FROM PARASITES TO PUPPIES:
TOWARDS NONINVASIVE DIAGNOSIS OF
PEDIATRIC SARS-COV-2 AND MIS-C

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June 7, 2021
OUTLINE

• Case presentation
• Timeline/recognition of MIS-C
• NICHD PreVAIL klds program
• Our studies: improving diagnosis of SARS-CoV-2 and MIS-C in kids
CASE (MAY 2020)

• History: 5 yo healthy child admitted to CHOP with fever and low BP
• 4d ago - F to 104, no other symptoms
• 3d ago - rash on thighs, spreading to abdomen and soles of feet
• 2d ago - nonexudative conjunctivitis (red eyes), cracked lips, N/V/diarrhea
• in ED: ? neck stiffness, fluid-responsive hypotension (low BP)
• Admitted to intensive care unit at CHOP

• Previously healthy child
• Social history: lives in community with high levels of transmission of COVID-19, no sick contacts, “hadn’t left the house in 1 month”
PHYSICAL EXAM:

Gen: fussy, consolable, asking for something to drink
HEENT: slight conjunctivitis, moderate resistance to neck flexion
LAD: no cervical, axillary, or inguinal adenopathy
Chest: clear no WOB
CV: hyperdynamic, RRR no M, well perfused
Ab: full, nontender, mild hepatomegaly, no splenomegaly
Skin: Rash, characterized by 2-3 mm erythematous blanching macules scattered on abdomen and inner thighs with larger confluent patches on thighs, decreased towards extremities, no linear areas, spares underwear area and no evidence of dermographia. No petechiae or purpura.
Neuro: normal tone, good movement of all extremities, alert and fussy but normal affect
Extrem: no joint effusions or redness
Blatz et al, OFID 2021

with parental consent
with parental consent
DIAGNOSIS: MULTISYSTEM INFLAMMATORY SYNDROME IN CHILDREN (MIS-C), ASSOCIATED WITH SARS-COV-2 INFECTION

• ECHO: normal biventricular function, trivial mitral and tricuspid regurgitation, and echo-bright coronary arteries (with normal diameter)
• Labs: Positive SARS-CoV-2 NP PCR and serology
• treatment: IVIG (X2) and methylprednisolone, ABx
• supportive care
• rapid resolution of fever, shock, rash -> rpt ECHO normal
SARS-COV-2 AND COVID-19

• 4 Jan 2020: WHO reports cluster of atypical PNA in Hubei province, China
• 12 Jan: genetic sequence of SARS-CoV-2
• 22 Jan: confirmed human-to-human transmission
• 11 Mar: WHO "global pandemic"
MAR - APRIL 2020: SARS-COV-2 IS MILD IN KIDS

- 112* positive tests of 2385 patients < 21 years tested at CHOP = 4.7%
- 6/382 (1.6%) of pre-procedural or pre-admission testing

Clinical severity of all positive pediatric patients at CHOP

<table>
<thead>
<tr>
<th>Severity</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic</td>
<td>6 (5%)</td>
</tr>
<tr>
<td>Mild</td>
<td>97 (84%)</td>
</tr>
<tr>
<td>Moderate</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Severe</td>
<td>5 (5%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>6 (5%)</td>
</tr>
<tr>
<td>Death</td>
<td>1 (1%)</td>
</tr>
</tbody>
</table>
JUST WHEN YOU THOUGHT CHILDREN WERE PRETTY SAFE...WELCOME TO 2020:
FIRST REPORTS OF INFLAMMATORY DISEASE

Italy, UK explore possible COVID-19 link to child inflammatory disease

Emilio Parodi, Alastair Smout

5 MIN READ

PICS Statement: Increased number of reported cases of novel presentation of multi-system inflammatory disease

27 April 2020
15 children in New York City have developed a puzzling and serious inflammatory syndrome possibly linked to covid-19

The condition is similar to what doctors have observed in Europe

Multisystem Inflammatory Syndrome in Children (MIS-C) Associated with Coronavirus Disease 2019 (COVID-19)

Distributed via the CDC Health Alert Network
May 14, 2020, 4:45 PM ET
CDCHAN-00432
CLINICAL FEATURES AND DIAGNOSIS OF MIS-C

PIMS-TS

- Headache / Confusion
- Conjunctivitis
- Lymphadenopathy
- Cough / Dyspnea
- Abdominal pain
- Vomiting / Diarrhea
- Rash
- Hand / Feet swelling

LAB TESTS
- CRP / Ferritin
- Neutrophils
- LDH, Triglycerides
- Fibrinogen / D-Dimer
- Liver enzymes
- NT-pro-BNP / Troponin
- Lymphocytes
- Hemoglobin
- Thrombocytes
- Albumin

DIFFERENTIALS
- Toxic shock syndrome
- Bacterial / viral sepsis
- HLH (Hemophagocytic lymphohistiocytosis)

DEFINITION
① Fever, inflammation & organ dysfunction
② No indication of any other disease
③ Exposure to SARS-CoV-2 in the last 2-6 weeks &/or SARS-CoV-2 IgG positive
MIS-C CASES IN THE US (2/2020 - PRESENT)

• >3700 cases (as of 6/01/21)
• Peak of MIS-C ~4-6 weeks from peak of COVID-19
• Rare complication (~1:10,000 or less)
• lots of unanswered questions!

Daily MIS-C Cases and COVID-19 Cases Reported to CDC (7-Day Moving Average)

The graph shows the 7-day moving average number of COVID-19 patients and MIS-C patients with date of onset between February 19, 2020 and April 23, 2021.
CARING for Children with COVID
(Collaboration to Assess Risk and Identify Long-term outcomes for Children with COVID)

- Leverages resources and networks from 3 NIH ICs to capture data from hospitalized patients with MIS-C
- Trans-NIH effort through RADx-rad to enhance diagnostic and predictive efforts
Predicting Viral-Associated Inflammatory disease severity in children with Laboratory diagnostics and artificial Intelligence (PreVAIL kIds)

• Understand the spectrum of pediatric SARS-CoV-2 illness, rapidly diagnose and characterize MIS-C associated with SARS-CoV-2, and predict the longitudinal risk of disease severity after exposure to and/or infection by SARS-CoV-2

• Develop translational tools to assess disease severity

• Milestone-driven award

• https://www.nichd.nih.gov/newsroom/news/122120-prevail-kids

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Slide kindly provided by Bill Kapogiannis
PreVAIL klds: Mission and Vision

- Overarching Goals of PreVAIL klds
  - Address the natural history and pathobiology of the entire spectrum of SARS-CoV-2 infection in children.
  - Accurate risk stratification & prediction of health outcomes in children

- Shared goals (and resources) across RADx
  - RADx-Rad Programs & DCC
  - RADx-Up
  - RADx-ATP/Tech
  - Trans-NIH and HHS resources (CTSA, REACH, SEED)

- Shared mission with trans-NIH pediatric SARS-CoV-2 agenda
  - CARING for Children with COVID Collaborative
THE CLINICAL CHALLENGE: WHICH CHILD/TEEN WITH FEVER HAS MIS-C?

• High level of concern by front-line ED clinicians
• Missed cases that return
• Anchoring bias - delayed treatment for non-MIS-C conditions
• Does early treatment help? Therapeutic study needs a clear diagnostic algorithm
OUR SOLUTION

Multi-disciplinary expertise in diagnosis and management of MIS-C
People stink
>1800 VOLATILES IN NORMAL HUMANS

- Feces
- Urine
- Breath
- Skin
- Milk
- Blood
- Saliva

Other species and types:
- chloro biphenyls
- halogen containing
- nitrogen containing
- sulphur containing
- furans and ethers
- ketones
- aldehydes
- alcohols
- esters
- acids
- hydrocarbons

Source: de Lacy Costello J. Breath Research 2014
Breath is a window to the blood
Biomarker discovery pipeline

1. **Study design**
   - Healthy patients
   - Patients with disease

2. **Sample collection**
   - Breath, urine, and saliva

3. **Data acquisition**
   - Mass spectrometry

4. **Statistical analysis**
   - Identification of metabolites

5. **Biomarker identification**
Covid-19 has killed roughly similar numbers in the last year as malaria killed on average every year of the last century.

Covid-19

3*

Million
Deaths since it began

Malaria

300

Million
Deaths in 20th century

*as of April 2021

SOURCE: NATIONAL CENTRE FOR BIOTECHNOLOGY INFORMATION
Voluntary exhalation

Breath volatiles pumped over thermal desorption (TD) column

TD/GC-MS (USA)
Breath biomarker discovery/validation for pediatric malaria

Febrile children +/- falciparum malaria

Candidate breath biomarkers

Schaber et al, JID 2018

Independent validation (new cohort)

Normalized abundance (counts)

unpublished
Breath · Urine · Saliva
COVID-19 Biomarker Study

THE BUS COVID BIOMARKER STUDY
THE GOAL: RAPID, EASY, SIMPLE DIAGNOSTICS FOR SARS-COV-2 INFECTION

- Uncomfortable
- Time to result: 2h to days
- Special equipment and personnel needs
- Supply chains strained

- Simple, easy, noninvasive
- Fast (15 min)
- Established developmental pipeline
- Suitable for resource-limited settings
- No supply chain problem
Breath · Urine · Saliva
COVID-19 Biomarker Study

Patients aged 4-20y
Admitted to CHOP
SARS-CoV-2 positive/negative

GCxGC-MS for biomarker discovery
Working dog training (PennVet)
Cindy Otto DVM PhD
Director, PennVet Working Dog Center
VERY GOOD DOGS!


AVG SENSITIVITY = 82.2 +/- 10.0%
AVG SPECIFICITY = 97.8 +/- 4.4%

Essler et al, PLoS One 2021
GROWING EVIDENCE FOR DISTINCT ODORS OF SARS-COV-2

Dubai

Miami

Are Covid-Sniffing Dogs The Miami Heat’s New MVPs?

Robert Glatter, MD Contributor

I cover breaking news in medicine, med tech and public health

BREATH-BASED DIAGNOSIS OF SARS-COV-2 IN CHILDREN
BIOMARKER DISCOVERY (N=25)

Berna et al, submitted
https://www.medrxiv.org/content/10.1101/2020.12.04.20230755v1
BREATH-BASED DIAGNOSIS OF SARS-COV-2 IN CHILDREN
INDEPENDENT VALIDATION (N=24)

84% OVERALL ACCURACY
91% SENSITIVITY

Berna et al, submitted
https://www.medrxiv.org/content/10.1101/2020.12.04.20230755v1
Most discriminatory breath volatiles in adults:

- M1 Ethanal
- M2 Acetone
- M3 Acetone/2-Butanone cluster
- M4 2-Butanone
- M5 Methanol Monomer
- M6 Methanol Dimer
- M7 Feature 144
- M8 Isoprene
- M9 Heptanal
- M10* Propanol
- M11* Propanal

Ruszkiewicz, Dorota M., et al. 

Global change in breath aldehydes
THE GOAL: RAPID, EASY, SIMPLE DIAGNOSTICS FOR PEDIATRIC INFECTION

• Breath volatiles = a reliable non-invasive window to health and disease
• Validated biomarkers for malaria
• Proof-of-concept for SARS-CoV-2 infection

owlstonemedical.com
ONGOING WORK

• Specificity (once respiratory viruses are back...)

• Biological origin of SARS-CoV-2-generated metabolites?

• PreVAIL kIds study for diagnosis of post-SARS-CoV-2 MIS-C
CHOP PREVAIL KIDS: ONGOING STUDY

**Inclusion criteria:**
- Children > 2 yr (4+ for breath)
- T >38.0°C for 3+ days
- 2+ clinical/historical features suggestive of MIS-C

**Clinical screening labs** (per CHOP MIS-C pathway):
- CBC, CMP, CRP, ESR (clinically indicated)

**Metabolomics of non-invasive clinical samples** (breath, urine, saliva)

**Immunoprofiling** (cytokine profiling, flow cytometry, SARS-CoV-2 serology, banked specimen)

**continued clinical suspicion for MIS-C?**
- YES
  - additional testing
  - admission
  - multi-disciplinary consultation for MIS-C diagnosis (current gold standard)
- NO
  - Diagnosis: not MIS-C
    - (n=150)
  - Diagnosis: MIS-C
    - (n=30)

Single enrollment site: CHOP Emergency Department
PROJECT OVERVIEW: PREVAIL KIDS

- **EMR:** demographics, health survey, and laboratory data
- **Metabolomics** (Aim 1): breath, urine, saliva
- **Immunoprofiling** (Aim 2A)
- **Correlation analysis** (Aim 2B)
- **Machine learning** (Aim 2C)
- **Candidate MIS-C diagnostic biomarker or algorithm with "acceptable" target product profile characteristics**