the HHV6 positive patients were inadvertently started on ganciclovir. Development of a consensus statement is in place regarding releasing the result of a positive HHV6 from the ME panel only among immunocompromised children to prevent inappropriate antiviral therapy.

#23: Investigation of Phosphomannomutase as an Antimalarial Drug Target

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Background. Malaria continues to pose an enormous economic and global health threat, killing over 200,000 people annually, primarily children under the age of 5. With the constant barrier of antimalarial resistance and the rise of delayed clearance by artemisinin derivatives, it is important to identify new drug targets for antimalarial parasites. We study targetable metabolic pathways in the malaria parasite, Plasmodium falciparum, to guide such future drug development against this disease. In recent years, we have discovered that a large family of hydrolases, the Haloacid Dehalogenase (HAD) Superfamily of proteins, are implicated in regulating a variety of P. falciparum metabolic pathways, which can lead to changes in central carbon metabolism and drug resistance. We now turn our attention to a related HAD protein, the putative phosphomannomutase in these parasites, HAD5, responsible for the interconversion of mannose-6-phosphate and mannose-1-phosphate. This is an essential process for all stages of the parasite, and thus has potential as a broad antimalarial target. We examined the role of HAD5 in these parasites, and its potential to be chemically inhibited.

Methods. Recombinant protein was generated and purified for enzymatic assays to determine HAD5 activity and test inhibitor potency against HAD5 compared to recombinant human phosphomannomutase and in vitro (n=8–10) and in vivo (n=4–8) experiments. In parallel, CRISPR/Cas9 was used to generate inducible knockdown parasite strains to demonstrate this gene's essentiality and its role in parasite biology. Parasite growth was measured by flow cytometry and light microscopy. Immunofluorescence analysis (IFA) was used to track the parasite developmental stage on a microscopic scale.

Results. Inhibition of HAD5 was achieved in biochemical assays, with an IC50 of 68µM in our most potent compound, representing roughly 10-fold increased potency against the parasite protein compared to human orthologs. In culture, knockdown of HAD5 led to increased parasitemia and resistance to rosetting and reversion into red blood cells, culminating in parasite death. In IFA-visualized parasites, reinvaded-facilitating proteins were no longer anchored to parasite surfaces, accounting for the inhibition of the parasite life cycle.

Conclusion. In the search for new antimalarial targets, identifying proteins that are essential across multiple parasite life-stages while being distinct from human orthologs is necessary to block parasite transmission, cure symptomatic infection, and minimize off-target effects. HAD5 is an essential protein in malaria parasites that is expressed throughout the parasite's life cycle, and can be specifically targeted by inhibitors, giving it promise as a future drug target.

#25: Contemporaneous Evaluation of Kawasaki Disease and Multisystem Inflammatory Syndrome in Children Cases in Northern Virginia

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Background. Multisystem inflammatory syndrome in children (MIS-C) has a temporal association with SARS-CoV-2 (SARS-CoV-2) infection and can present similarly to Kawasaki disease (KD). After the Centers for Disease Control and Prevention issued a MIS-C case definition in May 2020, we implemented local diagnostic and management strategies to standardize the care for patients with MIS-C encouraging limited laboratorian evaluation of patients presenting with a febrile illness. We then sought to re-evaluate our diagnostic and management recommendations to ensure appropriate resource utilization for children with MIS-C and KD.

Methods. Patients with MIS-C and KD were identified via convenience sampling of Pediatric Infectious Diseases clinical records at Inova Children’s Hospital from May 1, 2020 to August 28, 2020. Manual chart review was done to extract clinical points of interest and the two cohorts were compared with descriptive statistics. Abdominal symptoms included pain, emesis, and diarrhea. Respiratory symptoms included shortness of breath, tachypnea, cough, and need for mechanical ventilation. Musculoskeletal symptoms included pain and edema. Neurologic symptoms included headache, dizziness, altered mental status, and irritability.

Results. 7 patients with KD and 14 patients with MIS-C were identified. No patients with KD had presenting hypotension and 9 patients with MIS-C had presented hypotension. Of the patients in KD and MIS-C, 7 (p = 0.05). Conjunctival infection, rash, abdominal symptoms, musculoskeletal symptoms, and neurologic symptoms were seen in some patients with KD and MIS-C with no statistically significant occurrence of these symptoms between the two cohorts. The median initial absolute lymphocyte count was 2,860/µL in KD cases whereas it was 1,325/µL in MIS-C cases (p < 0.01). The median platelet count was 367,009/µL in KD cases versus 193,000 in MIS-C cases (p = 0.03). The median initial C-reactive protein was 11.2 mg/dL in KD cases versus 23.2 mg/dL in MIS-C cases (p < 0.01). There was no statistically significant difference in the white blood cell count, erythrocyte sedimentation rate, alanine transaminase, B-natriuretic peptide, troponin I, or ferritin values between KD and MIS-C patients. Coronary artery dilation or prominence was seen in 4 patients with KD and in 8 patients with MIS-C (p > 0.99). There were no deaths.

Conclusions. Following national recognition of MIS-C we saw approximately 1 MIS-C case per week. Presenting hypotension, an absolute lymphocyte count less than 1400/µL, a platelet count less than 200,000/µL, and a CRP greater than 20 mg/dL best predicted MIS-C versus KD. The initial white blood cell count, alanine transaminase, erythrocyte sedimentation rate, B-natriuretic peptide, troponin I, ferritin, and initial C-reactive protein did not readily distinguish between MIS-C and KD. Thus, our diagnostic management recommending limited laboratory evaluation for non-toxic patients presenting with a febrile rash illness, fever and abdominal symptoms, or with conjunctival injection is reasonable.

#26: Investigating CMV Pathogenesis and Breast Milk Transmission In Premature Infants Who Acquire Symptomatic CMV Viremia

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Background. CMV can be transmitted to babies after birth through breast milk and such infections may lead to a “sepsis-like syndrome” with neutropenia, hepatitis, and viremia. CMV infections can be epidemiologically characterized by envelope glycoprotein (gB) genotypes - gB1, gB2, gB3 and gB4. The role of specific genotypes in pathogenesis and immune control of infection is incompletely characterized. These studies aimed to characterize viral and host correlates of breast milk transmission of CMV in a neonatal intensive care unit (NICU) setting.

Methods. 200 infants were enrolled in a prospective study of infants <1500 grams at birth in the NICU at the University of MN Masonic Children’s Hospital. Breast milk samples were available from 164 mothers, representing 184 infants (including twin pairs). We compared CMV IgM titer (Gold Standard Diagnostics Corp, CA) and CMV IgG titer (DiaMedix, FL) in non-viremic infants of seropositive mothers for whom we had samples. Eighty infants with eight infants with DNAemia. Positive CMV breast milk samples from 65 mothers were further characterized by a multiplex real-time PCR assay, to characterize and compare gB genotypes.

Results. The prevalence of DNAacta in the breast milk was 65/150 (43%). There were no differences for whom breast milk was present in breast milk samples.

Conclusion. Breast milk from CMV-seropositive lactating mothers in a NICU setting can lead to transmission of infection and development of symptomatic CMV disease, which may not be recognized by clinicians. The distribution of gB genotypes in breast milk is similar to that observed in other CMV epidemiological analyses, and multiple genotypes may be identified in lactating seropositive women. Since gB is a critical target in CMV vaccine design, understanding strain-specific transmission may have implications for understanding the impact of pre-pregnancy vaccination on breast milk transmission. Purified recombinant gB3 vaccines studied in animal trials are based on the Towne strain of CMV, gB1 genotype (DOI: 10.1128/JVI.01695-18). Future studies should examine the impact of strain-specificity of vaccination on circulation of CMV strains in infants with CMV infection.

#27: Comparison of RT-PCR Cycle Threshold Values from Respiratory Specimens in Symptomatic and Asymptomatic Children with SARS-CoV-2 Infection

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Background. Understanding viral kinetics of SARS-CoV-2 is important to assess risk of transmission, manage treatment, and determine the need for isolation and protective equipment. Children have been noted to have less severe illness than adults and may have less transmission potential. We sought to determine whether children deemed to be symptomatic had a difference in the PCR cycle threshold (Ct) value of respiratory samples from symptomatic children with SARS-CoV-2 infection.

Methods. This was a retrospective cross-sectional study to compare PCR Ct values of 728 children who tested positive for SARS-CoV-2 by respiratory samples collected over a 4-month period. The study was a single center review of patients who tested positive for SARS-CoV-2 by RT-PCR from a respiratory specimen at a large tertiary care children’s hospital. Inclusion criteria included children 0–18 years of age who tested positive for SARS-CoV-2 by RT-PCR from a respiratory specimen for whom clinical information was available in the electronic medical record.

Results. We analyzed 728 children who tested positive for SARS-CoV-2 by RT-PCR from a respiratory sample over a 4-month period and for whom data was available in the electronic medical record. Overall, 71.2% of infected children were symptomatic. The mean Ct value for symptomatic patients (Ct mean 19.9, SD 6.3) was available in the electronic medical record. Overall, 71.2% of infected children were symptomatic.
significantly lower than asymptomatic patients (Ct mean 23.5, SD 6.5) (P value < 0.001, CTN=2.6 - 4.6). The mean PCR Ct value was lowest in children less than 5 years of age.

**Conclusions and Relevance.** In this retrospective review of children who tested positive by RT-PCR for SARS CoV-2, the mean Ct was significantly lower in symptomatic children and was lowest in children under 5 years of age.

#28: Rapid, Non-Invasive Detection of Invasive Bartonella Infections in Pediatric Patients Using the Karius Test, A Next-Generation Sequencing Test for Microbial Cell-Free DNA in Plasma

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**Background.** Bartonella henselae and Bartonella quintana are the etiologic agents of cat scratch disease (CSD) and "trench fever", respectively. Both are important causes of culture-negative endocarditis and fever of unknown origin (FUO). The diagnosis of Bartonella infections is limited by (1) the nonspecific, protein manifestations of the disease and its broad differential diagnosis; (2) the fastidious nature of Bartonella spp., leading to rare detections with traditional culture based methods; (3) the insensitivity and poor specificity of Bartonella serologies. Rapid, non-invasive diagnosis of Bartonella through next-generation sequencing (NGS) of plasma microbial cell-free DNA (mcfDNA) offers a means to overcome these limitations. Here we describe the diagnosis of 23 Bartonella infections in children from August 2017 – December 2020 using plasma mcfDNA NGS.

**Methods.** The Karius Test (KT), developed and validated in Karius' CLIA certified/CAP accredited lab, detects mcfDNA in plasma. After mcfDNA is extracted and NGS performed, human reads are removed, and remaining sequences are aligned to a curated database of > 1400 organisms. Sequences from organisms present above a statistical threshold are reported and quantified in molecules/µL (MPM). Clinical information was included from data submitted with the requisition or obtained at the time of reporting from clinical consultations with the provider.

**Results.** KT detected Bartonella henselae mcfDNA in 22 cases and Bartonella quintana in 1. Detects included 10 cases of endocarditis (7 prosthetic valve), 12 cases of CSD/FUO, and a single case of osteomyelitis. Glomerulonephritis was reported in 5 the cases of endocarditis. Six cases had splenic involvement; three had hepatic involvement. History of cat exposure was elicited in 8 cases. The mean MPM was highest for prosthetic valve endocarditis (mean 47,277 +/- 67,526) followed by native valve endocarditis (3,881 +/- 2,458), FUO/CSD (1,922 +/- 3,416), and osteomyelitis (119 +/- 0) (p<0.05). Three subjects had serial mcfDNA monitoring. Predictable declines in mcfDNA were observed in response to therapy in all three patients. The duration of positive Bartonella mcfDNA signal ranged from 22-42 days (30.7, +/- 10.3).

**Conclusion.** Open-ended, plasma-based NGS for mcfDNA provides a rapid, non-invasive method to diagnose diverse clinical manifestations of invasive pediatric Bartonella infection against a competing broad differential diagnosis. The quantification of mcfDNA may further help in differentiating the various clinical syndromes caused by Bartonella. Finally, serial monitoring to trend MPMs may serve as an indicator of burden of infection, provide a means to monitor treatment efficacy and ultimately help define the length of therapy for optimal outcomes.

### Table 1. Pediatric Cases of Bartonella Infections Diagnosed by Plasma-based Microbial Cell-Free DNA Next-Generation Sequencing.

| Clinical Manifestation | n % | Bartonella mcfDNA MPM | Mean SD | GN | Hepatic | Splenic | LN | Cat exposure |
|------------------------|-----|-----------------------|---------|----|---------|--------|----|-------------|
| Total                  | 23  | 20,269                | 41,693  | 100% | 3       | 6      | 5  | 3           |
| Endocarditis           | 10  | 38,610                | 52,702  | 35.5%| 5       | 0      | 0  | 2           |
| Native                 | 3   | 3,881                 | 47,273  | 13.6%| 2       | 1      | 0  | 1           |
| *Prothetic             | 1   | 47,273                | 1      | 34.5%| 3       | 0      | 2  | 0           |
| FUO/CSD                | 12  | 1,922                 | 67,426  | 30.4%| 2       | 2      | 5  | 3           |
| Osteomyelitis          | 2   | 3,416                 | 47,273  | 38.4%| 1       | 1      | 0  | 1           |

All detections are Bartonella henselae except for one case of *Bartonella quintana* (prosthetic valve endocarditis). MPM=molecules/µL; GN=glomerulonephritis, LN=lymph node involvement, FUO=fever of unknown origin, CSD=cat scratch disease.

### Figure 1. Anti-LukAB human mAbs were tested in a 96-well neutralization assay using HL-60 cells against a number of clinically relevant variants of LukAB. A subset of the mAbs tested, including MRSA-448 and MRSA-463, neutralized all the tested variants, indicating the presence of a broadly conserved epitope that can be targeted on LukAB.

### #33: Single-cell RNA sequencing analysis of Zika virus infection in human stem cell-derived neuroprogenitor cells and cerebral organoids

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**Background.** The molecular mechanisms underpinning the neurologic and congenital pathologies caused by Zika virus (ZIKV) infection remain poorly understood. It is also unclear why congenital ZIKV disease was not reported prior to the recent 2015-2016 epidemics in France Polynesia and the Americas, despite evidence that ZIKV virus has actively circulated in parts of Africa and Asia since 1947 and 1966, respectively.

**Methods.** To advance the understanding of ZIKV infections of the central nervous system in human stem cell-derived