PHENOTYPE HEMOCHROMATOSE NEONATALE
FOIE ET SURCHARGE EN FER FŒTALE ET PERINATALE: QUELS DIAGNOSTICS ?

SYNTHESE

Dr Sophie COLLARDEAU-FRACHON, MD PhD
Centre de Pathologie Est
Hôpital Femme-Mère-Enfant
CHU de Lyon, France
sophie.collardeau-frachon@chu-lyon.fr

Hôpitaux de Lyon

SoFFœt
Société Française de Fœtopathologie

Université Claude Bernard Lyon 1

Diagnostic de GALD/ HNAI

1/Extrahepatic iron overload is not specific of GALD

Iron in oral mucosal salivary glands

and /or MRI pancreas low signal intensity

- not always present in GALD cases
- can be present in non GALD cases

2/ Be careful with C5b9 immunohistochemistry

-specially on liver biopsy sample

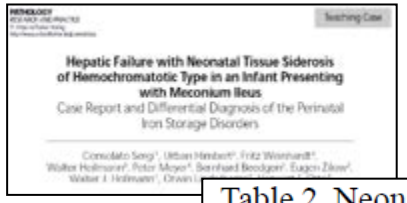
- is not specific of GALD

3/ Always think about other differential diagnoses

- importance of autopsy
- importance of liver histological examination

1/Extrahepatic iron overload is not specific of GALD

NH is a phenotype: several diseases can present with hepatic +/- extrahepatic siderosis



Hepatic Failure with Neonatal Tissue Siderosis of Hemochromatotic Type in an Infant Presenting with Meconium Ileus

Case Report and Differential Diagnosis of the Perinatal Iron Storage Disorders

Table 2. Neonatal disorders associated with iron accumulation

1. **Primary neonatal iron accumulation**
   - Familial type (autosomal recessive inheritance) [a]
   - Sporadic type

GALD

2. **Secondary neonatal iron**

Association with GALD

2.1. Syndromic
- Edward syndrome (Trisomy 18) [b]
- Down syndrome (Trisomy 21, OMIM 190685) [c]
- Zellweger syndrome (Cerebro-hepato-renal syndrome, OMIM 214100) [d]
- Tricho-hepato-enteric syndrome (Trichorrhexis blastysis, OMIM 222470) [e]
- Donohue syndrome (Leprechaunism, OMIM 246200) [f]
- Renal tubular dysgenesis syndrome (OMIM 267430) [g]

2.2. Non Syndromic
- Intrauterine infection [h]
- Exposure to toxic agents (e.g. pyrrolizidine) [i]
- Δ^4-3-oxosteroid 5β-reductase deficiency [j]
- Hereditary tyrosinemia (OMIM 276700) [k]
- Hemolytic disease (positive Coombs reaction) [l]
- Exogeneous iron overload [m]
- Congenital heart disease [n]
- Neonatal lupus erythematosus syndrome [o]
- Bowel obstruction [p]

myofibromatosis

Can be secundary to chronic fetal distress

- Mitochondrial cytopathies
  - DGUOK mutations
  - GRACILE syndrome
- Transaldolase deficiency
- Familial Hemophagocytic lymphohistiocytosis (FHL)
- Martinez-Frias syndrome
- 16p duplication

Infections
- **Parvovirus B19**
- CMV
- HSV
- Echovirus
- Adenovirus
- Enterovirus
- Orthomyxovirus A or B
- Respiratory syncytial virus
- Rubella
- Chlamidya psittaci
- Coxiella burnetii
- Mycoplasma pneumoniae
- S aureus
- Toxoplasma gondii
- E Coli

Phénotype HN: 4 nouveaux cas….

<u>Neonatal anemia: 1 case</u>
Congenital dyserythropoietic anemia type 1 (CDAN1)

CDG1a (PMM2): 1 case

Congenital leukemia & chromosomal breakage syndrome (Bloom syndrome): 1 case

FHL/XIAP: 1 case

Never reported to date

Diseases with NH phenotype

13 groups of etiology (literature and our cases)

Infections

Alloimmune diseases
GALD
anti-red cells (Rhesus or private antigens)

Neonatal anemia
Congenital dyserythropoietic anemia

Mitochondrial cytopathies
DGUOK, Gracile

Chromosomal anomalies
T21, T18, 16p

Congenital proliferation
Leukemia
FHL

Metabolic disorders
TALDO
Zellweger
Tyrosinemia*
CDG1a

Maternal autoimmune disorders
Neonatal lupus*

Neonatal diabetes
Martinez-Frias
Donohue

Congenital heart disease

Tricho-hepato-enteric syndrome

Exogeneous
Transfusions
Toxic agents

Neonatal cholestasis
Delta 4-3 oxo

Maternal autoimmune disorders and NH

Pediatric and Developmental Pathology 15, 450–470, 2012
DOI: 10.2350/12-02-1155-OA.1

French Retrospective Multicentric Study of Neonatal Hemochromatosis: Importance of Autopsy and Autoimmune Maternal Manifestations

Sophie Collardeau-Frachon,[1*] Sophie Heissat,[2] Raymonde Bouvier,[1] Monique Fabre,[3] Julien Baruteau,[4] Pierre Broue,[4] Marie-Pierre Cordier,[5] Dominique Debray,[6] Hanna Debiec,[7] Pierre Ronco,[7] and Vincent Guigonis[8]

dysimmunity manifestations in half of the mothers

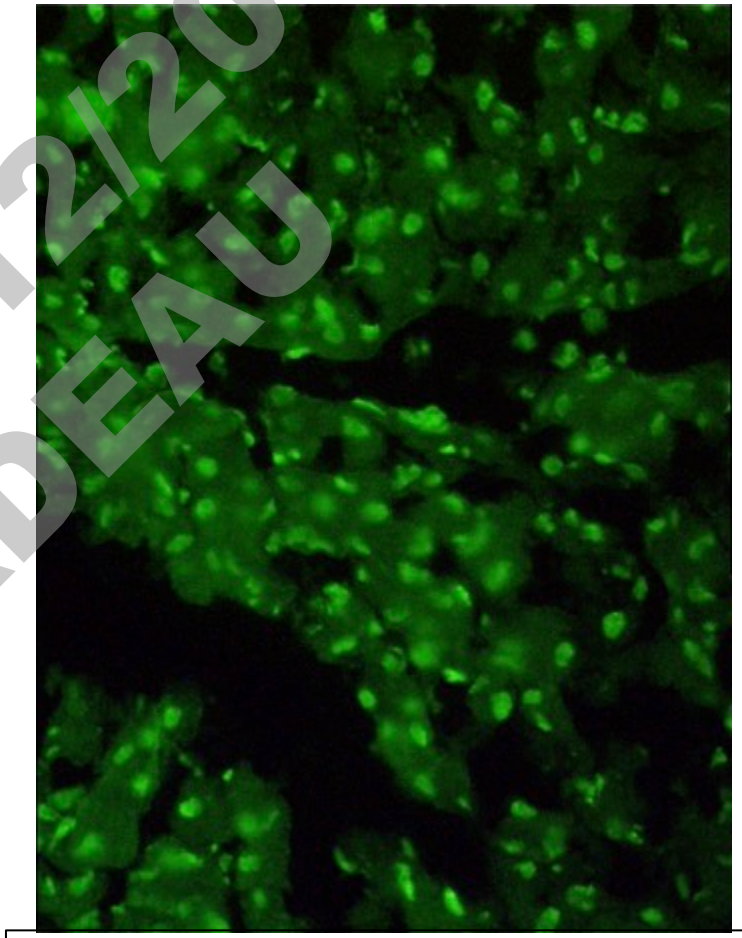
5 mothers : personal history of autoimmune disease:

- systemic erythematous lupus (1 case)
- hypothyroidism (2 cases)
- peripheral vascular disease (1 case)
- autoimmune hepatitis (1 case)

9 mothers without clinical autoimmune disorder: antinuclear autoantibodies at time of delivery

Characterization of the autoantibodies in 4 cases:

- anti-Ro/SSa and anti RLa/SSB in 1 case
- anti-mitochondria and anti-phospholipid in 2 cases
- and anti-cardiolipid in 1 case



Indirect immunofluorescence study :nuclear staining of hepatocytes and endothelial cells

Maternal autoimmune disorders and NH

Some NH cases could be a possible consequence of autoimmune disorders in the mothers with transfer of autoantibodies through the placenta

Schoenlebe J, Buyon JP, Zitelli BJ, Friedman D, Greco MA, Knisely AS. Neonatal hemochromatosis associated with maternal autoantibodies against Ro/SS-A and La/SS-B ribonucleoproteins. Am J Dis Child 1993;147:1072–1075.

1 case

Classification and genetic features of neonatal haemochromatosis: a study of 27 affected pedigrees and molecular analysis of genes implicated in iron metabolism

Alison L Kelly, Peter W Lunt, Fernanda Rodrigues, P J Berry, Diana M Flynn, Patrick J McKiernan, Deirdre A Kelly, Giorgina Mieli-Vergani, Timothy M Cox

2 / 8 mothers with anti-Ro/SSa and anti R-La/SSB antibody

Primary Biliary Cirrhosis-Specific Antimitochondrial Antibodies in Neonatal Haemochromatosis

Clinical and Developmental Immunology

2013

Daniel S. Smyk,[1] Maria G. Mytilinaiou,[1,2] Tassos Grammatikopoulos,[2] A. S. Knisely,[1] Giorgina Mieli-Vergani,[1,2] Dimitrios P. Bogdanos,[1,3] and Diego Vergani[1]

AMA in the mother& the child

Diseases with NH phenotype

Fetus or at birth

Infections

Alloimmune diseases
GALD
anti-red cells (Rhesus or private antigens)
anti-platelets

Neonatal anemia
Congenital dyserythropoietic anemia

Mitochondrial cytopathies
DGUOK, Gracile

Chromosomal anomalies
T21, T18, 16p

Congenital proliferation
Leukemia
FHL

Metabolic disorders
TALDO
Zellweger
CDG1a
tyrosinemia

Maternal autoimmune disorders

Neonatal diabetes
Martinez-Frias
Donohue

Tricho-hepato-enteric syndrome

Congenital heart disease

Exogeneous
Transfusions
Toxic agents

Neonatal cholestasis
Delta 4-3 oxo

Hepatic iron overload characteristics

epithelial

Alloimmune diseases
GALD
anti-red cells (Rhesus or private antigens)

Neonatal anemia
Congenital dyserythropoietic anemia

Neonatal diabetes
Martinez-Frias
(Donohue)

Maternal autoimmune disorde
Neonatal lupus

Tricho-hepato-enteric syndrome

Neonatal cholestasis
Delta 4-3 oxo

macrophages

Congenital heart disease

Exogeneous
Transfusions
Toxic agents

mixte

Chromosomal anomalies : 5 cases
T21 (L)
T18
(16p)

Infections
Parvo B19 (L)
CMV
HSV1
HSV2
Enterovirus
E Coli

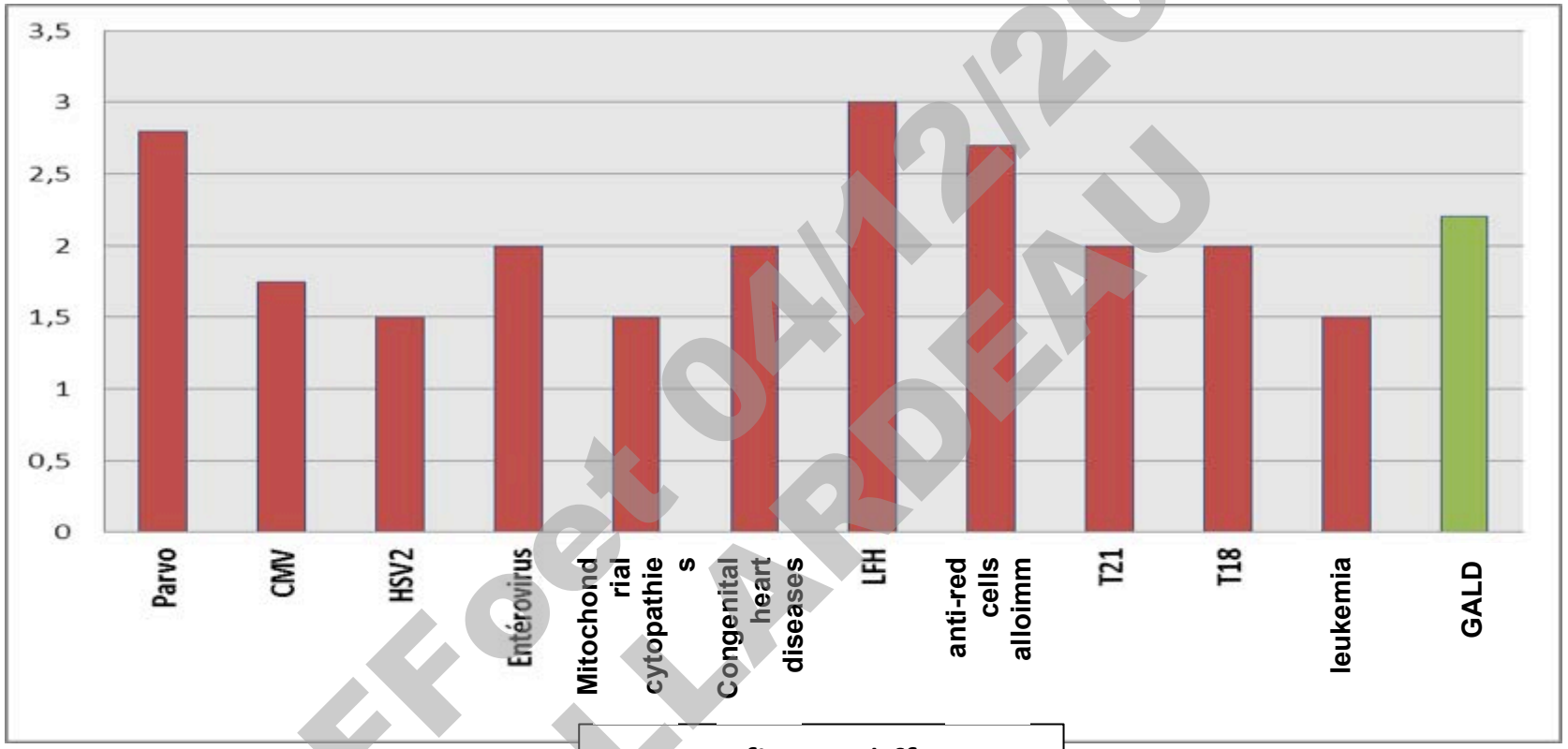
Congenital proliferation
Leukemia
FHL

Metabolic disorders
TALDO
Zellweger
Tyrosinemia
CDG1a

Mitochondrial cytopathies : 4 cases
DGUOK
Gracile

Hepatic iron overload characteristics: Perls score

1+ = <25%, 2+ = 25%-50%, 3+ = 50%-75%, 4+ = >75% stained parenchyma

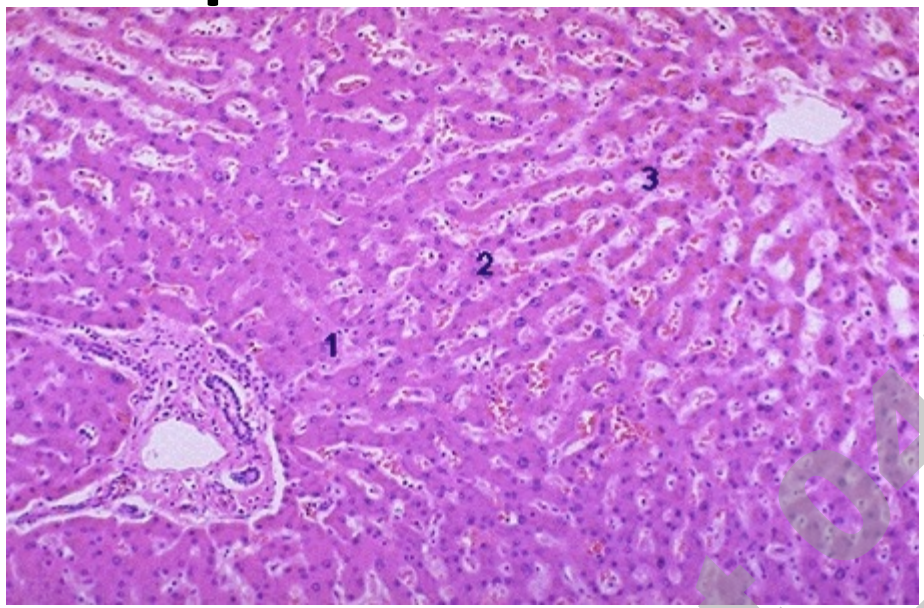


No significant difference

Average non GALD cases =2,4
Fetus : 2,4
Neonates: 2,3

Average : GALD cases= 2,1
Fetus: 2,9
Neonates: 2,1

Hepatic iron overload characteristics: location



Either diffuse or focal
- HSV2
- CMV
- T21
- Congenital heart disease
- leukemia

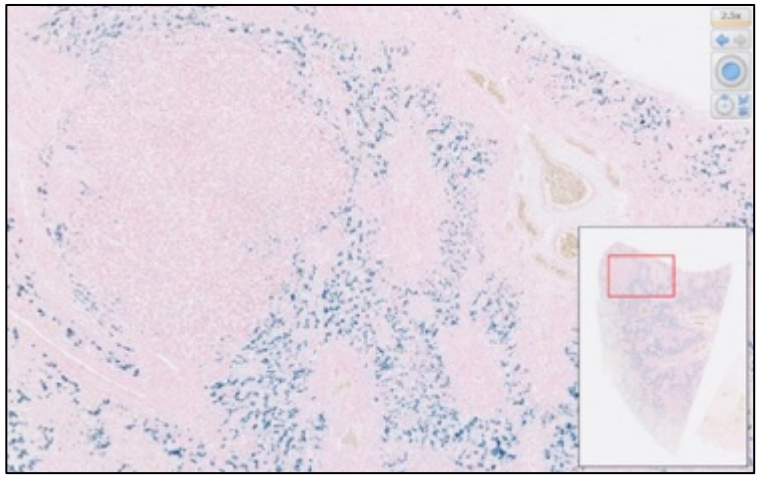
Mainly Z1
- HSV1

Mainly Z2
- Delta 4-3 oxo

Heterogeneous
- GALD
- DGUOK
- FHL

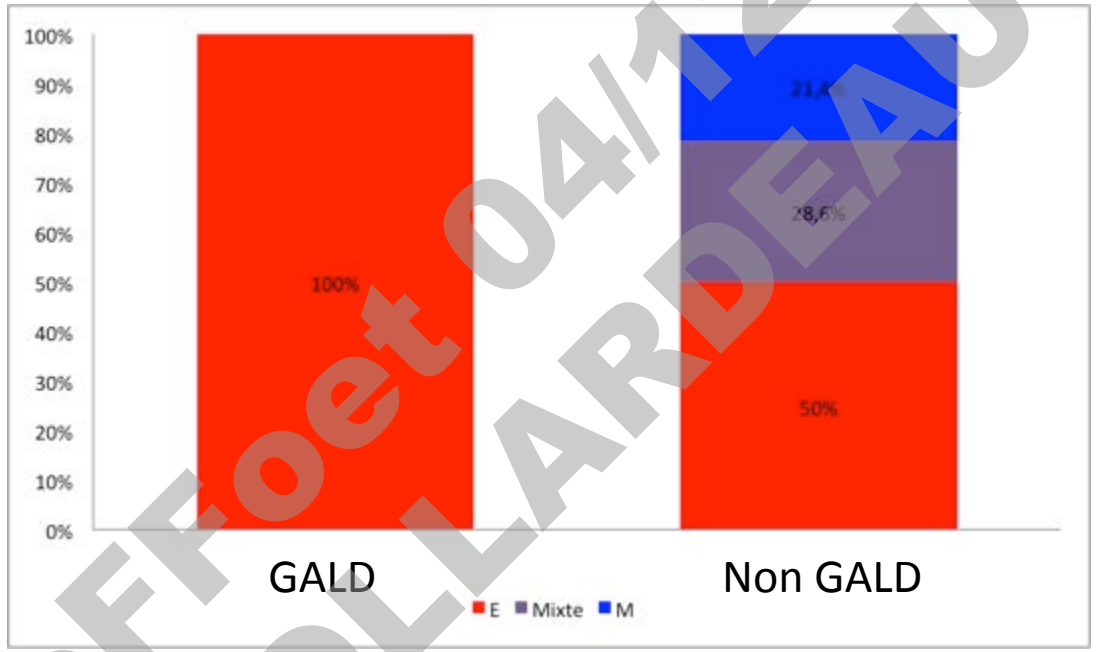
Diffuse : all zones
z1=z2=z3 same intensity
- Gracile
- E Coli
- CDAN1

Diffuse : all zones
z1>z2>z3
- Transaldolase
- T18
- anti-Rh alloimm
- Martinez-Frias
- CDG1a



Results : extrahepatic iron overload

- 91,3% GALD (since 26 GW)
- 75,5% non GALD (since 17 GW: parvovirus)



Perls positivity in macrophages spleen, lymph nodes, bone marrow do not favor the diagnosis of GALD
Look systematically for hemosiderosis on these tissues

Extrahepatic iron overload

only seen after Perls staining

localization varied:

- depending on the age
- within the same sibship

Sometimes:

- **only seen in a few organs**
- **with a mild intensity** (high-power magnification is required)

Results : extrahepatic iron overload

Absence

- GALD: 2 cases (fetuses 27GW)
- 1/ 4 CMV (21 GW)
- 3/5 parvo (18, 24 GW)
- Gracile (neonate 4 days)
- Leukemia: 22 GW
- ½ T18 (neonate 5 days)
- Congenital heart disease : 1/5 (neonate 52 days)

Don’t reject the diagnosis of GALD
if absence of extrahepatic iron overload
especially in young fetuses

Extrahepatic iron overload

Infections
Parvo
CMV
HSV
E.Coli

Alloimmune diseases
GALD
anti-red cells (Rhesus or private antigens)

Neonatal anemia
Congenital dyserythropoietic anemia

Mitochondrial cytopathies
DGUOK, Gracile

Chromosomal anomalies
T21, T18, 16p

Congenital proliferation
Leukemia
FHL

Metabolic disorders
TALDO
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Maternal autoimmune disorders

Neonatal diabetes
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Congenital heart disease

Tricho-hepato-enteric syndrome

Exogeneous
Transfusions
Toxic agents

Neonatal cholestasis
Delta 4-3 oxo

Reticuloendothelial system not spared

Infections
Parvo (L)
CMV
HSV2
E.Coli
enterovirus

Alloimmune diseases
GALD
anti-red cells (Rhesus or private antigens)

Neonatal anemia
Congenital dyserythropoietic anemia

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Exogeneous
Transfusions
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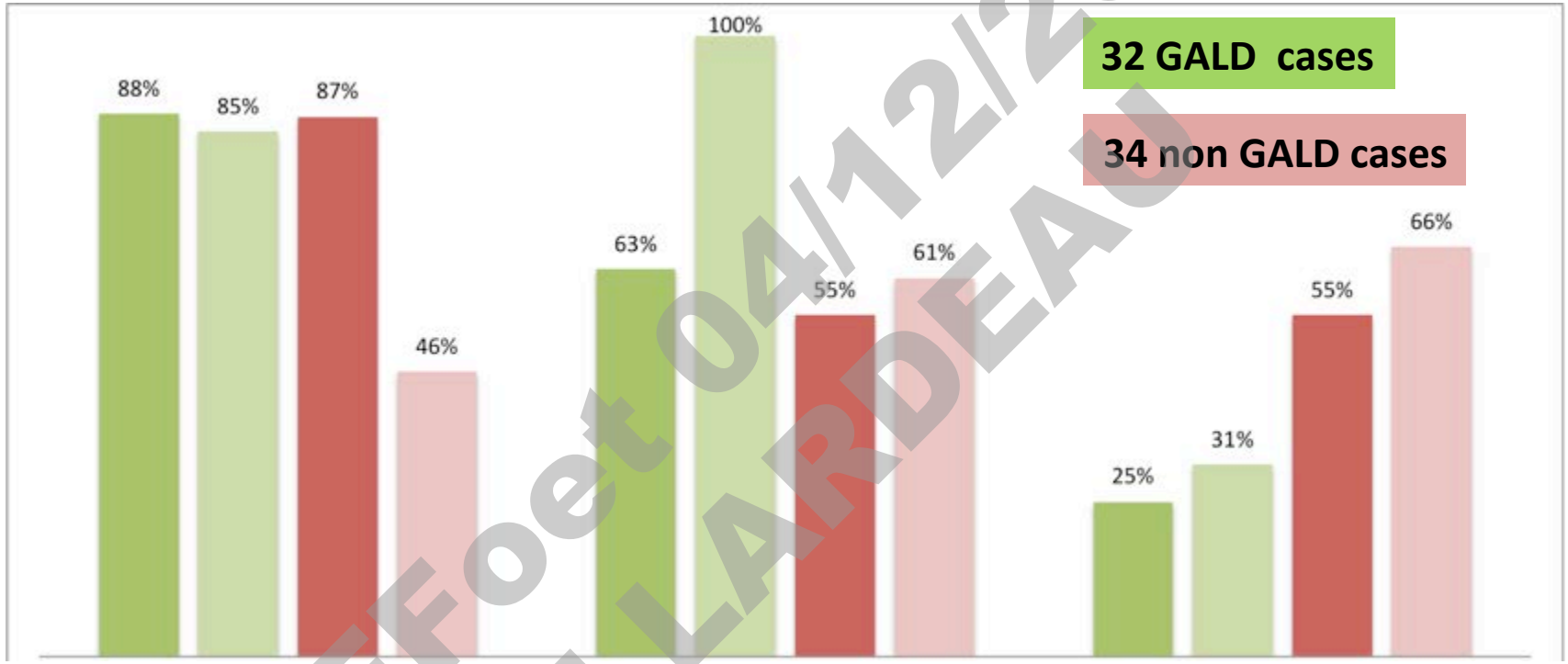
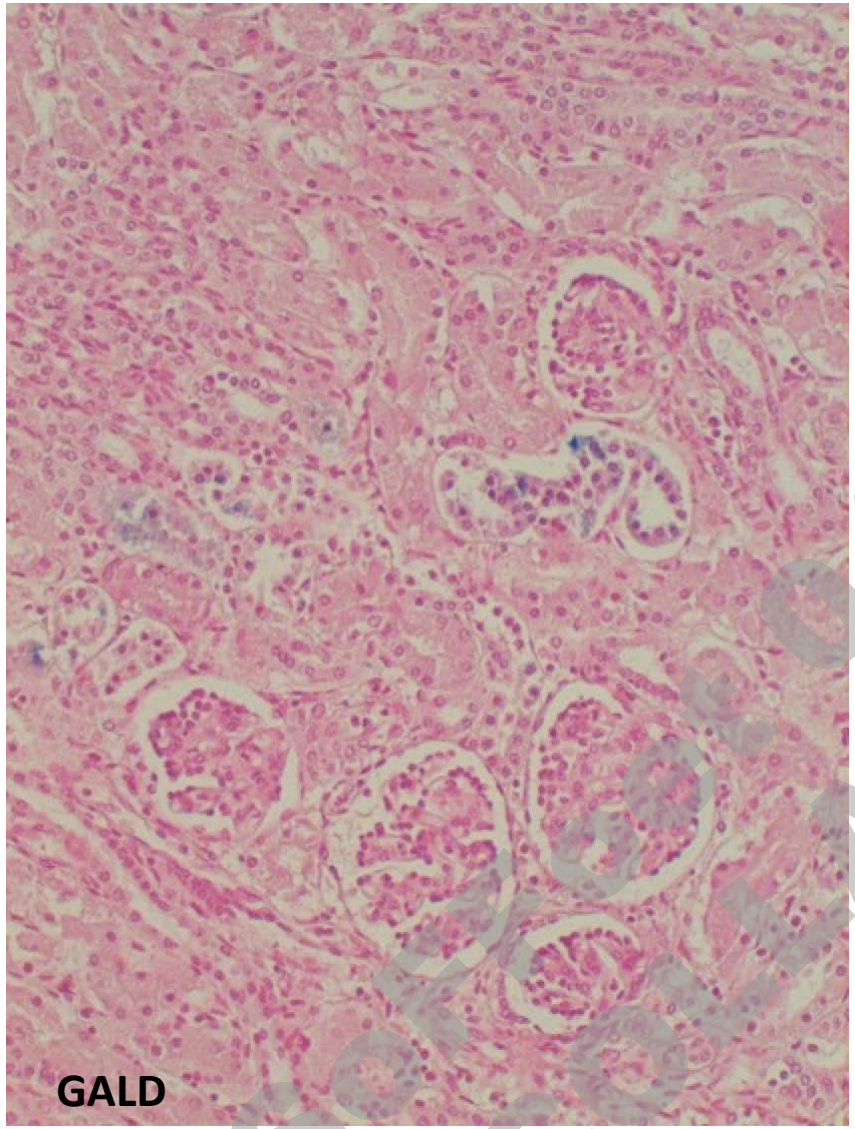
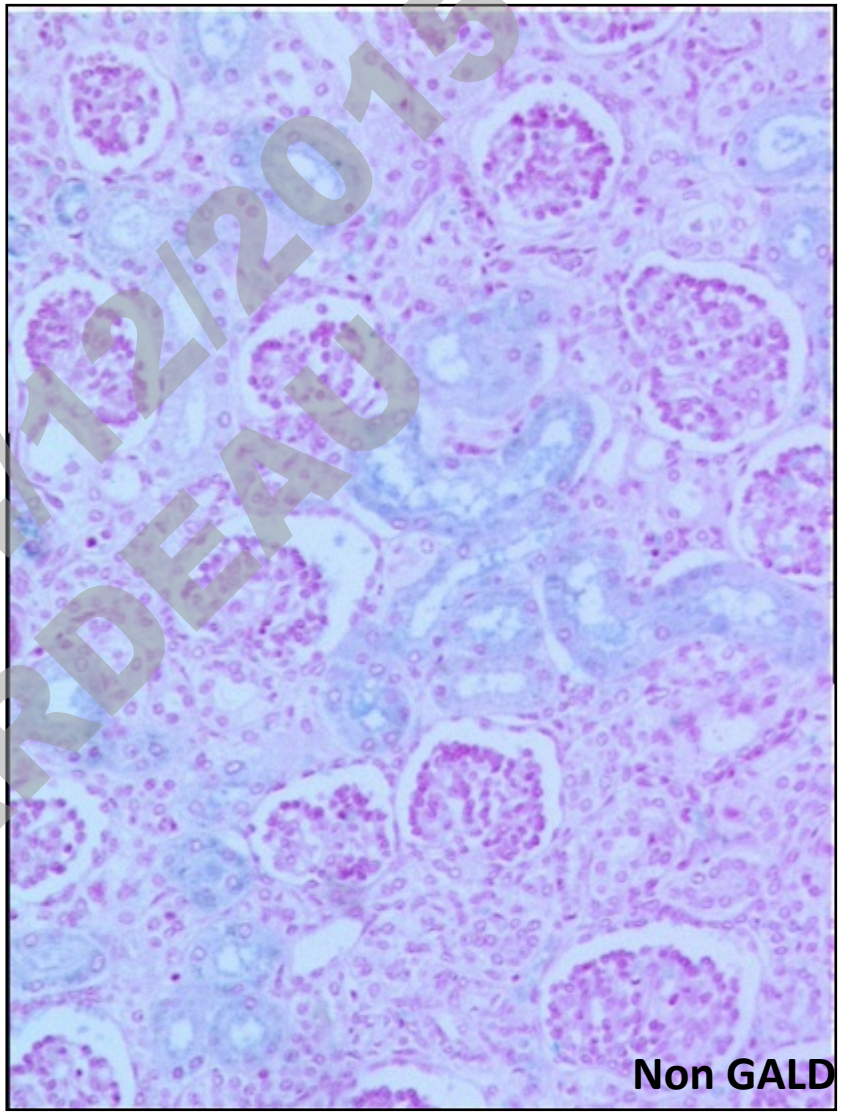
Extrahepatic iron overload in GALD and non GALD autopsy cases



GALD fetuses: 10 | GALD neonates: 22 | Non GALD fetuses:14 | Non GALD neonates: 20





Usually within a few proximal tubules

Extrahepatic iron overload in GALD and non GALD autopsy cases

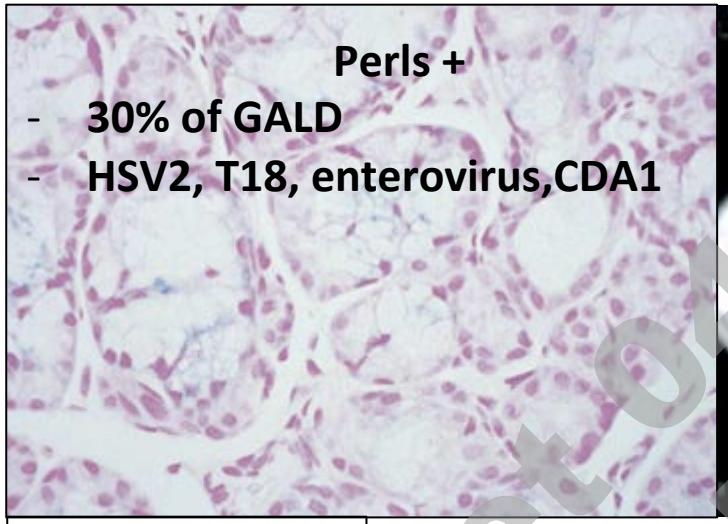
- Para tracheal glands
  - Parvo, enterov, transaldo, bile ac def, leukemia, CDAN1
- Adrenal
  - Parvo, CMV, HSV2, CDG1a, T21
- Thymus
  - Parvo, HSV2, cong heart def
- Duodenum
  - Parvo, T18, T21, CDAN1
- Stomach
  - Parvo, HSV2, E Coli, T18, CDG1a, leukemia, CDAN1
- Myocardium
  - HSV2, E Coi, T21, bile ac def
- Minor salivary glands (lip)
  - HSV2, enterov, CDAN1, T18

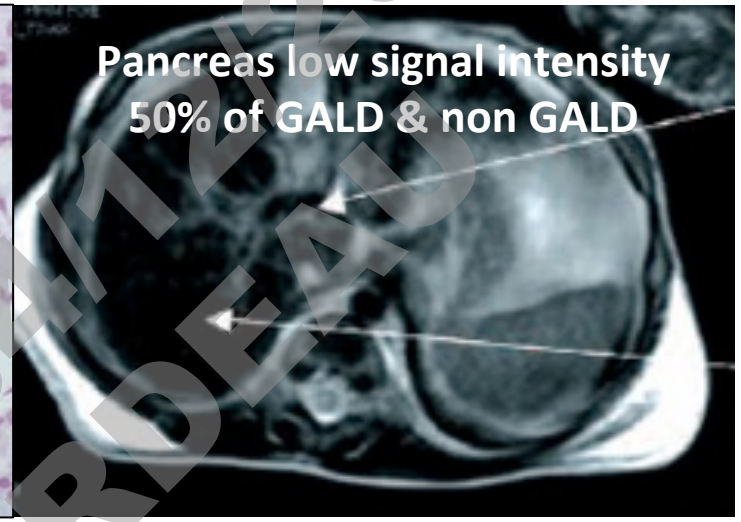
Influence of liver lesions on extrahepatic iron overload

cases with	severe liver fibrosis	
AND	GALD	Non GALD
extrahepatic iron hemosiderosis	93%	66%

	Influence of Perls score Intensity of liver hemosiderosis			
	Perls≥ 3		Perls≤2	
cases with extrahepatic iron hemosiderosis	GALD	Non GALD	GALD	Non GALD
	83%	64%	94%	77%

"**Siderosis of the buccal glands and other extrahepatic tissues is not seen in neonatal diseases other than NH/GALD**" (Whitington PF. Fetal and infantile hemochromatosis. Hepatology 2006;43:654–60.): **not true**





Literature
- T21
- DGUOK
- Lupus
- 16p
- Delta 4-3 oxo

Iron in oral mucosal salivary glands and/or pancreas T2 low signal intensity on MRI can lack in GALD

2: C5b9 immunohistochemistry

anti-C5b9 neoantigen, Quidel, San Diego, CA
dilution: 1/150

Group 2: non GALD cases with NH phenotype: 25/45 cases

- 2/5 parvo: 5%
- 3 / 5 CMV: <5%
- **2 HSV1: 90%**
- **1/2 HSV2: 80%**
- **1 /2 Enterovirus:90%**
- **1/3 DGUOK: 80%**
- **3 /3 Allo-imm anti Red B cells: 80%**
- 2/2 T18 < 5%
- **1/3 T21: 90%**
- 3/5 heart: 5%
- 1Taldo : 20%
- **1D4-3oxo: 90%**
- 2: leukemia <5%
- 1 GRACILE<5%
- 2/3 FLH<5%
- 1CDA I
- 1 Martinez-Frias
- 1CDG1a
- 2 E coli

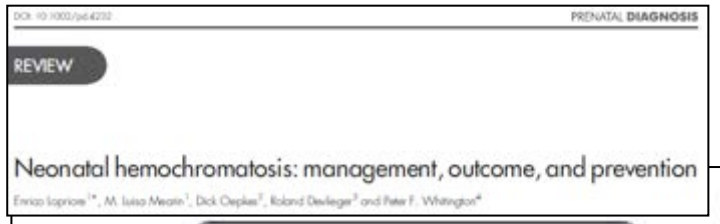
Group 1: 20/32 GALD cases expressed in all cases but
>75% in 40%
<75% in 60%
Average: 64%

Group 3: liver diseases without siderosis: 15 cases

- **2 Giant cell hepatitis with autoimmune hemolytic anemia: 90%**
- **1Gaucher : 80%**
- 2 BA: 0%
- 2 Alagille: 0%
- 1 A1AT: 0%
- 2 PFIC1: 20%
- 2 PFIC2:0%
- 2 Tyrosinemia: 0%
- **1 Cystic fibrosis: 75%**

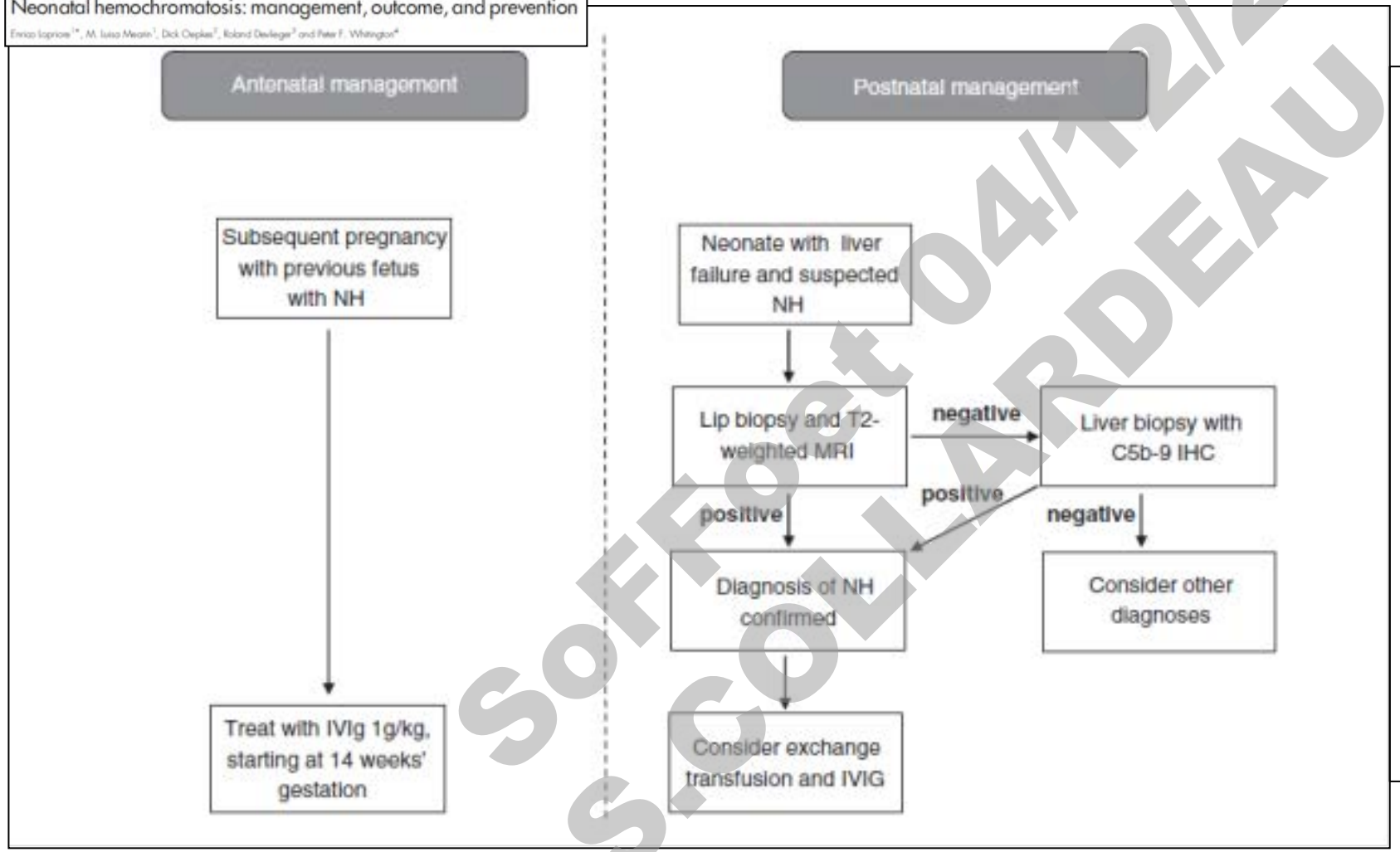
Group 4: 5 control cases
Normal liver: 0%
4 fetus
14 GW
22 GW
25 GW
27 GW
1 neonate
38 GW + 4 days

GALD: diagnosis and management


PRENATAL DIAGNOSIS

REVIEW

Neonatal hemochromatosis: management, outcome, and prevention



GALD diagnosis based on :

- **demonstration of ex[illegible]en side**

OR

- **positive C5b-9 on or in hepatocytes**

Definitive diagnosis of GALD

- **Still remains difficult**
  - Heterogeneity of liver hemosiderosis & C5b9 expression
- **Often a diagnosis of exclusion**
- **Importance of correlations**
  - clinical, biological, radiological and pathological findings
- **Easier on autopsy**

Definitive diagnosis of GALD
Importance of clinical & biological data

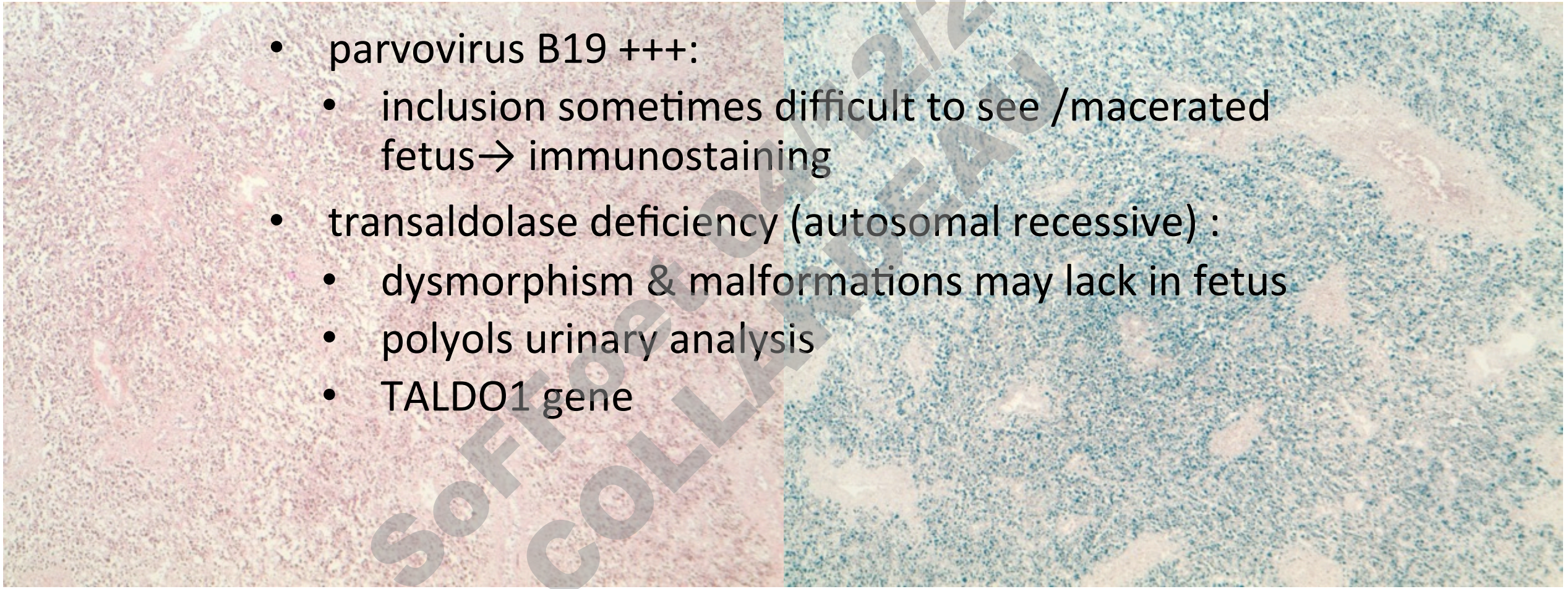
- 1st pregnancy? Previous cases of child or fetal loss
- Mother auto-immunity?
- Consanguinity?
- Dysmorphism, malformations?
- Onset of symtoms? Immediately after birth or not?
- Karyotype?
- Infections?
- Lactate/pyruvate ratio >20 → mitochondrial respiratory chain disorders
- LFT: low GGT → delta 4-3 oxo

Definitive diagnosis of GALD autopsy is very hellpful

- **All organs can be examined and sampled**
  - Look for other macroscopic and microscopic malformations
  - Don’t forget to take bone marrow sample
  - Placenta : signs of infections or dysimmunity
  - Renal tubular dysgenesis: more frequent in GALD cases?
- **Allows to do Perls staining on multiple organs**
  - extrahepatic iron overload is not limited to pancreas and labial salivary glands
  - must be systematic on liver, thyroid, pancreas , kidneys, spleen +/- lymph nodes and bone marrow
- **Allows to take samples** for biochemical, genetic, molecular investigations
  - Frozen samples : thymus (lymphocytes), liver, muscle, kidneys, lungs, placenta
  - skin biopsy: fiboblasts culture

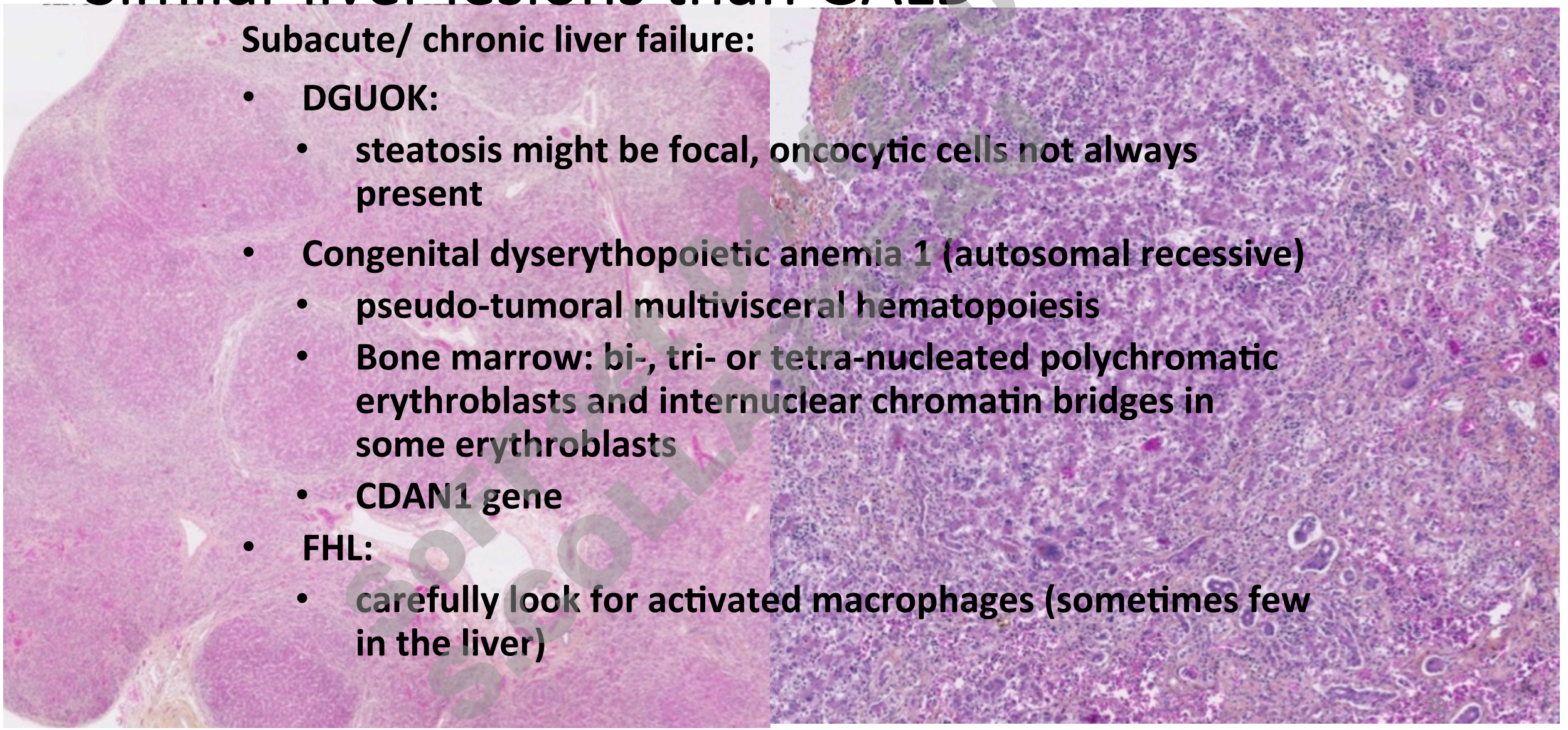
Similar liver lesions than GALD

Acute fetal liver failure:



- parvovirus B19 +++:
  - inclusion sometimes difficult to see /macerated fetus→ immunostaining
- transaldolase deficiency (autosomal recessive) :
  - dysmorphism & malformations may lack in fetus
  - polyols urinary analysis
  - TALDO1 gene

Similar liver lesions than GALD



Subacute/ chronic liver failure:

- **DGUOK:**
  - **steatosis might be focal, oncocytic cells not always present**
- **Congenital dyserythopoietic anemia 1 (autosomal recessive)**
  - **pseudo-tumoral multivisceral hematopoiesis**
  - **Bone marrow: bi-, tri- or tetra-nucleated polychromatic erythroblasts and internuclear chromatin bridges in some erythroblasts**
  - **CDAN1 gene**
- **FHL:**
  - **carefully look for activated macrophages (sometimes few in the liver)**

perspectives

- Étude du phénotype: autres maladies non encore recensées
  - Perls systématique sur tout foie nécrosé ou d'architecture anormale
  - Pb des investigations complémentaires pas toujours effectuées
  - Il nous manque des cas: Zellweger, Donohue, Martinez-Frias, sd THE, 16p
- Mécanismes physiopathologiques
  - GALD existe-t'elle vraiment?
  - Ag hépatique?
  - Autres allo-immunisations?
    - Ag privés érythrocytaires?
  - Autres facteurs impliqués dans la voie du complément
  - Piste auto-immunité: étude sérum maternel
    - Pb de administration des IgIV: plus possible pendant la G
    - Après G quand cas index dépisté: 3, 6, 9, 12 mois, 2 ans

besoin

- De vous +++
  - CR
  - Toutes les lames
  - Blocs foie, reins, rate, thyroïde, pancréas, placenta
- De sous (piste via comité de délivrance des IgIV, AMFE)
  - Base de données
  - Expériences/ techniques complémentaires
  - Site internet: infos des familles, cliniciens

Articles et présentations

- Cours IPPA Fontainebleau sept 2015
- 2 présentations orales Australie Nov 2015
- 5 articles en cours:
  - 2 thèses
  - 2 case report
  - 1 orphanet
- Collaboration
  - Européenne: Belgique, Suisse, Espagne, Portugal
  - Internationale: Children Hospital Of Philadelphia

Merci!

Membres de la SOFFOET

CHOP

Béatrice & Estelle

Centre de pathologie Est

Service de génétiques

Biochimie métabolique CBPEst