L'epatite di interesse pediatrico

Giuseppe Indolfi

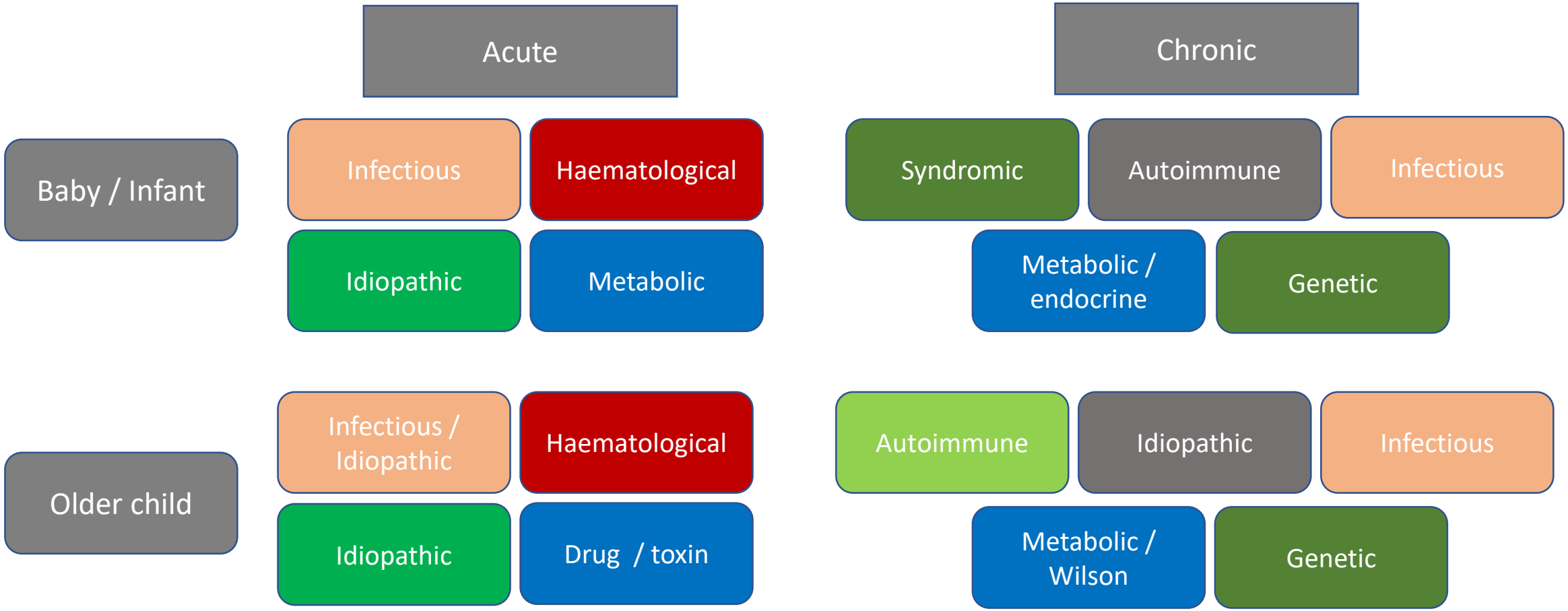
Department NEUROFARBA, University of Florence

Meyer Children's Hospital, IRCCS

Disclosures

Nothing to disclose

Hepatitis in children: aetiology



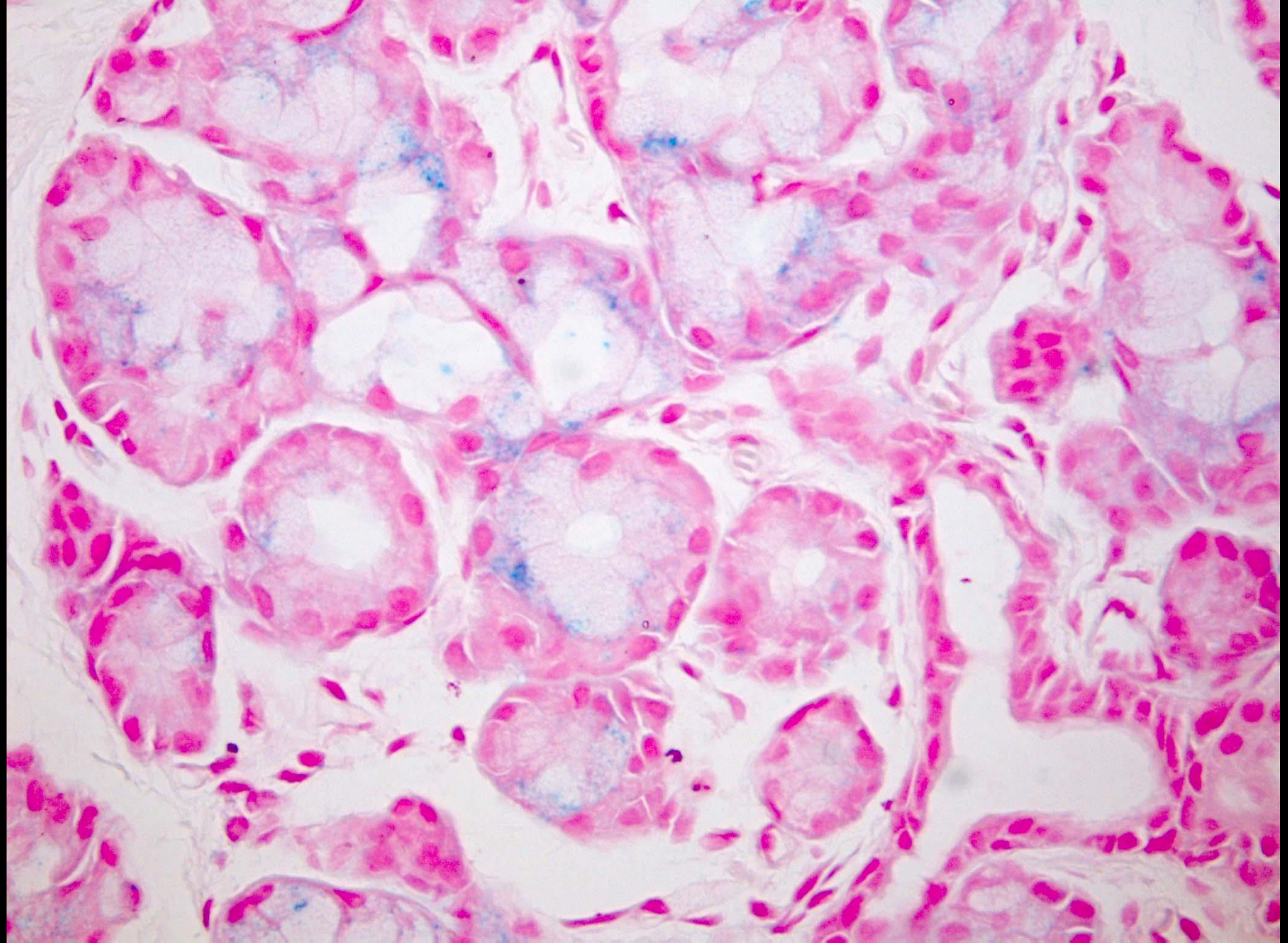
Meyer
UNIVERSITÀ DEGLI STUDI FIRENZE

Hepatitis in neonates

Alloimmune

Neonatal haemocromatosis – Gestational alloimmune disease

- Alloimmune disorder
- Iron studies – high ferritin, low transferrin and high iron saturation
- Often slightly elevated transaminases but jaundice, hypoglicemia
- Salivary gland biopsy – presence of extrahepatic iron
- double exchange transfusion, IVIG, liver transplantation



Indolfi, Transpl Int 2014

Research article JHEP|Reports

Genetic

Opinion paper on the diagnosis and treatment of progressive familial intrahepatic cholestasis

Patrick McKiernan,[1,*] Jesus Quintero Bernabeu,[2] Muriel Girard,[3] Giuseppe Indolfi,[4,5] Eberhard Lurz,[6] Palak Trivedi[7,8,9]

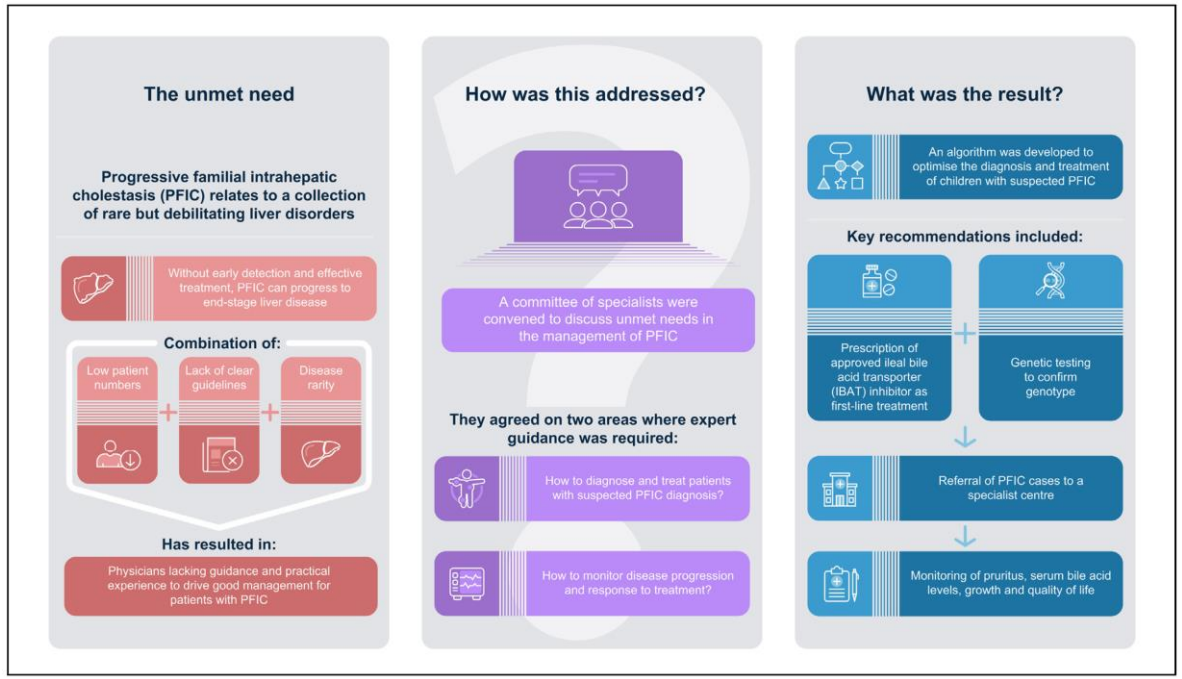


Table 1. Overview of genes associated with PFIC and their related histological and phenotypic characteristics.[1–14].

Protein deficiency	Mutated gene	Histological characteristics	Phenotypic characteristics
FIC15	*ATP8B1*	• Canalicular cholestasis • Giant cell transformation • Ductular paucity • Lobular disarray	• Early infancy • Low GGT • Jaundice • Pruritus • Hepatosplenomegaly • Hearing loss • Diarrhoea • Poor growth
BSEP6	*ABCB11*	• Canalicular cholestasis • Hepatocellular disarray • Lobular and portal fibrosis	• Early infancy • Low GGT • Hepatocellular carcinoma/cholangiocarcinoma • Jaundice • Pruritus • Hepatomegaly • Poor growth
MDR37	*ABCB4*	• Portal fibrosis • Bile duct proliferation • Mild giant cell hepatitis	• Later in childhood/young adulthood • High GGT • Hepatocellular carcinoma/cholangiocarcinoma • Jaundice • Pruritus • Hepatosplenomegaly • Portal hypertension • Reduced bone density
TJP28	*TJP2*	• Intracellular cholestasis • Giant cell transformation	• Early infancy • Low GGT • Hepatocellular carcinoma/cholangiocarcinoma • Jaundice • Pruritus • Rapid progression
FXR9	*NR1H4*	• Intralobular cholestasis • Hepatocellular ballooning • Giant cell transformation • Fibrosis	• Early infancy • Normal GGT • Rapid progression • Vitamin K independent coagulopathy • Hyperammonaemia
OSTα-OSTβ10	*SLC51A*	• Not reported	• Early infancy • High GGT • Diarrhoea
USP5311	*USP53*	• Not reported	• Early infancy/later in childhood • Low GGT • Pruritus • Hypocalcaemia • Hearing loss
KIF1212	*KIF12*	• Not reported	• Early infancy/later in childhood • High GGT
MYO5B13	*MYO5B*	• Hepatocellular cholestasis • Portal and lobular fibrosis • Giant cell transformation • Abnormal BSEP and MDR3 staining	• Early infancy • Low GGT • Hepatomegaly • Pruritus • Diarrhoea • Poor growth • Microvillus inclusion disease and intestinal failure
LSR14	*LSR*	• Not reported	• Early infancy • Low GGT • Pruritus
WDR83OS11	*WDR83OS*	• Not reported	• Later in childhood • Microcephaly • Dysmorphic facies • Genital abnormalities

GGT, gamma-glutamyltransferase; n.a., not applicable; PFIC, progressive familial intrahepatic cholestasis.

McKiernan P, J Hep Rep 2024

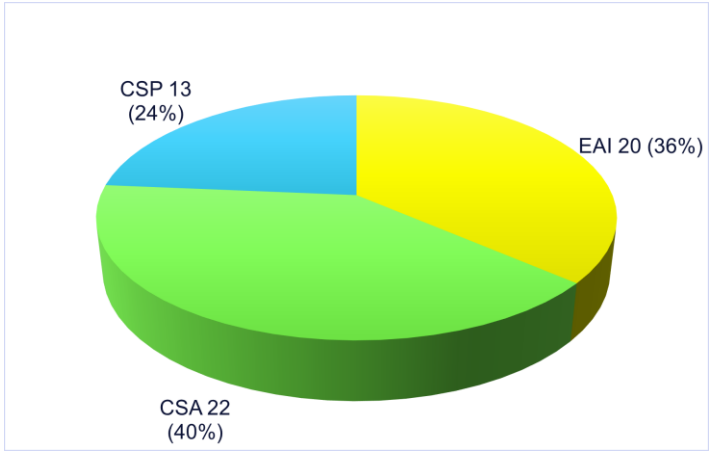
Meyer

UNIVERSITÀ DEGLI STUDI FIRENZE

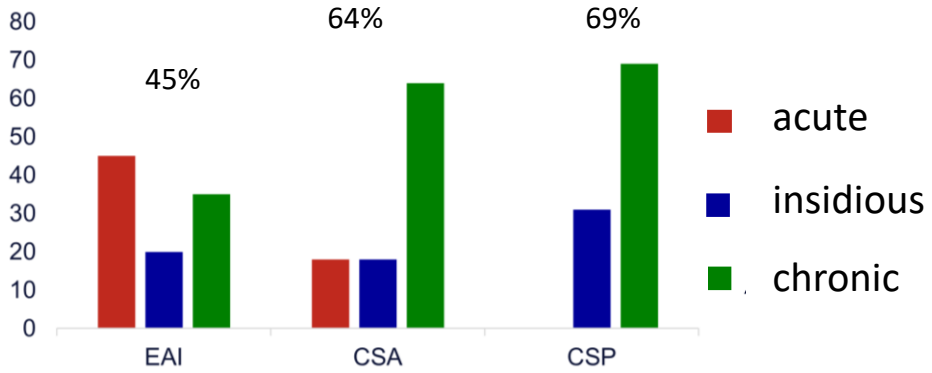
Autoimmune

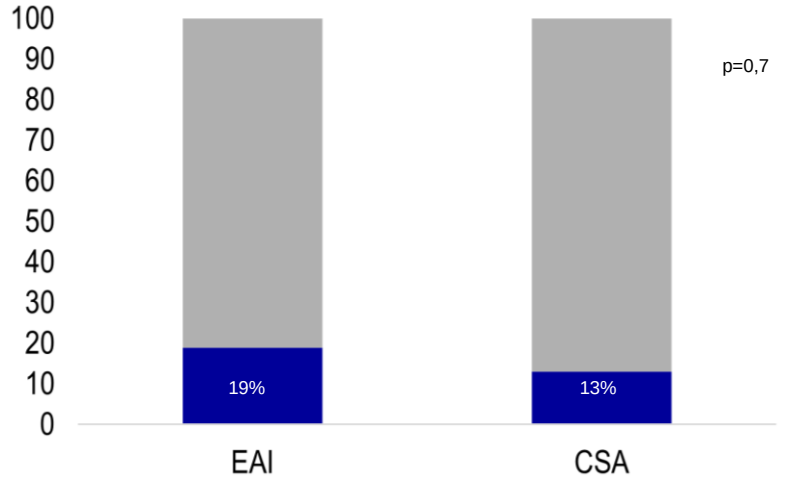
Hepatitis in children: autoimmune

Diagnosis n 55

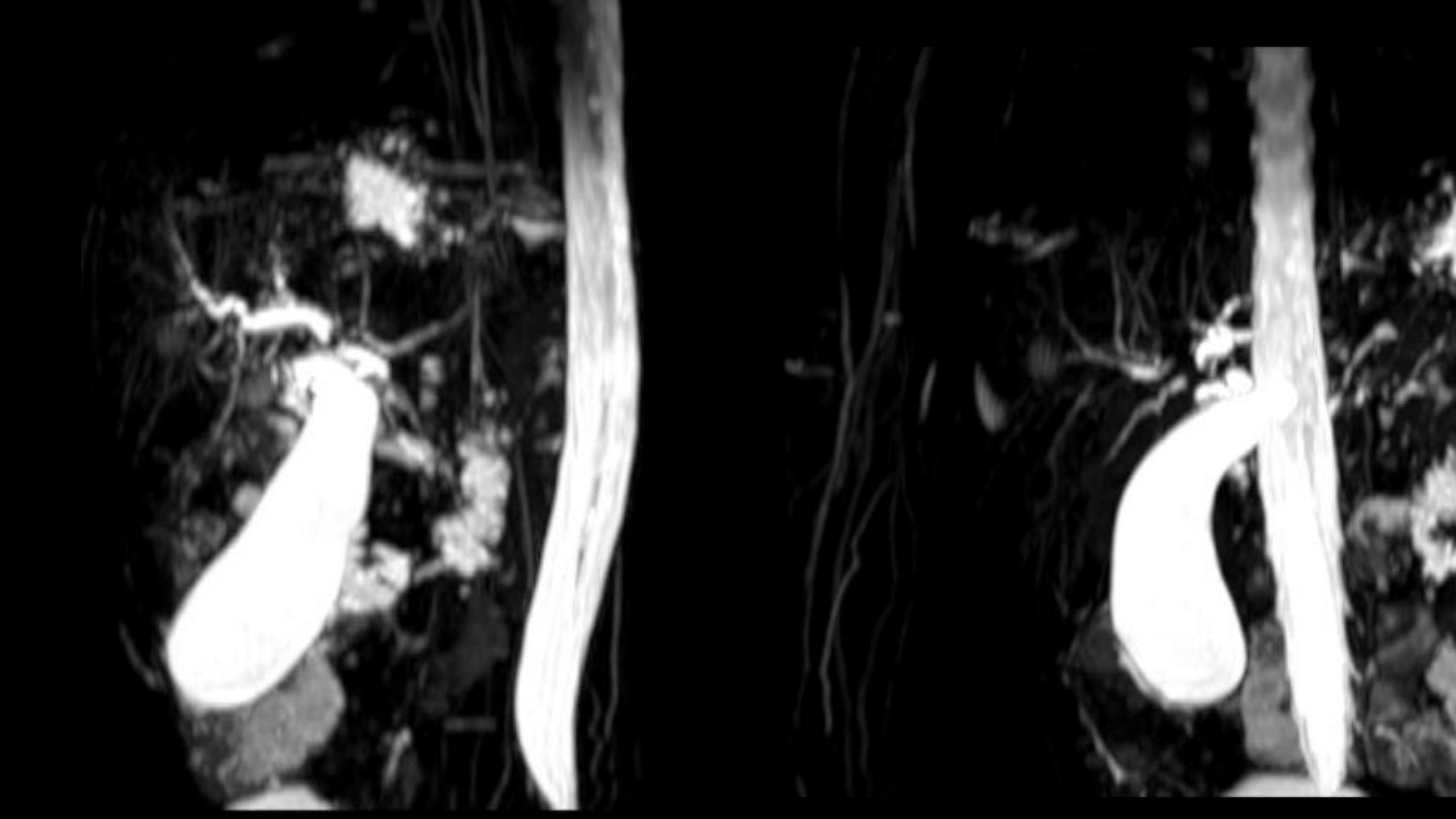


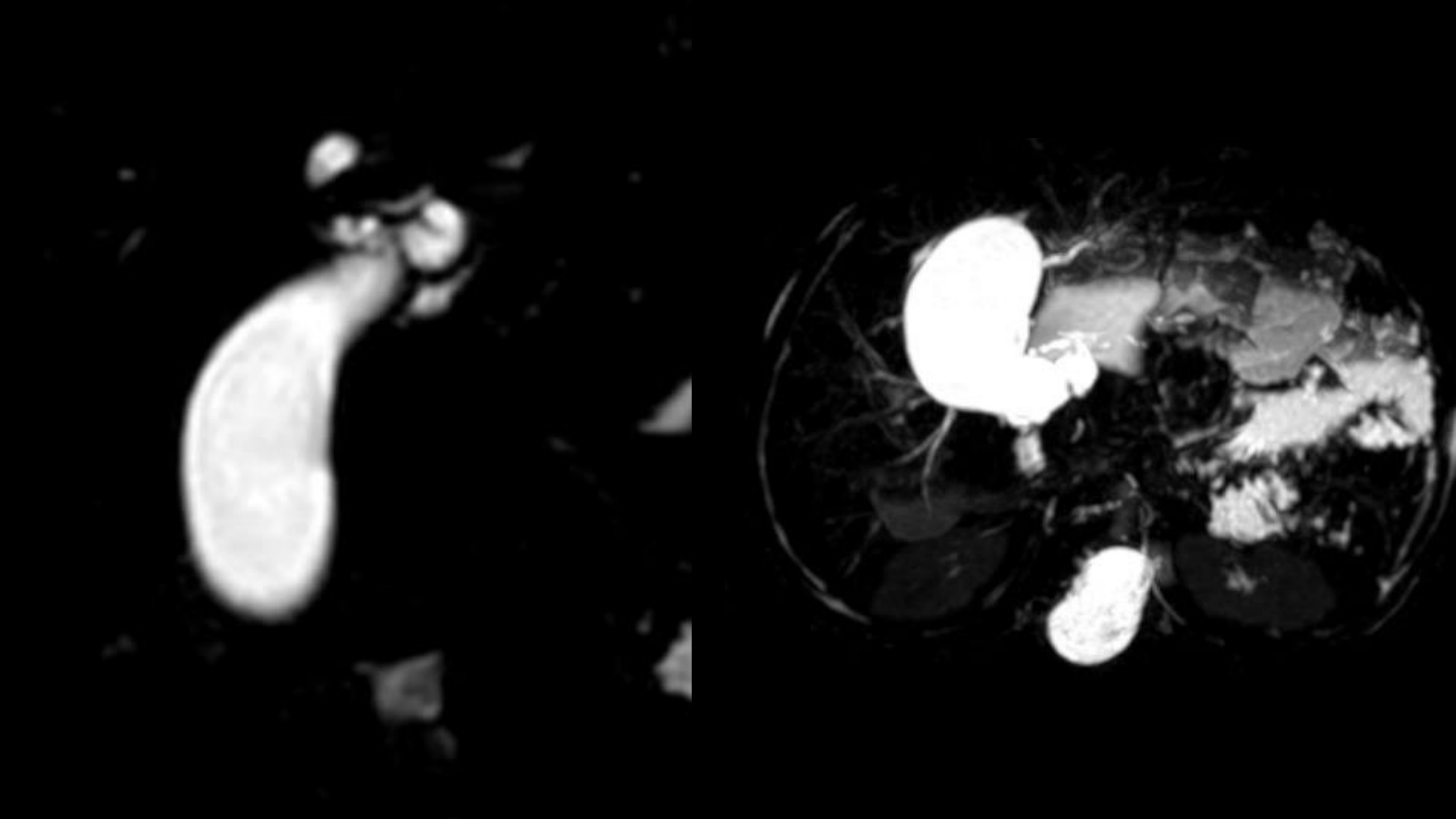
Age at diagnosis





off therapy





Hepatitis in children: chronic infections

Infectious

WHO updated guidelines

Treat all adults and adolescents (aged ≥12 years) with chronic hepatitis AND:

- Evidence of fibrosis (≥ F2) or cirrhosis (F4) based on
  - clinical criteria or
  - APRI score of ≥ 0.5 or
  - transient elastography value of ≥8.3 Kpa*, regardless of HBV DNA or ALT levels

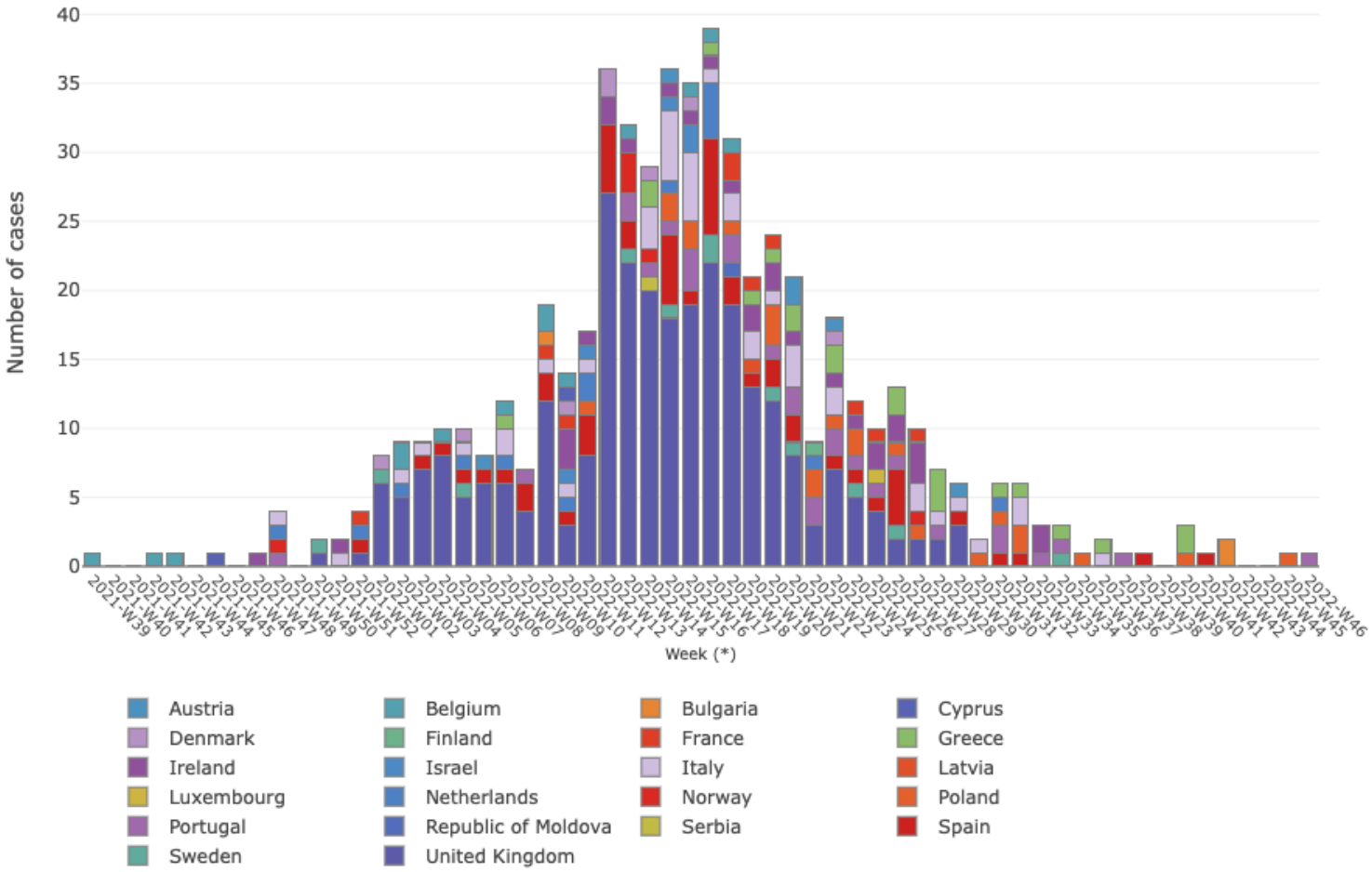
- **HBV DNA >2000 IU/ml** and an **ALT level above ULN** (30 U/L for men and 19 U/L for women)

- Presence of co-infections, family history of liver cancer or cirrhosis, co-morbidities

- Persistently abnormal ALT levels (in absence of access to an HBV DNA assay), regardless of APRI score

Hepatitis in children: infectious/idiopathic

Infectious / Idiopathic



Indolfi G, JPGN 2022

Acute Hepatitis of Unknown Origin: the Outbreak

WHO/ECDC Surveillance Summary (25 November 2022)

- 572 cases of acute hepatitis of unknown aetiology reported by 22 countries
    - 100 (27% of 371 cases with available information) admitted to ICU
    - 24 (7.5% of 320 cases with available information) received liver transplant
    - 7 (1.7% of 405 cases with available information) died

US CDC (17 August 2022)

- 358 patients under investigation
    - 22 (6%) received liver transplant
    - 13 (4%) died

WHO (12 July 2022)

- 1010 cases from 35 countries in five WHO Regions
    - 46 (5%) received liver transplant
    - 22 (2%) died

Meyer

UNIVERSITÀ DEGLI STUDI FIRENZE

Hepatitis in children: infectious/idiopathic

Infectious / Idiopathic

WHO updated guidelines

Treat all adults and adolescents (aged ≥12 years) with chronic hepatitis AND:

- Evidence of fibrosis (≥ F2) or cirrhosis (F4) based on
  - clinical criteria or
  - APRI score of ≥ 0.5 or
  - transient elastography value of ≥8.3 Kpa*, regardless of HBV DNA or ALT levels

- **HBV DNA >2000 IU/ml** and an **ALT level above ULN** (30 U/L for men and 19 U/L for women)

- Presence of co-infections, family history of liver cancer or cirrhosis, co-morbidities

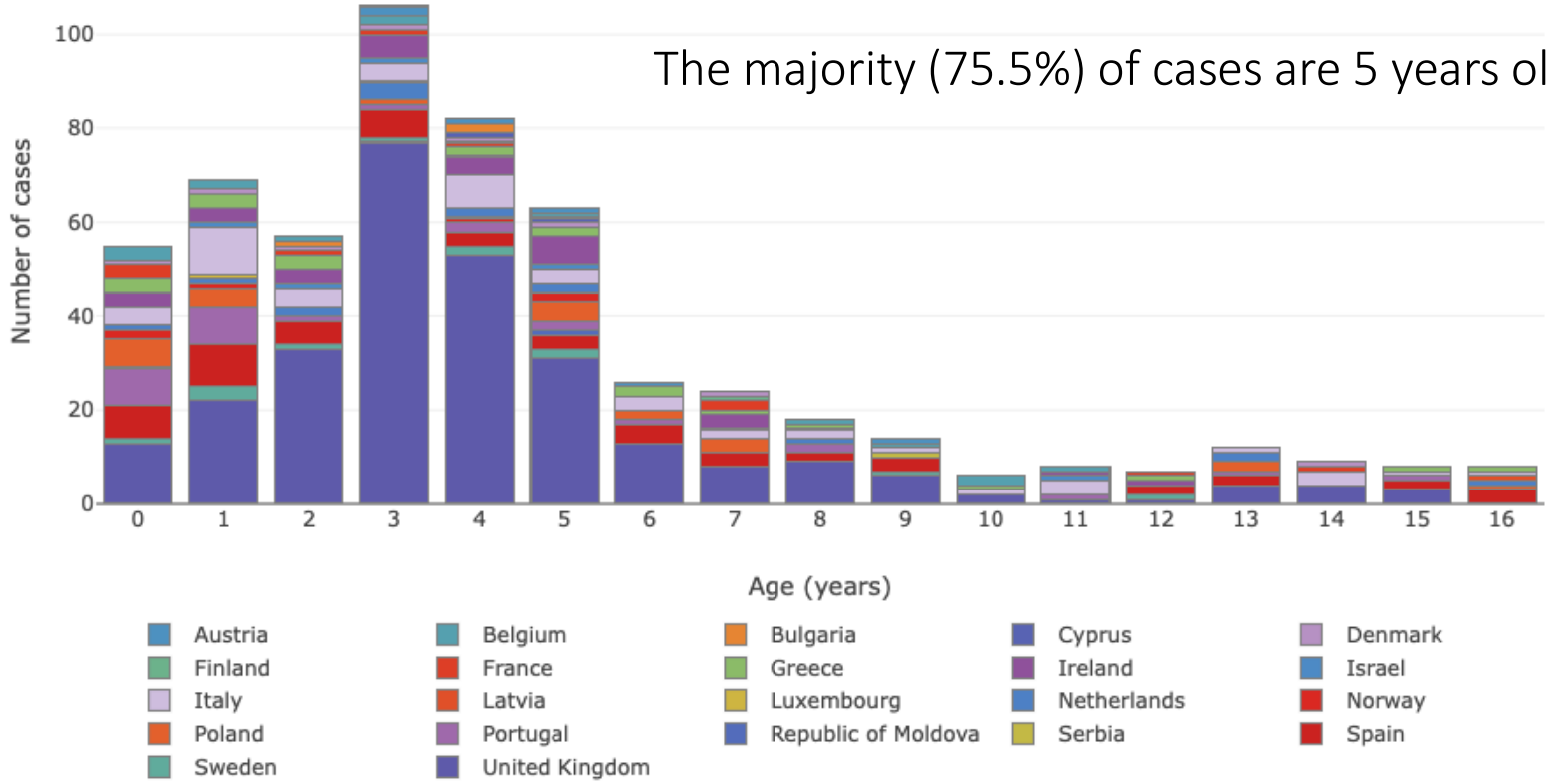
- Persistently abnormal ALT levels (in absence of access to an HBV DNA assay), regardless of APRI score

Indolfi G, JPGN 2022

Meyer

UNIVERSITÀ DEGLI STUDI FIRENZE

Number of cases by age in years, reported from countries in the WHO European region as of 24 November, 2022



The majority (75.5%) of cases are 5 years old or younger

Source: Cases reported from countries in the WHO European region according to the WHO/ECDC case definition to the ECDC European Monitoring System (TESSy)*. Note that the UK local case definition includes only cases younger than 16 which means cases aged 16 are unlikely to be reported to TESSy from the UK

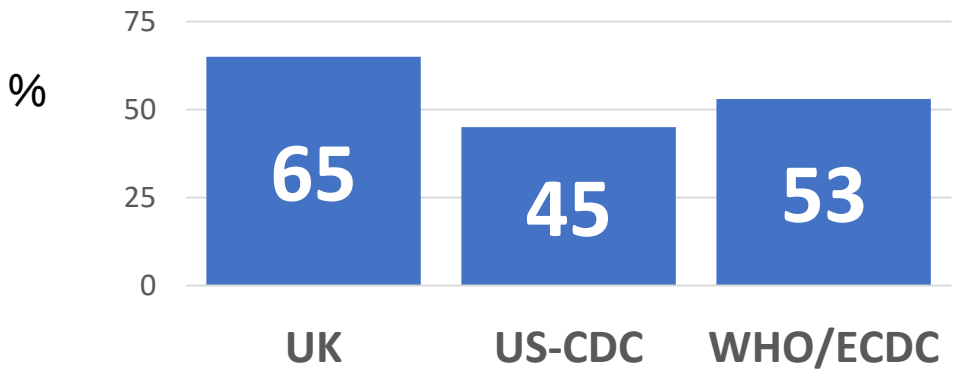
Meyer

UNIVERSITÀ DEGLI STUDI FIRENZE

Aetiology

- temporal and geographical clustering
- non specific prodromal signs and symptoms

adenovirus



UK Health Security Agency update 28 July;
US-CDC Technical report update 17 Aug
www.ecdc.europa.eu/en/hepatitis/joint-weekly-hepatitis-unknown-origin-children-surveillance-bulletin update 30 Sept

Adenovirus F41

- 35/77 (45%) UK

SARS-CoV-2 PCR

- 34/196 (17%) UK
- 39/378 (10.3%) WHO/ECDC

SARS-CoV-2 Serology

- 68/108 (63%) WHO/ECDC

Meyer
UNIVERSITÀ DEGLI STUDI FIRENZE

Pathogenesis

nature

https://doi.org/10.1038/s41586-023-05948-2

Accelerated Article Preview

Adeno-associated virus 2 infection in children with non-A-E hepatitis

Genomic investigations of unexplained acute hepatitis in children

Adeno-associated virus type 2 in US children with acute severe hepatitis

Methods

Target sequencing approach

Sequencing only **a specific region of a genome** with or without viral enrichment based sequencing

Metagenomics (untargeted approach)

Analysis of **all nucleic acids** contained within an environmental sample to discover which virus might be present

In situ hybridisation

Precise localization of a specific segment of nucleic acid **within a histologic section**

Methods

Immunohistochemistry

Antibodies are used to check for specific localization of certain antigens in a sample of tissue

Metatranscriptomic

To study **gene expression** of microbes within natural environments (metatranscriptome) and **quantify their expression levels**

Proteomics

The study of the entire set of proteins produced by the cell, i.e. by an organism

The Glasgow study

Metagenomic
Target sequencing approach
Target enrichment NGS
Metatranscriptomic
In situ hybridisation

32 cases

- metagenomic and metatranscriptomic
  - plasma (n=9), liver biopsies (n=4), throat swabs (n=6), faecal samples (n=7) and a rectal swab (n=1)

74 control subjects selected from DIAMONDS study and NHS GG&C repository

- group 1 - 13 age-matched healthy children
- group 2 - 12 children with PCR-confirmed HAdV infection and normal transaminases
- group 3 - 33 children with raised transaminases HAdV PCR negative
- group 4 – 16 Scottish children attending hospital contemporaneously with the hepatitis cases

64 Scottish platelet apheresis donors (host genetics and HLA typing)

Ho, Nature 2023

Meyer

UNIVERSITÀ DEGLI STUDI FIRENZE

The Glasgow study

Metagenomic
Target sequencing approach
Target enrichment NGS
Metatranscriptomic
In situ hybridisation

Acute hepatitis of unknown origin is an immune-mediated process

- a CD_4+ T-helper cell-mediated immunopathological response triggered by exposure to AAV2 infection is highly likely
- AAV2 helper virus(es) were not identified as most clinical samples were obtained more than 20 days after initial symptom onset

AAV2 is not a bystander

- AAV2 was not detected in a control group of children with HAdV infection who had normal liver function.

Ho, Nature 2023

Meyer

UNIVERSITÀ DEGLI STUDI FIRENZE

The London study

Metagenomic
Metatranscriptomic
Target sequencing approach
Proteomic
Immunohistochemistry

38 cases

- metagenomic and metatranscriptomic
  - 5 livers explants
  - 6 whole blood samples (from 5 non transplanted cases)
- PCRs
  - 28 non transplanted cases (liver, blood, stool, nasopharyngeal samples)

82 control subjects selected from PERFORM and DIAMONDS studies

- 65 immunocompetent children
  - 13 healthy
  - 52 fever/hepatitis/critical care
  - 17 immune-suppressed children with hepatitis

Morfopoulou, Nature 2023

Meyer

UNIVERSITÀ DEGLI STUDI FIRENZE

The London study

Metagenomic
Metatranscriptomic
Target sequencing approach
Proteomic
Immunohistochemistry

Acute hepatitis of unknown origin is an immune-mediated process

- **immunohistochemical** data showed that liver samples were enriched in adaptive immune cells and immune-related proteins, but contained no detectable HAdV or AAV2 proteins, or viral particles

AAV2 is not a bystander

- abnormal **AAV2 replication enabled by HAdV and HHV-6B** may have triggered immune-mediated hepatic disease in genetically and immunologically predisposed children

Morfopoulou, Nature 2023

Meyer

UNIVERSITÀ DEGLI STUDI FIRENZE

The US study

Metagenomic
Target sequencing approach
Target enrichment NGS

16 HAdV positive cases

- metagenomic and target sequencing approach
  - 27 samples (21 whole blood, 2 plasma, 1 liver tissue, 1 nasopharyngeal swab and 2 stool sample(s)

- **113 controls**
- 69/113 controls (61.0%) were collected over the same time frame as the cases

- 42 patients (37%) without hepatitis
- 30 (26.5%) patients with acute hepatitis (ALT > 100 U/L) of defined aetiology
- 23 (21%) patients with acute gastroenteritis (12 with positive HAdV stool testing),
- 18 (16%) blood donors

Servellita, Nature 2023

Meyer
UNIVERSITÀ DEGLI STUDI FIRENZE

The US study

Metagenomic
Target sequencing approach
Target enrichment NGS

In acute hepatitis of unknown origin AAV2 is not a bystander

- A significant association between coinfection by AAV2 and **one or more hepatotropic viral pathogens** and the clinical manifestations of severe acute hepatitis

- a direct causal link has yet to be confirmed

Servellita, Nature 2023

Meyer

UNIVERSITÀ DEGLI STUDI FIRENZE

Pathophysiology

AAV

Helper viruses

Genetic predisposition

Abnormal immune response

The three studies cannot rule out the possibility that COVID-19 is a contributory factor, because the timing of the hepatitis outbreak meant that antibodies against the SARS-CoV-2 virus were prevalent in all groups

https://doi.org/10.1038/d41586-023-00570-8

Medical research

Childhood hepatitis outbreak under scrutiny

Frank Tacke

Since 2022, more than 1,000 cases of childhood hepatitis with no known cause have been reported. The discovery of adeno-associated virus 2 in the blood and livers of such children might provide an explanation.

Paediatricians first began to report a concerning wave of acute severe hepatitis – a disease involving liver inflammation – in children in spring 2022. This spate of cases, which now numbers more than 1,000 (see go.nature.com/42dpfv4), had no known cause, unlike most cases of paediatric hepatitis. The cases were often serious, with 7.3% of affected children needing a liver transplant[1]. Writing in *Nature*, three groups now independently provide evidence that infection by adeno-associated virus 2 (AAV2) is linked to this wave[2–4]. Their work is likely to spark debate, and could influence disease management.

Two previous analyses[5,6] of this wave of acute severe hepatitis found an association with human adenovirus (HAdV), particularly the HAdV-F41 strain. However, this result has puzzled clinicians because, although adenovirus-induced hepatitis is a known phenomenon, it is rare and usually restricted to people with severely compromised immune systems. Moreover, the studies identified various HAdV strains, even in children from the same geographical region, implying that a single strain could not explain these hepatitis outbreaks. Furthermore, adenovirus-induced hepatitis is characterized by virus-derived structures, called inclusions, in the nucleus – something that has not often been detected in the current outbreak[7].

Controversy therefore remained around whether the outbreak was caused by HAdV or another virus. The previous studies identified HAdV using targeted genetic-sequencing approaches. By contrast, the current studies involved an untargeted approach called clinical metagenomics, in which all nucleic acids from a sample are sequenced, to discover which viruses might be present. All three identified AAV2 – a virus known to replicate in the liver, but not known to cause hepatitis[8].

In the first of the papers, Ho *et al.*[2] report high levels of AAV2 in blood and liver samples from 26 (81%) out of 32 affected children in the United Kingdom. By contrast, the researchers detected only low AAV2 levels in 5 (7%) of 74 members of a control group consisting of healthy children, children who had HAdV infection but not hepatitis, or children who had other diseases or other forms of acute hepatitis. The authors found that 93% of the affected group carried a gene variant that predisposes an individual to autoimmune diseases involving immune cells called T cells. Liver biopsies were available for five infected children. Analysis using a tissue-staining approach (histology) revealed AAV2 in abnormally swollen liver cells (hepatocytes) surrounded by T cells, suggesting a causative role of AAV2 in acute hepatitis.

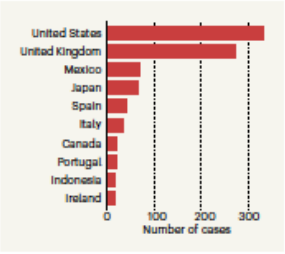


Figure 1 | Hepatitis outbreak around the world. As of July 2022, 35 countries had reported cases of severe acute paediatric hepatitis that had no known cause, according to the World Health Organization (see go.nature.com/42dpfv4). Numbers for the 10 countries with most reported cases are shown here. Three groups[2–4] now provide evidence that adeno-associated virus 2 has a role in this outbreak.

In the second study, also from the United Kingdom, Morfopoulou *et al.*[3] report high levels of AAV2 in 27 of 28 affected children, and only low levels of AAV2 in a control group, which included immunocompromised children who had acute hepatitis due to other causes, and children with normal immune systems who had fever. The authors used several techniques to demonstrate that the disease was not due to direct damage to hepatocytes (known as hepatotoxicity) caused by AAV2 alone. Because low levels of HAdV and human herpesvirus 6B (HHV-6B) were detected in the liver in most cases, the authors speculate that these viruses might enable AAV2 replication, leading to damage to hepatocytes.

In the third paper, Servellita *et al.*[4] report similar findings from the United States. They detect AAV2 in 13 of 14 cases, compared with only 4 of 113 controls. All 14 cases also tested positive for HAdV. In the 13 children infected with AAV2, the authors found co-infection with a 'helper' virus that might promote AAV2 replication – either Epstein–Barr virus (EBV) or HHV-6. Thus, most of the cases had a triple infection (AAV2, HAdV and either EBV or HHV-6). The fact that these authors examined only children who were infected with HAdV might explain why they detected more concomitant viral infections than did the other groups.

All three studies make the same observation of AAV2 in children with unexplained acute hepatitis. That the studies took place on two continents adds to their value, given the global nature of the outbreak (Fig. 1). However, a note of caution is needed – these studies were all conducted retrospectively, rather than being gold-standard prospective trials, in which subjects are selected for study, with data being subsequently collected over time. The case numbers are relatively small, and the number of liver samples even smaller. The studies report limited clinical information (such as ethnic background, whether the children were born by caesarean section and whether they could have been exposed to viruses during day care), which is needed to reveal potential factors or co-factors in disease development. Such data would be essential to exclude the possibility that AAV2 is a harmless bystander.

Direct evidence for how AAV2 might cause hepatitis is limited. Ho and co-workers' genetic and histological analyses imply that the disease is rooted in abnormal immune responses, rather than in a hepatotoxic role for AAV2. Morfopoulou and colleagues provide support

Meyer

UNIVERSITÀ DEGLI STUDI FIRENZE

Marco, 2 years old

- fever, pharyngitis → amoxicillin

- fever, vomiting → Emergency Department local hospital
  - jaundice, hepatomegaly
  - ALT 1846 IU/L; direct bilirubin 4 x nv
  - INR 4.48 – vitamin K 10 mg intravenous → INR 4.7
  - Ferritin 13.000 micrg/L

- diagnostic work-up
  - autoantibodies, IgG
  - metabolic work up
  - exposure to drugs and toxins
  - screening for infections (including A-E, SARS-CoV-2, adenovirus, herpes viruses)

November 2023

Marco, 2 years old

- Acute hepatitis and acute liver failure of unknow aetiology
  - **Adenovirus positive (blood)**
  - **HHV6 positive (blood)**
- Referred to a liver transplant centre
- Recovered without transplantation

Meyer

UNIVERSITÀ DEGLI STUDI FIRENZE

28 December 2024

Emilia, 4 years old

- Fever (paracetamol and ibuprofen)

- 24 h later haematemesi and melena
    - ALT 12.000 IU/L
    - INR 2,79 – vitamin K 10 mg intravenous → INR 3
    - Ferritin 8000 microg/L
    - H1N1 positive

- 36 h
    - Acute kidney failure
    - Encephalopathy

- died 48 hours later

Meyer

UNIVERSITÀ DEGLI STUDI FIRENZE

Conclusions

Challenges

- clinical and aetiological (rather than epidemiological**) phenotype**

- Complexity of the paediatric age