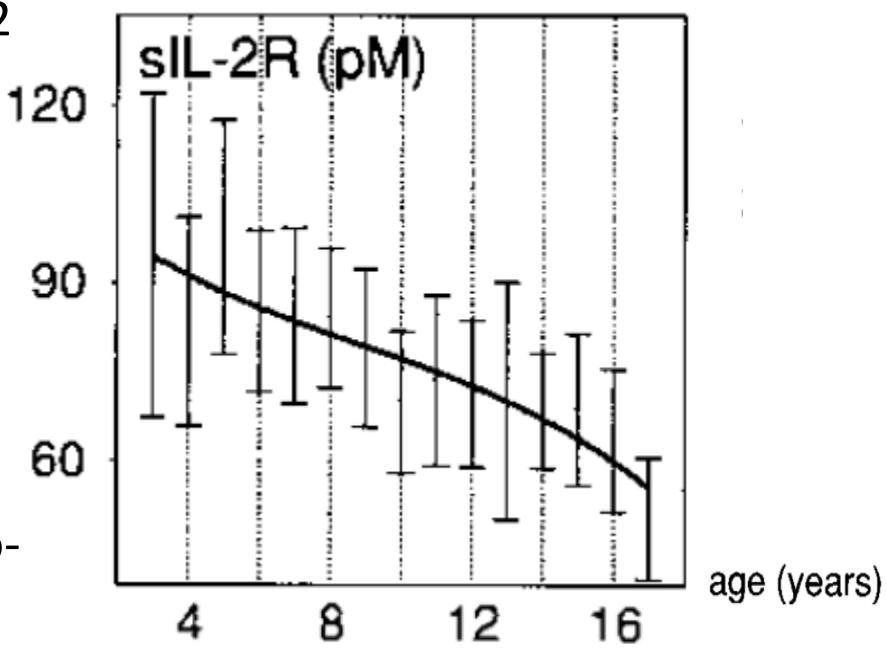
Hypothèse: profil de glycosylation de la ferritine peuvent varier entre les différents états pathologiques, reflet de mécanismes physiopathologiques sous-jacents distincts?

Merci!

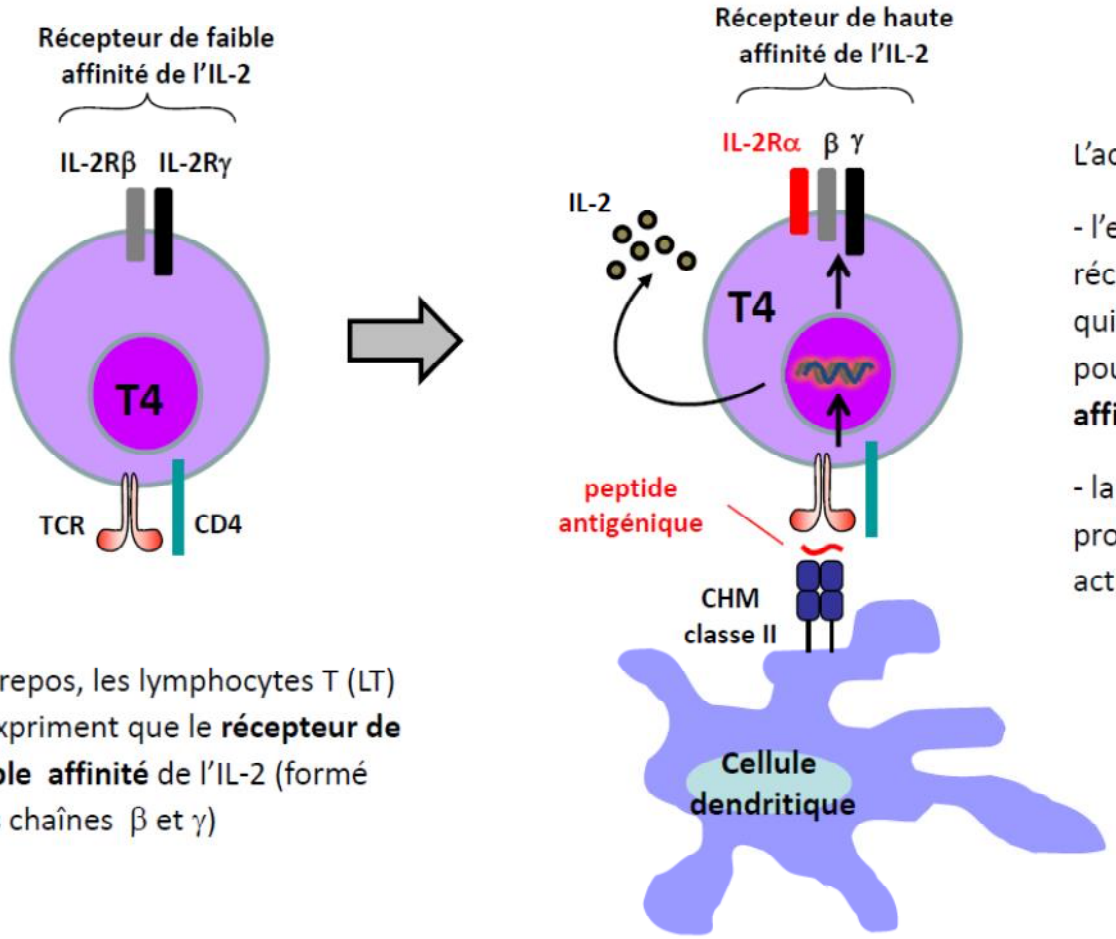


CD25 soluble

- CD 25 = chaine α du récepteur de l'IL-2
- Rôle physiologique?
- Reflet de l'activation des cellules T
- Considéré comme marqueur d'activité des cellules T dans les HLH
- ↑ leucémies lymphoides, lymphome
  rejet de greffe aigu
- Corrélé à l'activité de pathologies auto-immunes (PR, SLE)



Clinical and diagnostic laboratory immunology 1998;5: 28-32



Au repos, les lymphocytes T (LT) n'expriment que le **récepteur de faible affinité** de l'IL-2 (formé des chaînes β et γ)

L'activation du LT par l'Ag entraîne :

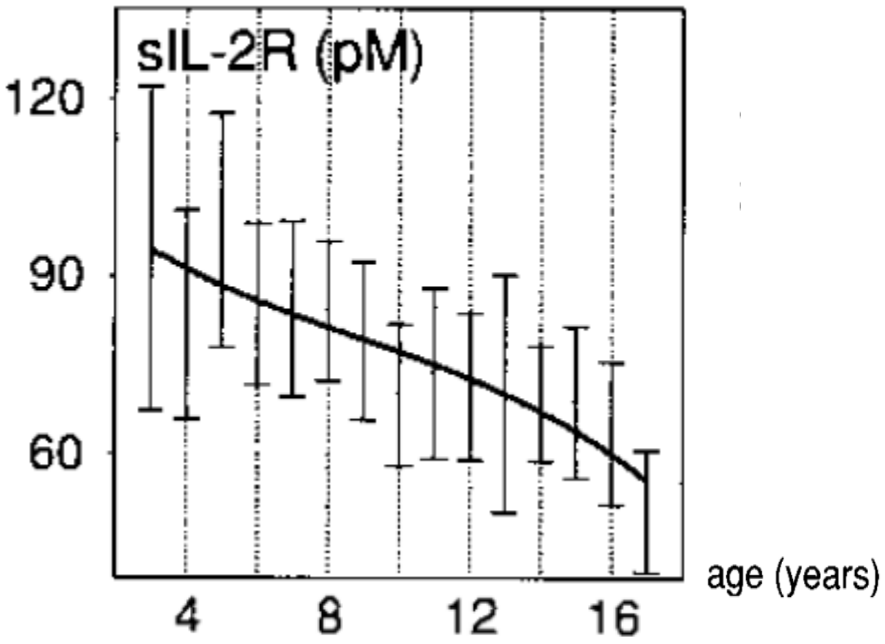
- l'expression de la chaîne α du récepteur de l'IL-2 à la membrane qui s'associe alors aux chaînes β et γ pour former le **récepteur de haute affinité** de l'IL-2,

- la synthèse d'IL-2 qui induit la prolifération uniquement des LT activés.

http://www.med.univ-montp1.fr/enseignement/masters_LMD/M1/Immunopathologie/Cytokines.pdf

CD25 soluble élevé

- CD 25 = chaine α du récepteur de l'IL-2
- Rôle physiologique?
- Reflet de l'activation des cellules T
- Considéré comme marqueur d'activité des cellules T dans les HLH
- ↑ leucémies lymphoides, lymphome
  rejet de greffe aigu
- Corrélé à l'activité de pathologies auto-immunes (PR, SLE)



Clinical and diagnostic laboratory immunology 1998;5: 28-32

CD25 soluble élevé

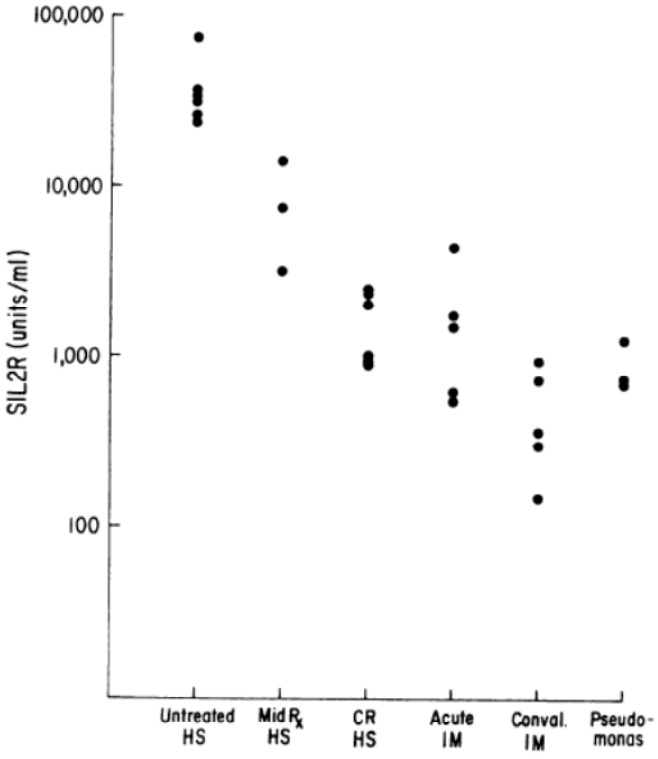


Fig 1. Comparison of SIL2R levels in patients with hemophagocytic syndromes (HS), untreated, on treatment, and in complete clinical remission (CR) with infectious mononucleosis (IM) and *Pseudomonas* infections.

Blood 1989;73:2128

HLH-2004 diagnostic guidelines

A) Molecular diagnosis
B) Diagnostic criteria
- Fever
- Splenomegaly
- Cytopenia (at least 2 of 3 lineages: hemoglobin <90 gm/l, platelets <100 × 10^9/l, neutrophils <1.0 × 10^9/l
- Hypertriglyceridemia and/or hypofibrinogenemia (triglycerides ≥265 mg/dl, fibrinogen ≤1.5 gm/l
- Hemophagocytosis BM, spleen, or lymph nodes
- Low or absent NK cell activity
- Ferritin ≥500 ng/ml
- Soluble CD25 ≥2400

Diagnostic rule

The diagnosis of HLH can be established in presence of a molecular diagnosis consistent with HLH or by meeting five of eight clinical and laboratory diagnostic criteria

Sensibilité 93%
Specificité 100%

Schneider M, ULM, Germany, personal Communication.

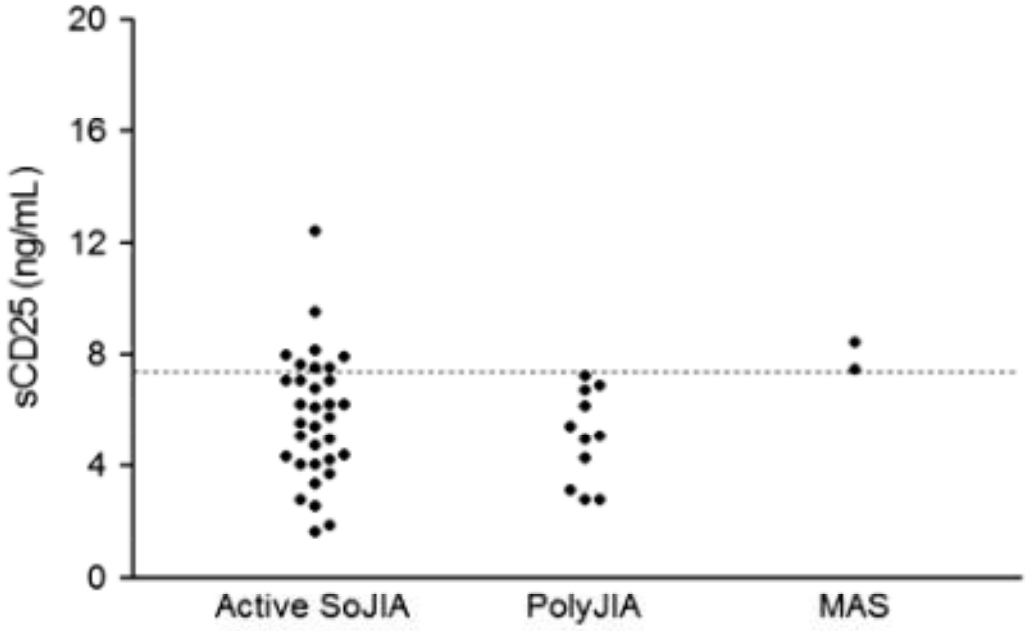


Figure 1 Scatter plot showing levels of sCD25 in three groups of patients. Active systemic onset juvenile idiopathic arthritis (SoJIA) (33), polyarticular JIA (11) and macrophage activation syndrome (MAS) (2) patients. The line represents the value above those seen in polyarticular JIA.

Biomarqueur pour identifier les formes subcliniques de MAS chez les patients atteints de sJIA?

International Journal of Rheumatic Diseases, 2014;17:261

Preliminary diagnostic guidelines for MAS complicating sJIA

Laboratory criteria

- Decreased platelet count (≤262 × 10^9/l)
- Elevated levels of AST (>59 U/l)
- Decreased WBC count (≤4.0 × 10^9/l)
- Hypofibrinogenemia (≤2.5 g/l)

Clinical criteria

- Central nervous system dysfunction
- Hemorrhages
- Hepatomegaly (≥3 cm below the costal arch)

The diagnosis of macrophage activation syndrome requires the presence of at least two laboratory criteria or the presence of at least one laboratory criterion and 1 clinical criterion. Bone marrow aspiration for evidence of macrophage hemophagocytosis may be required only in doubtful cases

Ravelli criteria 2005

CD163

- Recepteur « scavenger » de l'Hb (complexe hemoglobine-haptoglobine) exprimé sur la membrane des macrophages et monocytes
- Forme soluble correspondant au domaine extracellulaire est relarguée par protéolyse (induit stress oxydatif et stimuli inflammatoire)
- Fonction?
- Biomarqueur de l'activation des macrophages dans les HLH, sepsis et atteintes hépatiques

Table 2. Characteristics of the 42 enrolled patients*

	Study group		
	Hemophagocytic syndrome confirmed (n = 14)	Hemophagocytic syndrome not confirmed (n = 7)	Control group (n = 21)
Female, no. (%)	5 (36)	5 (71)	10 (48)
Age, years	54 (40–63)	66 (53–72)	62 (44–81)
No. of criteria evocative of hemophagocytic syndrome	5.5 (5–6)	3 (3–3)	2 (1–2)
Highest temperature, °C	39.0 (38.9–39.9)	39.0 (38.2–39.2)	38.4 (38.0–39.2)
Hepatomegaly, no. (%)	10 (71)	2 (29)	2 (10)
Splenomegaly, no. (%)	12 (86)	1 (14)	2 (10)
Ferritin, μg/liter	3,344 (2,074–7,334)	555 (464–1,420)	451 (126–929)
Glycosylated ferritin, %	10 (3–14)	40 (36–47)	36 (26–49)
Leukocytes, $\times 10^9$/liter	2.5 (1.9–5.2)	4.0 (3.4–9.0)	10.0 (6.1–12.5)
Neutrophils	1.7 (0.9–3.1)	2.8 (2.7–7.5)	5.8 (3.6–8.6)
Lymphocytes	0.5 (0.4–1.0)	0.8 (0.6–1.0)	1.5 (1.0–2.3)
Hemoglobin, gm/dl	8.8 (8.0–10.4)	9.3 (8.2–11.1)	11.0 (9.4–12.0)
Platelets, $\times 10^9$/liter	87 (64–124)	116 (70–145)	390 (307–532)
Fibrinogen, gm/liter	1.8 (1.5–2.1)	5.1 (3.1–5.7)	6.0 (5.3–6.7)
CRP, mg/liter	106 (61–140)	111 (60–140)	130 (89–160)
Triglycerides, mmoles/liter	3.12 (2.71–3.60)	1.23 (1.11–1.46)	1.21 (1.00–1.56)
Sodium, mmoles/liter	132 (131–134)	137 (132–141)	139 (134–140)
ALT, IU/liter	65 (35–181)	22 (20–30)	16 (14–39)
AST, IU/liter	101 (37–155)	29 (19–36)	21 (16–29)
Bilirubin, μmoles/liter	22 (10–60)	12 (8–25)	12 (10–15)
Albumin, gm/dl	27 (23–31)	28 (22–31)	28 (24–31)
LDH, IU/liter	1,326 (620–2,509)	452 (430–680)	420 (359–510)
Prothrombin time, %	73 (62–96)	74 (64–81)	84 (72–94)
Histologic hemophagocytosis, no. (%)	14 (100)	1 (14)	ND
In-hospital deaths, no. (%)	6 (43)	1 (14)	3 (14)

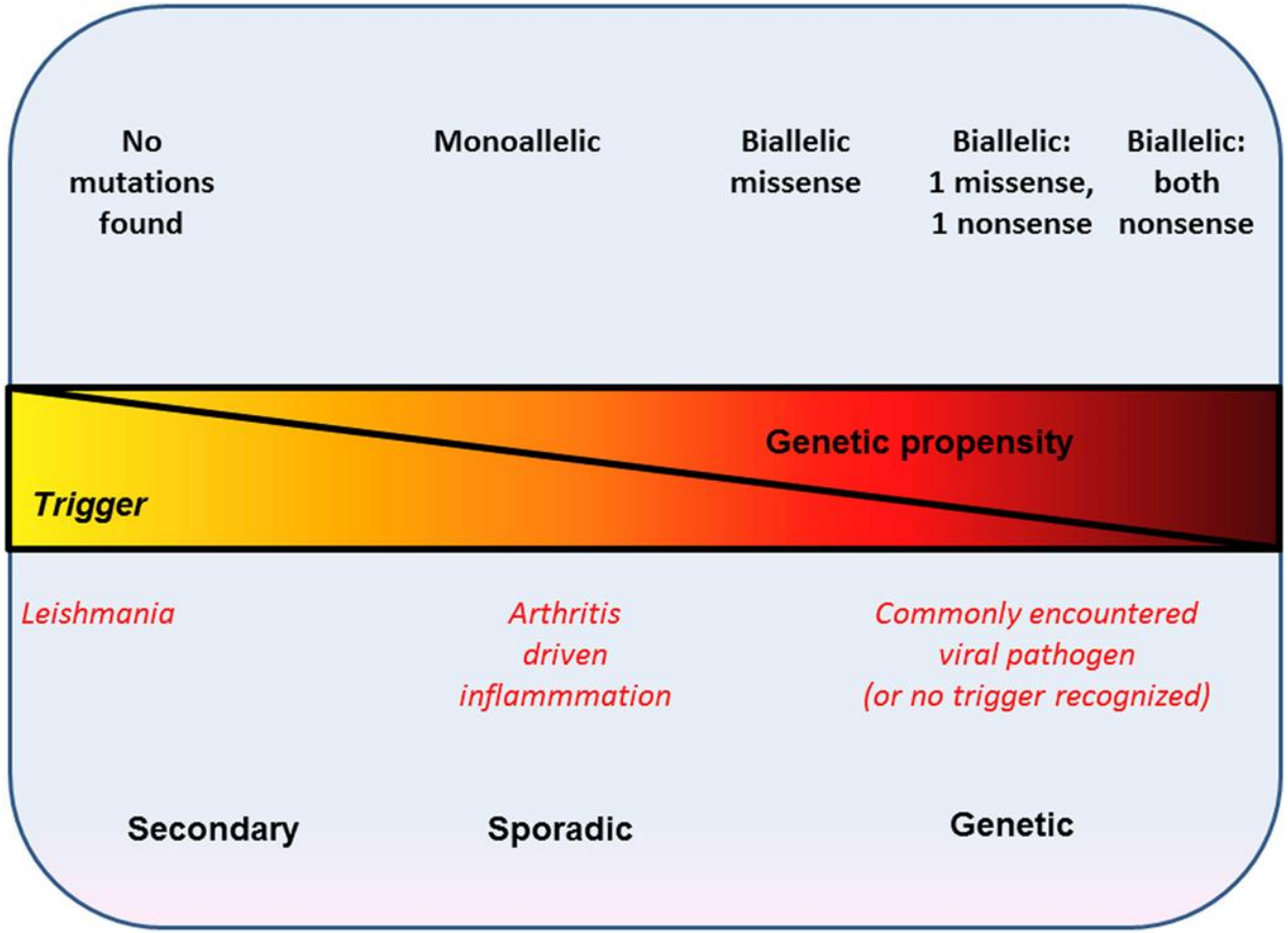
* Except where indicated otherwise, values are the median (interquartile range). Normal values were as follows: ferritin (women) 20–150 μg/liter; ferritin (men) 30–300 μg/liter; leukocytes 4–10 $\times 10^9$/liter; hemoglobin 12–16 gm/dl; platelets 150–450 $\times 10^9$/liter; fibrinogen 1.5–3.5 gm/liter; C-reactive protein (CRP) 0–5 mg/liter; triglycerides 0.65–1.85 mmoles/liter; sodium 135–145 mmoles/liter; alanine aminotransferase (ALT) <30 IU/liter; aspartate aminotransferase (AST) <35 IU/liter; bilirubin <19 μmoles/

Table 2 Common clinical manifestations and laboratory abnormalities: MAS, AOSD, cAPS and septic shock

	Septic shock	cAPS	AOSD	MAS
Hyperferritinemia	+ [15,39]	[71%] [8]	[70 to 89%] [40,41]	[87 to 100%] [42]
Range of ferritin levels (ng/mL)*	21 to 2,210 [15]	250 to 2,875 [8]	223,6 to 54924 [43]	994 to 189,721 [44]
Hypercytokinemia	+ [45]	+ [46], [47]	+ [48]	+ [49-53]
Infection as a trigger	[100%] [54]	+ [46]	+ [41]	+ [55]
Fever	+ [54]	+ [56]	[82 to 100%] [41]	[78 to 94%] [42]
Multiorgan involvement	[100%] [54]	[100%] [46]	+ [41]	+ [14,55,57]
Hepatomegaly	Rare [14]	NR	[42%] [41]	[61 to 88%] [42]
Splenomegaly	Rare [14]	NR	[22 to 65%] [41]	[45 to 59%] [42]
Hemophagocytosis	+ [14]	NR	+ [3,40]	[81%] [42]
Thrombocytopenia	+ [14], [54]	[46%] [58]	-	[89%] [42]
Anemia	+ [54]	Hemolytic anemia [35%] [58]	[68%] [41]	[67 to 82%] [42]
Leukopenia	+ [14], [54]	NR	-	[39 to 56%] [42]
Neutropenia	+ [54]	NR	-	+ [14,55,57]
Neutrophilia	+ [54]	+ [56]	[81%] [41]	-
Macrophage activation	+ [14]	NR	+ [59]	+ [14,55,57]
Low/absent NK activity	+ [14]	NR	+ [60]	+ [14,55,57]
Sol. IL-2R >2,400 U/ml	+ [14]	NR	+ [48]	+ [14,55,57]
Abnormal liver function tests	+ [54]	+ [56]	[73%] [41]	[94%] [42]
HyperTG	+ [14]	NR	NR	[77 to 100%] [42]
Coagulopathy	+ [54]	DIC [15%] [58]	Rare [41]	+ [55]
Hypofibrinogenemia	+ [14], [54]	[15%] [58]	Rare [41]	[78 to 89%] [42]
ESR/CRP (↑ or ↓)	↑ [54]	↑ [46]	↑ [99%] [41]	ESR ↓ [79 to 92%] [42] CRP ↑ [61]

[%], percentage of association reported in the literature; +, positive association but not precise percentage reported; -, not associated; NR, no reported association. *CRP* C reactive protein, *DIC* disseminated intravascular coagulation, *ESR* elevated sedimentation rate, *hyperTG* hypertriglyceridemia, sol. IL-2R, soluble IL-2 receptor.
* There is only our study on cAPS and it is a small cohort, and there are only a few studies on ferritin levels in sepsis, so the values of ferritin in these two conditions may be underestimated.
Table 2. All four conditions are life-threatening events in which an uncontrolled and immune response, triggered in most cases by infectious agents, leads to a severe hyperinflammation. There is evidence of hypercytokinemia and hyperferritinemia during the symptomatic period of the diseases. With the exception of the cAPS, for which there is no information in the literature, there is an impaired or absent function in natural killer (NK) and cytotoxic T cells.

La mise en évidence d'un trigger infectieux, cancéreux ou rheumatologiques n'exclut pas la mise en évidence d'anomalie génétique



Journal of Allergy and Clinical Immunology DOI: (10.1016/j.jaci.2015.06.048)

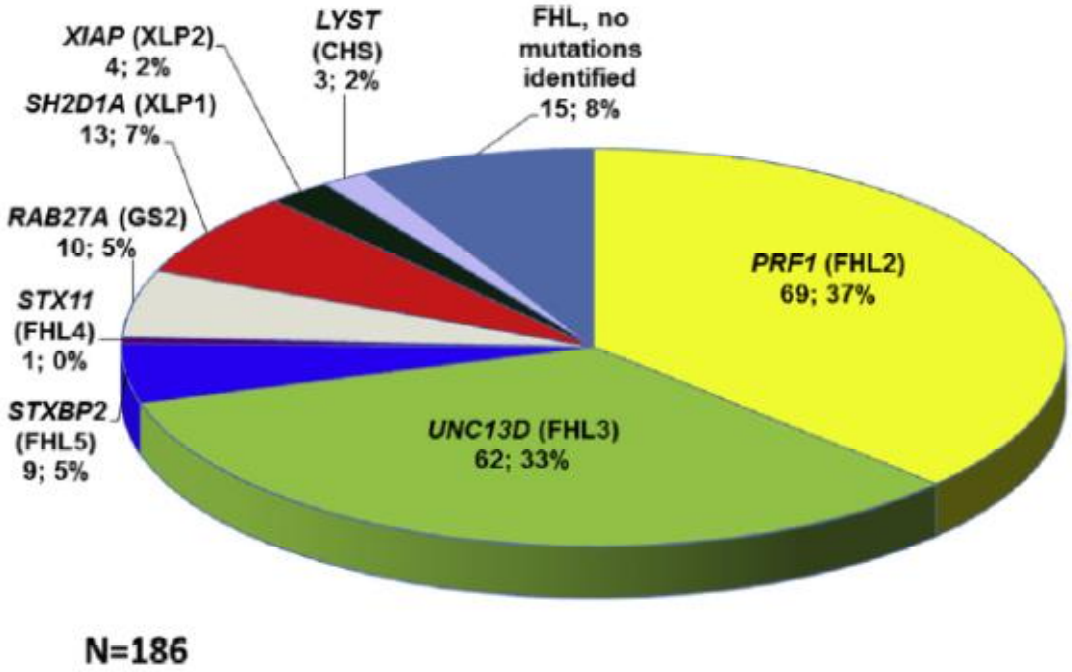
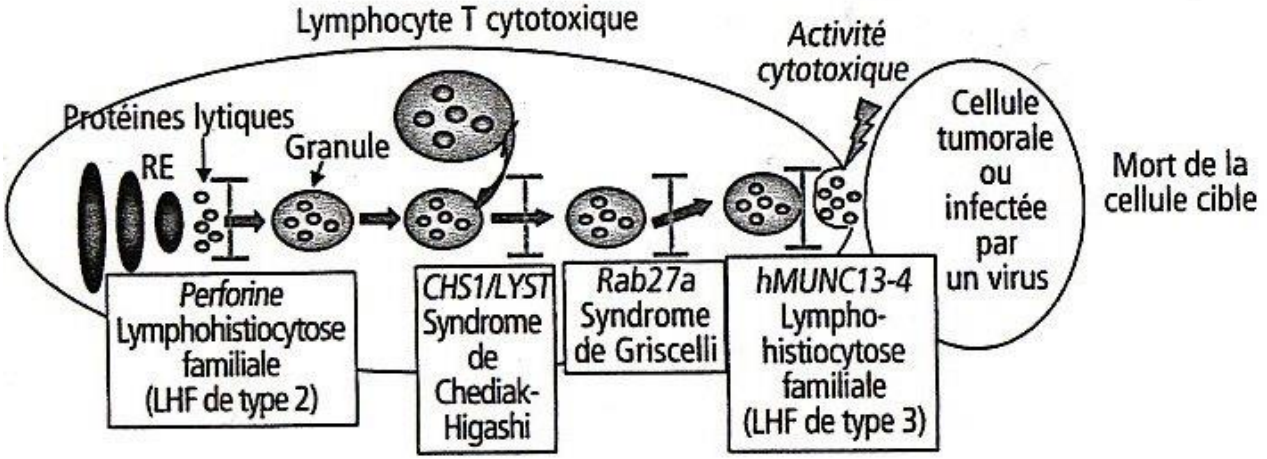


FIG 3. Breakdown of the different genetic subtypes in 171 patients with FHL or FHL-related disease. For each subtype, the name of the gene, the abbreviation of the disease subtype, the absolute number, and the percentage are shown. Furthermore, we include as FHL one subgroup of 15 patients with either familial recurrence and/or refractory/recurrent disease despite specific therapy and/or repeatedly documented severe functional defect in degranulation or cytotoxicity assays (see Table E3).

Quelles en sont les causes?

Héréditaire →défaut primaire de l'activité cytotoxique des cellules T et NK



Différentes anomalies moléculaires ont été identifiées et sont impliqués dans les étapes de la voie d'exocytose des granules cytotoxiques, synthèse des protéines lytiques contenues dans ces granules

Å Tests fonctionnels

Defects of killing par les cellules T et NK identifiés par les tests appropriés

Mesure de l'expression de proteine (CMF expression perforine)

Granule release assay (CD107a)

Cytotoxicity assay

Mutation analysis

Immunodéficience prédisposant au HLH

Reflet de l'activation de la réponse immunitaire

Anomalies pathologiques dû à la réponse immune inadéquate

Table 3. Diagnostic and classification criteria for macrophage activation syndrome.

HLH-2004 diagnostic guidelines	Preliminary diagnostic guidelines for MAS complicating sJIA	Preliminary diagnostic guidelines for MAS complicating jSLE	Classification criteria for MAS complicating sJIA
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AST: Aspartate aminotransferase; BM: Bone marrow; HLH: Hemophagocytic lymphohistiocytosis; jSLE: Juvenile systemic lupus erythematosus; LDH: Lactate dehydrogenase; MAS: Macrophage activation syndrome; NK: Natural killer; sJIA: Systemic juvenile idiopathic arthritis; WBC: White blood cells.

Cron R et al. Expert Rev Clin Immunol 2015

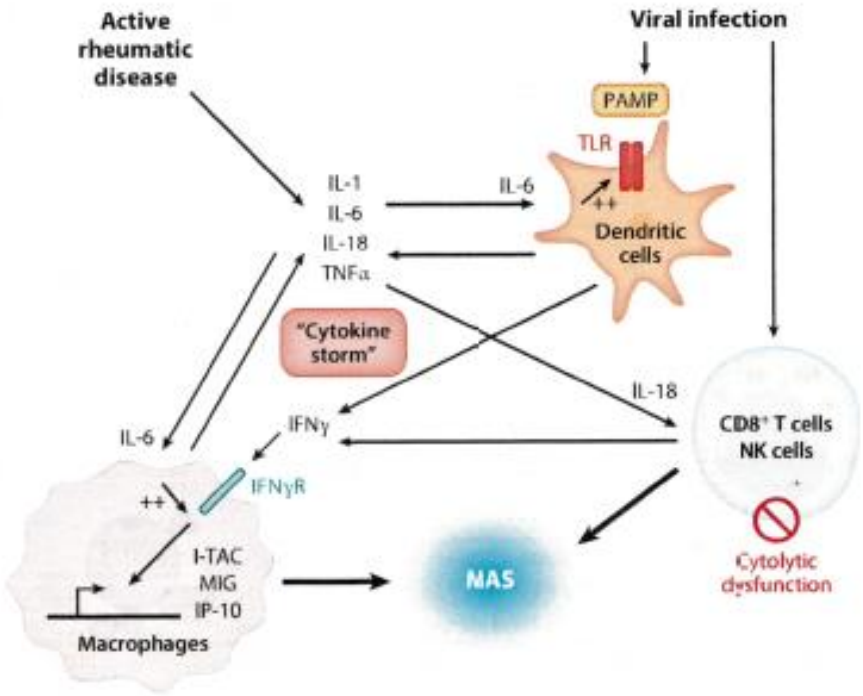

Figure 1

"Cytokine storm" and the development of macrophage activation syndrome (MAS). MAS can develop in the setting of high systemic juvenile idiopathic arthritis (SJIA) disease activity, which is associated with increased levels of cytokines including IL-1, IL-6, IL-18, and TNFα. MAS can also be triggered by viral infections, wherein pathogen-associated molecular patterns (PAMPs) are recognized by toll-like receptors (TLRs) and trigger further secretion of inflammatory cytokines. The proinflammatory environment including elevated IL-6 can enhance signaling through TLRs. Infection also leads to activation and proliferation of $CD8^+$ T cells and NK cells, including secretion of IFNγ. Defects in the cytolytic activity of these lymphocytes also contribute to hyperinflammation. Increased IL-18 levels further drive IFNγ production by these activated lymphocytes. This surge in IFNγ leads to activation of macrophages that acquire a proinflammatory phenotype and generate high levels of chemokines and cytokines. These activated macrophages, along with $CD8^+$ T cells, traffic to tissue including bone marrow and liver and lead to the cytopenias, liver dysfunction, and coagulopathy associated with MAS.

Schulert. Annu. Rev. Med. 2015

Immunodéficience prédisposant au HLH

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Anomalies pathologiques dû à la réponse immune inadéquate

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Adapted 4/5 criteria
Sens 35%, spec 100%

Sens 86% spec 86%

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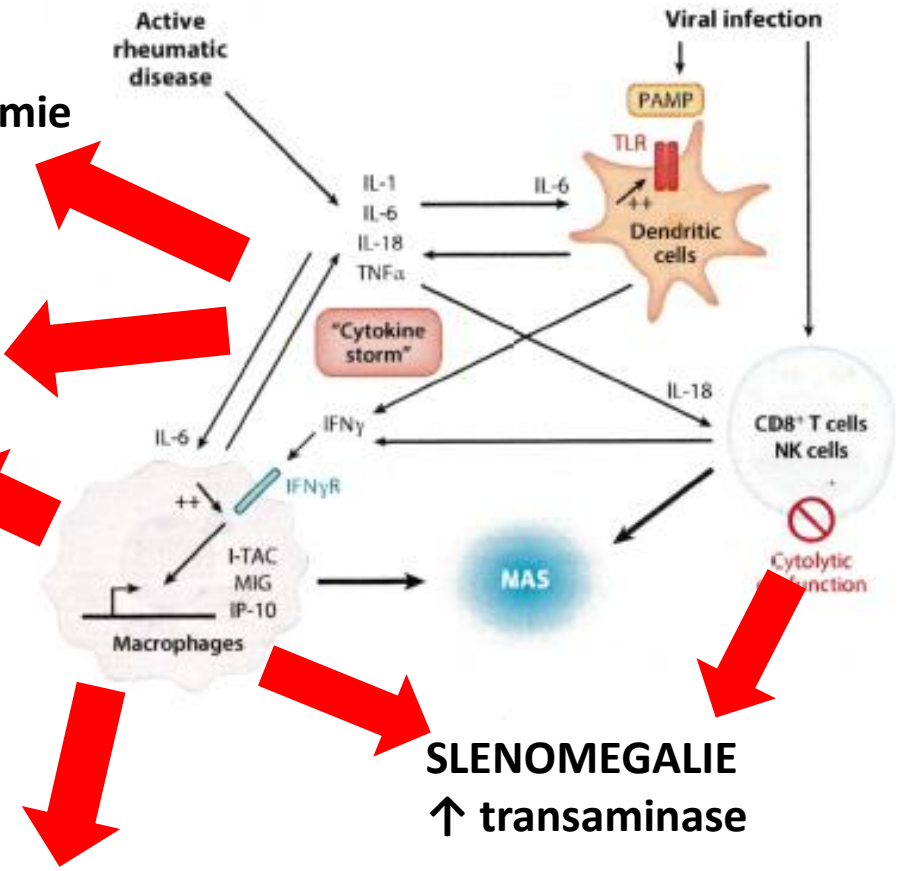
FIEVRE

Hypertriglycéridémie
(inhibition de la lipoprotéine lipase)

Cytopénie



Soluble CD25 ↑

SLENOMEGALIE
↑ transaminase

Hyperferritinémie (macrophage activation GDF15?)
Hypofibrinogénémie (plasminogen-activator)
Hémophagocytose
CD163 ↑

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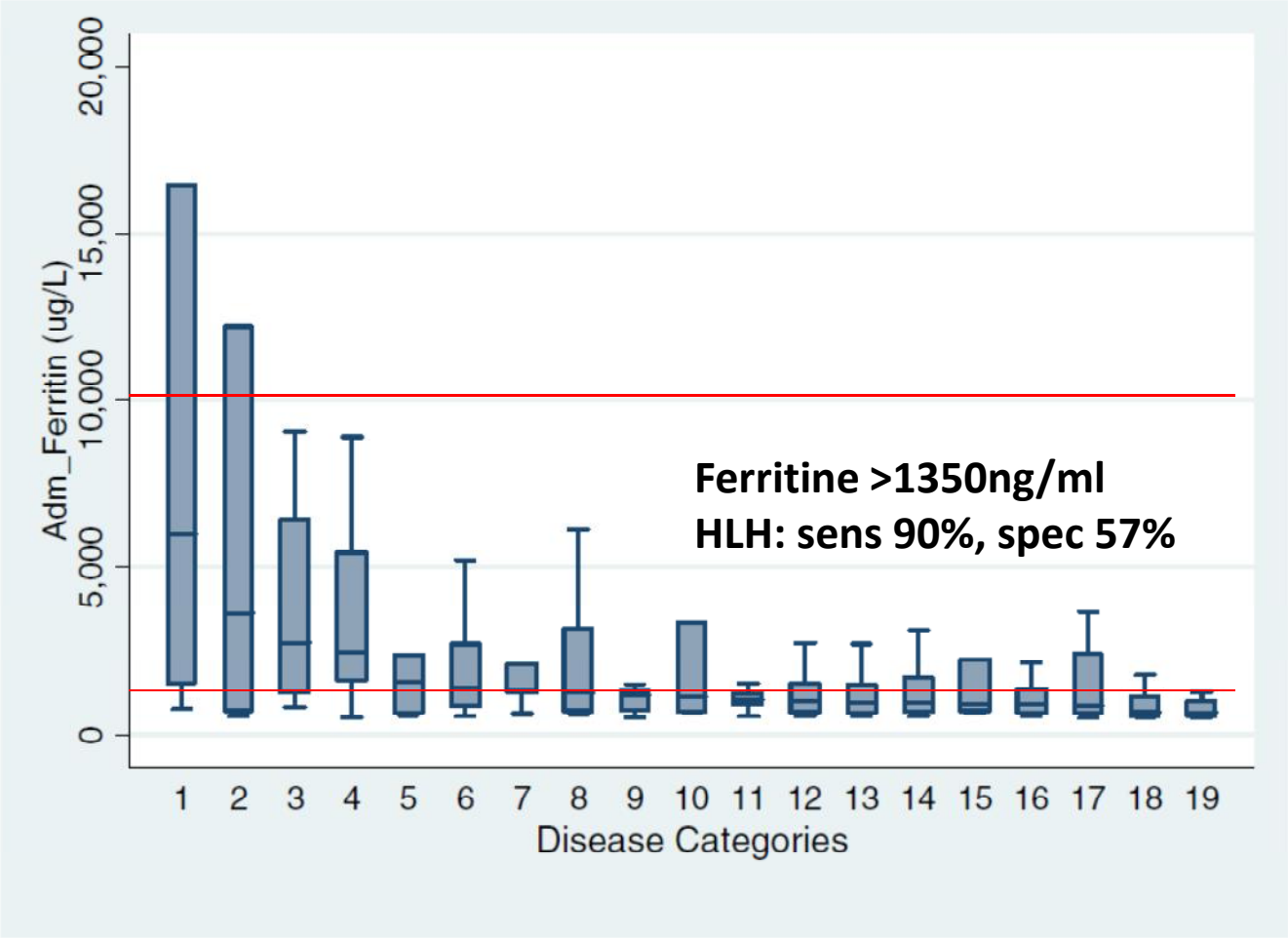

Disease Categories
1-HLH
2-Unknown
3-Shock
4-Stem cell transplant
5-Heart disease
6-Chronic transfusion
7-Immunodeficiency
8-Liver disease
9-Cystic fibrosis
10- Bone marrow failure
11-Prematurity
12-Malignancy
13-Viral infection
14-Bacterial infection
15-Solid organ transplant
16-Trauma
17-Autoimmune disease
18-Hemoglobinopathy
19-Renal disease

Pediatr Blood Cancer 2008; 50:1227-35