ESTIMATING THE ASSOCIATION BETWEEN ACUTE SARS-CoV-2 AND FEBRILE SEIZURE IN CHILDREN USING THE CANADIAN IMMUNIZATION MONITORING PROGRAM-ACTIVE (IMPACT)

by

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Dalhousie University is located in Mi'kma'ki, the ancestral and unceded territory of the Mi'kmaq. We are all Treaty people.

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Abstract

Introduction: Most children with acute SARS-CoV-2 infection exhibit mild symptoms, but neurological complications are also reported. The association between febrile seizures, acute SARS-CoV-2 and other viral infections was not well characterized during the COVID-19 pandemic.

Objective: To estimate the association between hospitalization for febrile seizure and acute SARS-CoV-2 or influenza or respiratory syncytial virus (RSV), or enterovirus/rhinovirus infection in children aged <7 years presenting to a Canadian Immunization Monitoring Program-Active (IMPACT) emergency department (ED) or hospitalized with febrile seizure from 1 Aug 2021 to 31 Dec 2022.

Method: Prospective active surveillance in 12 IMPACT pediatric tertiary care centers captured children <7 years with febrile seizures. Eleven centers were included in the analysis. Nurses screened ED and hospitalization records for cases of fever (temperature ≥38.0°C) and physician diagnosed febrile seizure. Cases that met the case definition underwent review of medical and immunization records and reporting to a central database. Pre-existing neurological conditions were excluded. The primary exposure was microbiologically confirmed SARS-CoV-2 infection within 10 days prior to febrile seizure or during the ED or hospital visit. Secondary exposures were influenza, RSV, or enterovirus/rhinovirus. The outcome was hospitalization for febrile seizure. The association was measured with a cohort study design and mixed-effects logistic regression.

Result: Among 3,306 subjects with febrile seizure over 50% of subjects were aged between 6 to 23 months. Across 11 centers, 595 (18%) patients required hospitalization. The median seizure duration differed significantly between hospitalized (5 minutes, IQR 2,15) and non-hospitalized (2 minutes, IQR 1,5) subjects. SARS-CoV-2 testing was performed for 520 (87%) hospitalized and 694 (26%) non-hospitalized cases ($p<0.001$), clearly indicating a high differential testing associated with outcome. Additionally, SARS-CoV-2 testing, and hospitalization for febrile seizure varied by IMPACT centres ($p<0.001$) and Omicron period ($p<0.001$). Consequently, our models were unable to confirm or reject the hypothesis even after adjusting for multiple confounders e.g., age at presentation, seizure history (in absence of any neurological conditions), presentation during Omicron period, IMPACT centre (random effect).

Conclusion: Limited testing in non-hospitalized patients, leading to extensive differential misclassification bias of exposure meant that the association between acute SARS-CoV-2 infection or other viruses and febrile seizure hospitalization could not be validly estimated, suggesting a need for a more systematic testing approach. However, this study revealed large variations in hospitalization and testing practices across IMPACT sites and over the COVID-19 pandemic. The reasons for, and the consequences of this variation should be explored in future research.

List of Abbreviations Used

AIC - Akaike Information Criterion

aOR - Adjusted Odds Ratio

AUC - Area Under the Receiver Operating Curve

BIC - Bayesian Information Criterion

CRF - Case Report Form

CNS – Central Nervous System

ED - Emergency Department

ICC - Intraclass Correlation Coefficient

ICD-10 - International Classification of Diseases, Tenth Revision

ICU - Intensive Care Unit

IMPACT - Canadian Immunization Monitoring Program-Active

IQR - Interquartile Ranges

LRT - Likelihood Ratio Test

MIS-C - Multisystem Inflammatory Syndrome in Children

MOR – Median Odds Ratio

OR – Odds Ratio

PHAC - Public Health Agency of Canada

RSV - Respiratory Syncytial Virus

RT-PCR - -Reverse Transcription Polymerase Chain Reaction

SARS-CoV-2 - Severe Acute Respiratory Syndrome Coronavirus 2

VIF - Variance Inflation Factor

VOC - Variants of Concern

WHO - World Health Organization

CHAPTER 1: INTRODUCTION

1.1 SARS-CoV-2 virus

Severe Acute Respiratory Syndrome Coronavirus 2 or SARS-CoV-2 virus is a novel form of human coronavirus that causes the disease called coronavirus disease 2019 (COVID-19). The initial pneumonia outbreak caused by the virus in Wuhan, China in December 2019 was later declared as a global pandemic by WHO on 11 March 2020 owing to the growing number of deaths and hospitalizations worldwide, as well as the unparalleled economic and socio-demographic consequences[1].

1.1.1. Structure

SARS-CoV-2, a member of coronavirus family, is an enveloped single-stranded RNA virus with a positive sense[2]. This virus has an RNA genome that holds its genetic data, and an envelope that protects the genome and gives the virus its form. Also, it has spike proteins that attach to specific proteins on human cells, enabling the virus to enter cell [3].

1.1.2. Evolution and Variants of Concern

SARS-CoV-2 evolves through genetic mutations that alter its characteristics, including its transmissibility, symptom severity, and resistance to vaccines and treatments. The spike protein of some variants may also be affected by mutations that impact its ability to bind to human cells or avoid the immune system. Variants such as Alpha, Beta, Gamma, Delta, and Omicron have been identified as variants of concern (VOC) by the WHO due to their potential impact on spread, severity, testing, treatment, and vaccination. These

VOCs were first detected in different countries between late 2019 and late 2021, and each has distinct subvariants. Omicron, in particular, has emerged as the dominant variant in many countries with its subvariants BA.1, BA.2, BA.3, BA.4, BA.5, B1.1.529. Monitoring the emergence and spread of these variants is essential to guide public health measures and the development of vaccines.

Canada saw multiple epidemic waves of higher viral transmission throughout the pandemic. Likewise, different VOCs remained the predominant ones during different tenures. According to Global Initiative on Sharing Avian Influenza Data, the ancestral strain and alpha variant was predominant during 1st April 2020 to 17th April 2021, Delta variant from 18th Apr 2021 to 31st Nov 2021 and the Omicron variant from 1st December 2021 to 31st December 2022[5]. Evidence shows that the Omicron variant is more transmissible and has a higher growth rate, attack rate, and basic reproduction number than other lineages[6]. It has been associated with a sharp increase in infections but appears to cause less severe disease than other variants. However, the increased number of cases associated with the Omicron variant has resulted in a cumulative excess of COVID-19-related hospitalizations compared to other variants[7].

1.1.3. Seasonality

At present, no established seasonal pattern exists for COVID-19 in Canada. Although a possible pattern may coincide with peak outbreaks of other respiratory viruses between January and March as forecasted by the Public Health Agency of Canada (PHAC)[8]. Several viruses have seasonal variations with higher transmission during colder, drier months. This is because low temperatures and low sunlight may increase the risk of

infection, particularly in countries in the northern hemisphere[9]. This could be due to human behavior only, such as spending more time indoors during the winter. However, much remains still unknown about SARS-CoV-2 virus's transmission pattern. Due to evolving variants and waning immunity from previous infection or vaccination the uncertainty around the seasonal pattern further increases[10].

1.1.4. SARS-CoV-2 infection

1.1.4.1. Transmission

Person-to-person spread is the primary means of SARS-CoV-2 transmission. Transmission can take place from individuals who are symptomatic, pre-symptomatic or even asymptomatic. Mode of SARS-CoV-2 transmission can be airborne, contact and droplet, orofecal, vertical or fomite[11]. SARS-CoV-2 virus enters host cell by binding its spike protein with the angiotensin-converting enzyme 2 receptors in the lungs and other tissues. The host's transmembrane serine protease 2 plays an important role for SARS-CoV-2 cell entry and subsequent replication[4].

1.1.4.2. Incubation period

The incubation period refers to the duration between exposure to a virus and the manifestation of symptoms caused by the virus. Previously, it was estimated that the incubation period for SARS-CoV-2 virus prior to the emergence of the Omicron variant was between 2 to 14 days, with a median of 4-7 days from exposure to the onset of symptoms. However, the Omicron variant has a shorter median incubation period of 2-4 days[12]. A meta-analysis has suggested that the incubation period of COVID-19 has gradually decreased from the Alpha to the Omicron variant, i.e., the average incubation

periods for each variant were reported as 5.00 days (Alpha), 4.50 (Beta), 5.10 days (Beta/Gamma), 4.41 (Delta), and 3.42 (Omicron)[13]. According to PHAC, although viral loads can be detected in the nose and throat within 24 hours of exposure, symptoms typically appear 2-4 days after exposure[12]. Understanding the incubation period of SARS-CoV-2 virus is important for predicting the symptom onset, disease management, and prevention.

1.1.4.3. Pathogenesis

SARS-CoV-2 initially targets the respiratory and vascular systems, with the infection progressing through two phases. In the early phase, the virus replicates and causes direct tissue damage, followed by an immune response in the late phase involving the recruitment of T lymphocytes, monocytes, and neutrophil recruitment which releases cytokines such as tumor necrosis factor-α (TNF-α), granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin-1 (IL-1), interleukin-6 (IL-6),), IL-1β, IL-8,IL-12 and interferon (IFN)-γ which act systemically to induce fever[4].

1.1.4.4. Symptoms

The symptoms of SARS-CoV-2 infection can vary depending on the infection phase, age group, and viral variant. The acute phase of SARS-CoV-2 infection can present with mild to severe symptoms such as fever, cough, and fatigue. Recovery can occur without medical intervention, but severe cases may require hospitalization[4]. Symptoms of acute infection overlap with that of other viral infections, such as influenza and other respiratory and enteric viral infections. The acute phase of SARS-CoV-2 infection typically lasts for a few days to a few weeks, although the exact duration can vary from person to

person. By contrast, long COVID refers to a range of symptoms that persist for several weeks or months after the acute phase of COVID-19. These symptoms can include fatigue, difficulty with concentration, shortness of breath, chest pain, joint pain, and depression, among others[14].

In children, the signs and symptoms (fever and cough) of COVID-19 can be similar to those of other infectious and non-infectious conditions, including influenza, other viral upper respiratory infections, streptococcal pharyngitis and allergies[12]. Young children may be especially vulnerable to upper respiratory acute infection due to their small and relatively collapsible airways. In older adults, symptoms may present differently, with low-grade fever, cough, shortness of breath, loss of taste or smell, and fatigue and body aches. Sore throat, new-onset congestion, nausea, vomiting, or diarrhea may also be present in older adults[12,4].

With the Omicron variant, symptoms have been shown to be more likely upper respiratory in children such as runny nose, sneezing and sore throat than the pre-Omicron variants. During pre-omicron waves, many children were asymptomatic or only had mild symptoms such as fever (46-64%) and cough (32-56%). Duration of acute symptoms for those with the Delta variant was longer than those with the Omicron variant (mean duration 9 days vs. 7 days)[12].

1.1.4.5. Reinfection

It is possible to be infected with SARS-CoV-2 even after recovering from a previous infection[7]. During the pre-Omicron period the chance of reinfection was low within six to eight months of the initial infection[15-16]. However, with the emergence of Omicron the

hazard of reinfection increased (Hazard Ratio 1.75, 95% CI: 1.48 to 2.10) compared to pre-Omicron waves. Both immune evasion capabilities of variants e.g., Omicron and the waning immunity of infected individuals might contribute toward reinfection[17]. Although the risk of reinfection with Omicron subvariants BA.4 and BA.5 was lower following BA.1 or BA.2 infection than following infection with a pre-Omicron variants[18].

1.2. Epidemiology of COVID-19

COVID-19 was spread globally to over 676 million confirmed cases and 68 million deaths, while Canada has reported 4,777,664 cases of infection and 54,734 deaths as of October 2023[19-20]. However, these numbers likely underestimate the true impact of the virus, as only a fraction of cases are diagnosed and reported, as demonstrated by seroprevalence surveys i.e., the rate of prior exposure to SARS-CoV-2 exceeds the incidence of reported cases by approximately 10-fold or more in the United States and Euroupe[7]. Seroprevalence surveys involve testing a representative sample of individuals from a population to determine the presence of specific antibodies in their blood[7].

1.2.1. Infection trend in Canada

Understanding the infection trend i.e., the direction and pattern of change in the number of COVID-19 cases is critical for disease surveillance, outbreak identification, and preventive measures such as informing policy, initiating or removing restriction. Several factors influence the rate of SARS-CoV-2 infection, including the emergence of new variants, changes in population density, and public health measures, but not seasonality. Infection-acquired seroprevalence increased significantly from 5.4% (95% CI 4.3,6.8) in November 2021 (before the detection of Omicron in Canada) to over 73% in January 2023

following more than one year with Omicron as the dominant variant[21-22]. On the other hand, Western and Central provinces had over 74% mean seropositivity due to infection while Atlantic Canada had the lowest rate at 67%, as for December 2023[23]. The most recent findings from the EnCORE study, which measured SARS-CoV-2 seroprevalence in Montreal's children and adolescents, indicated that infection-acquired seroprevalence in children aged 2-19 years increased from 58.1% in September 2022 to 79.4% for the 2–4-year age group and 76.3% for the 5–11-year age group in June 2023[23-24]. The impact of the removal of public health control measures (such as the re-opening of school and daycare) on SARS-CoV-2 infection rate was complex and depended on multiple factors including the prevalence of COVID-19 in the community, the behavior of individuals outside of these settings and the specific measures implemented by schools and daycare centers to reduce transmission e.g., mask-wearing, ventilation[24-25].

1.2.2. Impact of COVID-19 in children

The Delta and Omicron variants of the SARS-CoV-2 virus caused a significant increase in the number of COVID-19 cases, hospitalizations, and severe illness among children and adolescents. In Canada, the weekly COVID-19 count among children aged 0-11 years started to increase rapidly in mid-November 2021, with the highest number recorded in January 2022 at 27,024[20]. Canadian seroprevalence studies conducted in Quebec from 26th January 2022 to 17th February 2022, and in British Columbia in March 2022 have shown that a significant proportion (ranging from 30% to 70%) of children below 5 years of age had contracted SARS-CoV-2 infection[26-27]. Recent data from a study using residual blood samples from pediatric emergency room patients in Quebec and Ontario (March to May 2023) indicated lower infection induced seroprevalence in children

under 2 years (39.0%) compared to older children, with 50.0% for 2-5 years and 63% for 5-10 years old[23]. However, these data may not be generalizable to other regions of Canada during the same period and the national COVID-19 seroprevalence estimates are unknown in children 5 years of age or younger.

Public Health Agency of Canada's (PHAC) report on COVID-19 disease severity by age group showed that the average monthly hospitalization rate per 100,000 population aged between six months and four years increased from 1.4 in the pre-Omicron period (March 2020-Dec 2021) to 15.9 in the Omicron period (Jan-March 2022). The same report revealed that in the pre-Omicron period, the average monthly Intensive Care Unit (ICU) admission rate was 0.1 per 100,000 population, and the death rate was 0.01 per 100,000 population in the same age group. These rates increased to 1.3 for ICU admissions and 0.27 for deaths per 100,000 population in the Omicron period[28]. Risk factors for severe disease among children hospitalized for COVID-19 were being younger than 1 year of age or having chronic comorbid conditions associated with neurologic or pulmonary disorders[29]. In children, risk factors for severe COVID-19 outcome are age, certain underlying medical conditions such as asthma, cancer, chronic lung disease, obesity, sickle disease, disabilities (e.g., Down syndrome)[12,30].

Between March 2020 and May 2021, 406 cases of multisystem inflammatory syndrome in children (MIS-C) were reported in children, with a median age of 5.4 years[31]. MIS-C are conditions associated with inflammation of gastrointestinal, cardiovascular, hematologic, mucocutaneous, and respiratory systems. It was found to be temporally associated with SARS-CoV-2 [32]. Approximately 30% of MIS-C cases required ICU or hemodynamic support, especially those with confirmed links to SARS-CoV-2[31].

1.3. COVID-19: Diagnosis

1.3.1. Types of testing

There are two types of tests approved by Health Canada for diagnosing active SARS-CoV-2 infection. **Nucleic acid-based test** or nucleic acid amplification test, also called molecular testing or reverse-transcription polymerase chain reaction (RT-PCR) detects the virus' genetic material. The sensitivity of PCR tests is higher than that of rapid antigen tests. The PCR test is the gold standard for diagnosing active SARS-CoV-2 infection in patients with symptoms[34]. **Antigen-based tests** detect specific proteins on the surface of the virus. Antigen tests may miss some positive cases, particularly when viral load is low or in the early stages of infection, as these tests work by detecting viral proteins only [35]. Sensitivity of diagnostic testing may vary by variant or subvariant. Antigen tests could be less sensitive for the Omicron variant compared to the Delta variant in nasal samples, especially in the first 1-2 days after infection[36-37]. However, rapid antigen tests are faster than standard PCR and can be used at the point of care e.g., by a health care professional or at home, making them useful for mass testing and screening of asymptomatic individuals[35]. There is another type of test called serology or antibody tests. These test for antibodies produced in response to a previous SARS-CoV-2 infection or vaccination and do not directly detect the virus[4,34].

1.3.2. Changes in testing pattern in Canada

The SARS-CoV-2 testing pattern in Canada evolved over the course of the pandemic, with changes in the type and frequency of testing reflecting changes in the epidemiology of COVID-19 in the country. Early in the pandemic in 2020, testing was focused on individuals who traveled to areas with known transmission of the virus, as well

as people with symptoms, close contacts of confirmed cases, and certain high-risk groups[34-37]. But, as the pandemic progressed and community transmission of the virus became more widespread in mid-2021, testing criteria were expanded to include a broader range of individuals, including those with mild or no symptoms, as well as those who were at higher risk of exposure due to their occupation or living situation to prevent further transmission of the virus[34,38]. By the end of 2021, the testing pattern shifted from relying primarily on PCR testing to using rapid antigen tests more frequently to identify and isolate cases faster, especially with the emergence of Omicron. Nova Scotia, for example, altered its testing strategy starting from 27th Dec 2021 considering the testing capacity stretched to its limit due to high number of infections driven by the Omicron variant as well as the ongoing vaccine rollout (e.g., pressure on healthcare resource). During the first half of 2022, rapid antigen tests or rapid PCR tests (for point of care testing in some health settings) were prioritized for those with symptoms or close contacts with COVID-19 patients. PCR testing was reserved for those at higher risk of severe disease, hospitalized patients (to guide not just treatment but also isolation), people who live and work in higher-risk group living settings (e.g., long-term care homes, shelters, group homes, correctional facilities, i.e., prisons) and frontline health care workers[39-40]. The rate and decision of testing and priority groups for RT-PCR varied among provinces and territories[42-43] and varied across hospitals. Nova Scotia, for example, halted the requirement of a PCR test to confirm a positive rapid test result after the arrival of Omicron[41]. Overall, the SARS-CoV-2 testing pattern in Canada has been shaped by a range of factors, including the changing epidemiology of the virus, availability of testing supplies, and the evolving understanding of the virus and its transmission.

1.4 Febrile seizure

1.4.1 What is febrile seizure?

Febrile means "feverish". Febrile seizures are convulsions that can happen when a young child has a fever above 100.4°F (>38.0°C)[44]. These are the most common types of childhood seizure, usually occurring between 6 months and 5 years of age, with peak incidence in the second year of life[45]. Although there are reports of first febrile seizures in children up to 7 years of age and at 3 months of age. Febrile seizure episodes are associated with a febrile illness, not caused by an infection of the central nervous system (CNS) or a previous unprovoked seizure[46]. In children of European background, 2% to 5% will experience 1 or more febrile convulsions before the age of 5 years. However, the overall incidence of febrile seizure in children is not known[46].

1.4.2 Types of febrile seizures

Based on clinical features, febrile seizures are classified as either simple or complex. Simple febrile seizures are defined as single, generalized tonic-clonic convulsions lasting <15 minutes and self-limited. On the other hand, features of complex febrile seizures are focal seizures, prolonged seizure (duration greater than 15 min) or multiple seizures occurring as clusters of episodes during the same 24-h period[47]. Complex febrile seizure comprises 10-35% of all cases[48].

1.4.3 Risk factors of febrile seizures

There are some known risk factors associated with febrile seizures, described below.

1.4.3.1 Viral infection with seasonality

Viral infections with seasonality are found to be the most common cause of the febrile illnesses associated with febrile seizure e.g., enteroviruses related gastrointestinal infection in summer, rotavirus, influenza A and B infection in winter and rhinovirus infection in spring-early autumn[44]. Seasonal coronaviruses along with influenza have contributed relatively more febrile seizure-related emergency room visits than other respiratory viruses[49].

1.4.3.2 Other risk factors

Family history of febrile seizures or epilepsy increases the risk of febrile seizures i.e., 25-40% of children presenting with febrile seizures have a family history of such[44]. The exact pattern of inheritance is uncertain, but recent studies have identified gene loci associated with febrile seizures on chromosomes 5, 8 and 19. Febrile seizures are also strongly age-dependent with the median age of first presentation between 17 and 23 months of age. Febrile seizures tend to occur more frequently in boys than in girls [49]. Underlying neurological deficits, such as cerebral palsy or neurodevelopmental delay, neonatal discharge from hospital at 28 days of age or later, low serum zinc and iron levels have also been associated with higher prevalence of febrile seizures in children. Studies have reported increased incidences of febrile seizure 1-3 days post influenza vaccination[50-51], 1-2 days post Diphtheria, Tetanus, acellular Pertussis, Polio and Hemophilus influenzae type b (DTaP-IPV-Hib) vaccination[50-52] and 8-14 days post Mumps Measles Rubella (MMR) vaccination[53] in children. Some environmental risk factors have been associated with increased febrile seizure incidence, including maternal smoking and stress[44]. A Canadian

case-control study demonstrated that the risk of febrile seizure incidence increases to 28% in children with any two risk factors[54]. Moreover, febrile seizure recurrence occurs in 30-50% of children. Each additional febrile seizure and low-grade fever increases the risk of further recurrence[44].

1.4.4 SARS-CoV-2 infection and febrile seizure

SARS-CoV-2 virus could be associated with febrile seizures in children. In a retrospective multicentered study using electronic health record data from March 1, 2020, to April 19, 2021, involving 8,854 children aged 0-5 diagnosed with COVID-19, 44 individuals were reported to have febrile seizures. The median age among these 44 COVID-19 patients with febrile seizures was 1.5 years, and 68.2% had simple febrile seizures while 31.8% had complex febrile seizures[55]. A single-centered study in Turkey on neurological manifestations of paediatric COVID-19 cases between March 11, 2020, and January 30, 2021, found that among 2,530 children with COVID-19 symptoms or contact history, 4.7% children tested positive in the PCR test for acute SARS-CoV-2 infection and needed inpatient care for febrile seizures[56]. Another single centered study in India involving outpatient and inpatient electronic medical records of children <16 years reported 14 cases of simple febrile seizure out of 988 children diagnosed with confirmed COVID-19 during June 2020-May 2021[57]. In a US retrospective study from July 1, 2020, to December 31, 2021, 2.7% of the 27,692 COVID-19 cases had febrile seizures. However, when employing a gender matched case-control design with logistic regression, and adjusting for age and race, odds ratio for febrile seizures in patients testing positive for SARS-CoV-2 was 0.96, p=0.949 [CI 0.81, 1.14]) compared to those who tested negative for SARS-CoV-2[58]. The emergence of SARS-CoV-2 Omicron variant likely increased the

incidence of febrile seizure in children aged 6 months and 5 years. A study involving five hospitals in Korea reported that until 2021, pre-Omicron variants were found to be associated with severe disease and a 20.5% hospitalization rate among the infected patients aged <5 years, but no febrile seizure was observed. However, after the Omicron surge, 16.5% of hospitalized patients (64 of 387) had febrile seizure, despite the overall dropping of hospitalization rate to 1.2% [59]. This result resembled findings of a study conducted in South Africa, which reported that 20% of hospitalized patients aged below 19 years with the Omicron variant experienced seizures[60]. Seizure can occur during the acute phase of the infection and even in the post-acute phase if the SARS-CoV-2 infection evolves into MIS-C[61-62].

Another important point was that two separate population-based retrospective observational studies conducted in Canada and Hong Kong found a disproportionate decrease in febrile seizure-related hospital admissions during the pandemic year of 2020 compared to the pre-pandemic years 2019-2020. The Canadian study reported a drop-in mean incidence rate of hospitalizations and ED per 100,000 population from 25 in 2015-2019 to 13.5 in 2020 among children aged 0-4 years[63]. The Hong Kong study reported that when compared to 2015-2019, seizure-related hospital attendances decreased (RR 0.379, 95% CI 0.245–0.588) in 2020 across all pediatric age groups, with a significantly larger decrease in the 0-6 years age group compared to the 7-18 years age group. The studies put forth the hypothesis that the observed reduction in seizure occurrences and changes in health-seeking behavior were due to the implementation of social distancing and hygienic precautions, which collectively lowered viral transmission[63-64].

1.4.5. Role of COVID-19 vaccines

COVID-19 vaccination might have a dual role in febrile seizure related hospitalization. Firstly, as COVID-19 vaccination protects children from severe illness and hospitalization from COVID-19, it was important to consider if COVID-19 vaccinated children were less likely to have febrile seizure than the unvaccinated child population[65-67]. Reports from systematic review and meta-analysis indicated that in Omicron cases, fever was more commonly observed among individuals who were not vaccinated as compared to those who were vaccinated[13]. On the other hand, similar to non-live vaccines (as explained in 1.4.3.2), as mRNA vaccines, COVID-19 vaccines might increase the incidence rate of febrile seizure 0-3 days after vaccination [70]. Febrile seizure was an adverse event of special interest (AESI) for COVID-19 vaccines as febrile seizure has been associated with other vaccines in children [70-73]. In short, COVID-19 vaccination could have a protective effect against febrile seizure caused by infection and it could be risk factor for vaccine-related febrile seizure. So, we needed to check both roles of COVID-19 vaccines.

Health Canada authorized the use of Pfizer-BioNTech Comirnaty in children 5 to 11 years of age on 19th November 2021 (2 dose primary series; 10 mcg per dose) [68]. Moderna Spikevax was authorized on 17th March 2022 for children 6 to 11 years of age (2 dose primary series; 50 mcg per dose) and on 14th July 2022 for children aged 6 months to 5 years (2 dose primary series; 25 mcg per dose)[68]. As of January 01, 2023, 52.7% of children aged 5-11 years and 9% of children aged 0-4 years had received at least 1-dose of COVID-19 vaccine[69].

1.5 Canadian Immunization Monitoring Program ACTive (IMPACT)

The Canadian Immunization Monitoring Program ACTive (IMPACT) is a national active surveillance network based in pediatric tertiary care hospitals that identifies cases of vaccine-preventable infectious diseases and adverse events following immunization that result in hospitalization[74-75]. Active surveillance involves actively searching for cases according to pre-defined case definitions to monitor the spread of diseases and the safety of vaccines within a community or population[76-79]. Gathering information on cases of selected infections (e.g., SARS-CoV-2, pertussis, influenza, invasive infections caused by *Haemophilus influenzae*, *Streptococcus pneumoniae and Neisseria meningitidis*), IMPACT helps to determine the burden and severity of these infections, measures the benefits of new vaccines, and supports planning and evaluation of immunization programs. Its strategic positioning makes it an ideal tool for surveilling emerging diseases and monitoring alterations in event rates or identifying novel signals of concern from vaccines. To determine the relationship between SARS-CoV-S infection and febrile seizure IMPACT monitored emergency department visits and hospitalizations for febrile seizure from August 2021 to December 2022. As of 2020, 13 pediatric centers across Canada conducted IMPACT surveillance. This accounted for over 90% of pediatric tertiary care beds [74-75]. IMPACT uses trained surveillance nurses supervised by volunteer pediatric clinicians who act as site investigators. These nurses screen hospital admission lists for surveillance targets. For potential cases they then review medical records and retrieve immunization records. Cases are reported electronically on standardized case report forms to the IMPACT data center [80]. IMPACT has the advantage of producing standardized, high-quality (e.g., high adherence to case definitions) and complete data (e.g., immunization history, laboratory results, comorbidities, or concomitant medications)[76-80]. IMPACT

disseminates study results through annual reports to funders, conference presentations, and peer-reviewed publications[80]

1.6 Study rationale

Rationale for the active surveillance at IMPACT were that there was a theoretical concern for an increase in febrile seizures following SARS-CoV-2 infection due to the historical correlation between febrile seizures and different respiratory tract infections[49]. Additionally, there was consideration that vaccination could protect against seizures by reducing infections or making infections less severe. However, we do not know if SARS-CoV-2 was associated with hospitalization for febrile seizures. We also do not know the association between influenza, RSV, enterovirus and rhinovirus infection and febrile seizure related hospitalization in the context of COVID-19 pandemic, although these viruses have been reported to be associated with fever and febrile seizure in children. This study aimed to establish the association between these acute infections and hospitalization for febrile seizures, adjusting for various confounders.

CHAPTER 2: OBJECTIVES

2.1. Hypothesis

Among children younger than 7 years of age presenting to IMPACT ED or admitted to hospital with febrile seizure, a microbiologically confirmed SARS-CoV-2 infection or an influenza or RSV or enterovirus/rhinovirus infection was associated with an increased likelihood of hospitalization.

2.2 Objectives

Objective 1: To describe the demographic and clinical features of children aged <7 years presenting to an IMPACT center for febrile seizure who were hospitalized or non-hospitalized from 1 Aug 2021 to 31 Dec 2022

Objective 2: To estimate the association between microbiologically confirmed acute SARS-CoV-2 infection and hospitalization for febrile seizure in children aged <7 years who presented to an IMPACT ED or were transferred/hospitalized with febrile seizure during the study period.

Objective 3: To estimate the association between influenza or RSV or enterovirus/rhinovirus infection and hospitalization for febrile seizure in children aged <7 years who presented to an IMPACT ED or were transferred/hospitalized with febrile seizure during the study period

CHAPTER 3: STUDY DESIGN AND METHODS

3.1. Study design and study subjects

This was a prospective active surveillance study of febrile seizures among children aged <7 years who presented at an IMPACT center between Aug 1, 2021, and Dec 31, 2022. We utilized a cohort study design to estimate the association between microbiologically confirmed SARS-CoV-2 infection, influenza, RSV, or enterovirus/rhinovirus infection, and hospitalization for febrile seizures. Following a cohort over a period to address the research question defined it as a cohort study[1,15]. It employed a hybrid design, combining elements of both prospective and retrospective cohort studies. For instance, information on exposure (i.e., infection status from testing at the IMPACT ED or inpatient care) and outcome status (i.e., hospitalized, or non-hospitalized with febrile seizures) was collected in real-time. Additionally, some information was gathered retrospectively, contributing to the confirmation of exposure or outcome status, such as parent-reported temperature measurements for fever and recent SARS-CoV-2 infection status.

3.2. Case definition

Children aged 0 to 6 years who visited ED or were hospitalized at an IMPACT center with any of the following inclusion criteria and without any of the exclusion criteria. The study population was specified as children under the age of 7, as febrile seizure is a clinical entity that typically occurs in children between 6 months to 6 years of age[44-45]. In older age groups, seizures with fever are more likely to indicate neurological pathology, rather than febrile seizure.

3.2.1 Inclusion criteria

- A physician diagnosed febrile seizure and a measured temperature of ≥38.0◦C in hospital or by parent if recorded in chart.
- Cases of status epilepticus (seizures lasting more or equal 30 minutes or multiples seizures without recovery in between) where fever was also recorded (unless there was a central nervous system (CNS) infection or other neurological condition as per exclusion criteria in 3.2.2)
- Cases where 'fever' and 'seizure' were separately documented (e.g., seizure in discharge records but fever was reported in patient's chart)

3.2.2 Exclusion criteria

- Febrile seizures in the context of a CNS infection/neurological conditions such as epilepsy, seizure disorder, meningitis, encephalitis, encephalopathy, brain abscess, infarct or stroke, cerebral hemorrhage, brain tumor, Acute Disseminated Encephalomyelitis (based on physicians' diagnosis).
- Febrile seizures occurring with a diagnosis of MIS-C.

3.3. Data collection

The study collected primary data from 12 IMPACT centers across eight Canadian provinces. Eleven centers were included in the analysis. At each center, data was collected by trained nurses, supervised by dedicated pediatric infectious disease clinicians who act as site investigators. Nurses screened hospital ED information system or ED visits lists and hospital admission lists for physician diagnosis of febrile seizure cases. Cases that met the case definition underwent review of medical records and immunization records (capturing

all vaccinations within the prior 30 days). The active monitoring approach involved collecting outcome data mostly in real-time although cases were also captured retrospectively through International Classification of Diseases, Tenth Revision (ICD-10) code searches, which gave us the confidence that no cases were missed. Cases were then entered into an electronic data capture system. Then the data went through multiple quality checking steps (e.g., multiple attempts to collect the missing information from sites, validating the outliers, ensuring the medical history information was correct and made clinical sense) before starting analysis[80-81].

The IMPACT national active sentinel surveillance platform was selected as the data source for studying febrile seizure for several reasons. It allowed us to understand why some patients ended up being hospitalized for febrile seizure while others were not. The detailed information collected from medical and immunization records at each center ensured the reliability and robustness of data regarding primary and secondary exposures along with the other risk factors of febrile seizure[80]. These risk factors helped us to generate relevant covariates to check and adjust their confounding effect on the association between exposure and outcome variable. Data from 11 centres in 8 provinces contributed to the generalizability of the study[80].

3.4. Ethical considerations

Each IMPACT centre obtained the necessary research ethics and hospital approvals, and we were granted a consent waiver because our study met the criteria for minimal risk (minimal potential harm or discomfort to study subjects) and because obtaining consent was impracticable. The sole potential harm associated with the study was

a breach of confidential information, mitigated through several measures. For instance, staff members are trained to maintain the confidentiality of personal information. The case report forms (CRFs) (see Appendix 1) did not contain any identifiable patient data, such as names or addresses. Instead, each febrile seizure CRF was assigned an IMPACT ID upon entry into the system. Data storage, sharing, and destruction are controlled through an internal quality management system. The database is hosted in a secure data facility with 24-hour security, internal and external backup measures, and disaster recovery plans in place. [81]

3.5. Variables

3.5.1 Exposure variable

Our primary exposure was microbiologically confirmed acute SARS-CoV-2 infection, while influenza or RSV or enterovirus/rhinovirus infection were secondary exposures of interest.

Acute SARS-CoV-2 infection was confirmed through positive RT-PCR or rapid antigen tests conducted at IMPACT outpatient settings, the ED, or during hospitalization. IMPACT did not record tests and results reported by parents, performed at home or in other non-IMPACT health facilities. The risk period was defined as 10 days prior, or during the days spent at ED or hospital for febrile seizure. The 10-day interval prior ED or hospital visit was specified considering the median time from exposure to symptom onset (4-7 days for delta variant and 2-4 days for omicron variant) and the mean duration of acute symptoms e.g., fever (9 days for Delta and 7 days for Omicron variant)[13]. Individuals who tested positive for SARS-CoV-2 more than 10 days but less than or equal to 90 days before

their visit to the ED or hospitalization for febrile seizure, along with those who tested negative for SARS-CoV-2 at the IMPACT center for febrile seizure related visit, were considered as not having an acute SARS-CoV-2 infection. This classification was based on the assumption that a positive test result more than 10 days prior makes it unlikely for the SARS-CoV-2 infection to be directly associated with the occurrence of the seizure. Typically, patients get tested or qualify for RT-PCR tests after serious acute symptoms emerge, such as high-grade fever leading to hospitalization.

We defined the exposure variable in two ways:

Definition 1: First, we defined it as a multi-level categorical variable, denoted as "**Acute Infection Type**". It encompassed ten levels:

a. acute SARS-CoV-2 (who were detected with acute SARS-CoV-2 only, but may or may not have been tested for other types of infection)
b. Influenza (who were detected with influenza only, but may or may not have been tested for other types of infection)
c. RSV (who were detected with RSV only, but may or may not have been tested for other types of infection)
d. Enterovirus/Rhinovirus (who were detected with either enterovirus or rhinovirus only, but may or may not have been tested for other types of infection)
e. Adenovirus (who were detected with Adenovirus only, but may or may not have been tested for other types of infection)
f. Other infection (who were not detected to have a, b, c, d, e but were detected for any other single infection e.g., *E.coli* or Parainfluenza or *Staphylococcus aureus,* but may or may not have been tested for all types of infection)

g. >1 concurrent infections with SARS-CoV-2 (a + (b/c/d/e/f) = >1)

h. >1 concurrent infections without SARS-CoV-2 (b+c+d+f= >1)

i. No detected infection (who tested negative for SARS-CoV-2 and were not reported to have or tested for b/c/d/e/f)

j. unknown infection (who were not tested for SARS-CoV-2 infection and were not reported to have or tested for b/c/d/e/f)

Definition 2: We identified significant limitations in the measurement of both our primary (SARS-CoV-2 infection) and secondary exposure (e.g., influenza, RSV, enterovirus/rhinovirus) of interest in our data. Regarding the primary exposure, a substantial number of subjects were not tested for SARS-CoV-2, resulting in a high percentage of missing information on acute SARS-CoV-2 infection status. For the secondary exposure (e.g., influenza, RSV, enterovirus/rhinovirus), IMPACT data only captured information on the presence of infections. Except for SARS-CoV-2, IMPACT did not record negative test results or information on untested individuals for these infections. Consequently, all categories of our definition 1 of the exposure variable likely had missing data on non-SARS-CoV-2 infections, making them not mutually exclusive.

We also considered the fact that every subject in this study visited the IMPACT center following a febrile seizure event. Our working assumption was that all cases involved infections of any type, whether detected or undetected. We acknowledged that exceptions were also possible but rare (e.g., where, instead of an acute infection, an autoimmune disease, inflammatory condition, or an evolving neurological condition played a role).

Given our data limitations and this working assumption, we introduced an alternative definition of our exposure variable that considered only subjects tested for acute SARS-

CoV-2 infection and disregarded any categorization against non-SARS-CoV-2 infection status. After excluding the unknown SARS-CoV-2 infection group, we had 37% of the subjects left for analysis based on the second definition. We defined the exposure variable as **'Acute SARS-CoV-2 infection'** with two categories:

a) Tested positive for acute SARS-CoV-2 with/without other infection.
b) Tested negative for acute SARS-CoV-2 with/without other infection.

Our objective was to determine if individuals testing positive for SARS-CoV-2 had a higher likelihood of hospitalization compared to those testing negative.

3.5.2 Outcome variable

The outcome variable was hospitalization for febrile seizure and the level of this variable was binary, i.e., hospitalized with febrile seizure versus non-hospitalized. Subjects who were escalated to inpatient care from ED or stayed in the short stay/holding unit and/or ED for >24 hours or who were already admitted to hospital when diagnosed for febrile seizure were considered as hospitalized. On the other hand, subjects who visited the ED and were discharged from ED/short stay/holding units within 24 hours or less were considered as non-hospitalized.

3.5.3 Covariates and other variables of interest

Based on a literature review, we initially developed a directed acyclic graph (DAG) illustrating factors that might influence the association between acute SARS-CoV-2 infection and febrile seizures (Figure 1) for better understanding. Subsequently, we created another DAG (Figure 2), narrowing down the risk factors that could impact hospitalization

for febrile seizures and for which we had data from IMPACT. From the DAG we ensured that these covariates do not come in the causal pathway between the exposure and outcome variables.

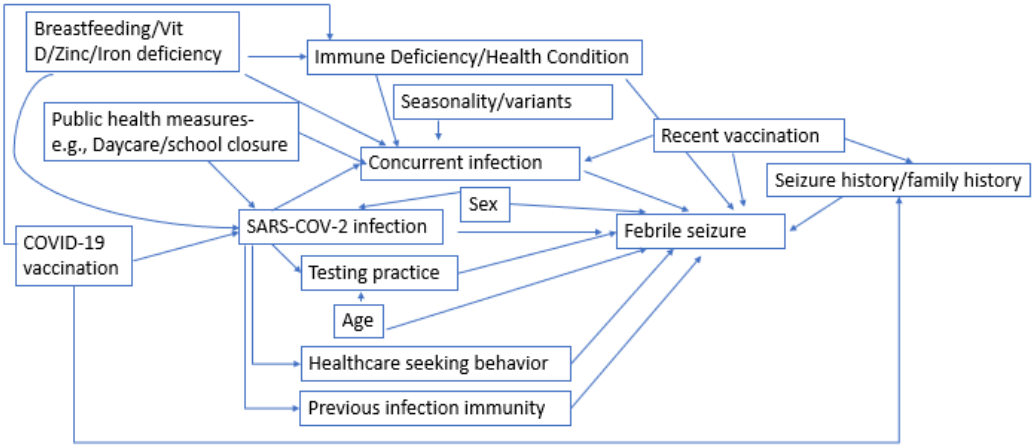

Figure 1: DAG for the association between SARS-CoV-2 infection and febrile seizure

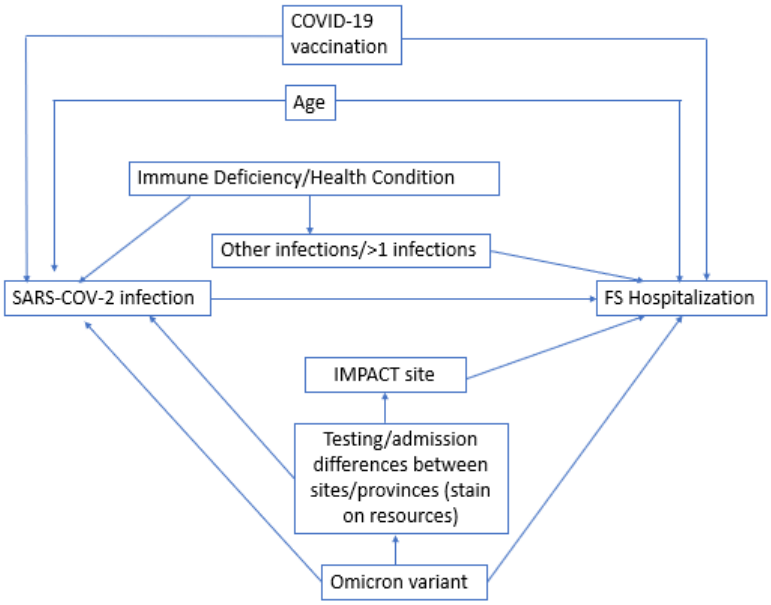

Figure 2: DAG for the association between SARS-CoV-2 infection and hospitalization for febrile seizure

Utilizing these DAGs, we identified covariates and other variables of interest (i.e., descriptive characteristics). The sorted covariates for adjustment in the multivariable regression model included age at presentation, IMPACT centre, underlying health condition, immunodeficiency, seizure history (history of febrile or afebrile seizure without any pre-existing neurological condition) and Omicron/pre-Omicron period (to adjust testing variation). Descriptive characteristics of interest were sex, vaccination in risk window for febrile seizure, COVID-19 vaccination, type of febrile seizure [simple (generalized, lasts <15 minutes, does not recur within 24 h) versus complex [focal features and/or lasts ≥15 min and/or >1 seizure in 24 h), outcome at discharge (3 level variable: recovered or improving /developed new or persistent comorbidity/ death), highest measured temperature (in Celsius), duration of seizure (minutes), duration of total hospital stay (days) and ICU admission requirement. Details of these variables are given in Table 1.

Adjustment of age, for example, was needed as hospitalization for febrile seizure is strongly age dependent[45,49]. The second important covariate was any health condition or immune deficiency that are risk factors for COVID-19 related hospitalization[30]. Table 1 gave a list of health and immunodeficiency conditions from our data that we considered for adjustment. Next covariate of interest was IMPACT centres. Adjusting for IMPACT centres accounted for provincial differences in public health measures as well as clustering effect of sites i.e., site differences in terms of SARS-CoV-2 testing in ED, type of test used, criteria for testing, admission criteria, referral population. Similarly, we aimed to account for the variation in health services caused by Omicron. After the arrival of Omicron variant, the rate of hospitalizations increased and SARS-CoV-2 testing protocols changed e.g.,

children became ineligible for RT-PCR testing at public health settings (see section 1.3.2 for details). These may have led to a change in healthcare seeking behavior (e.g., cautious parents rushing at hospital) impacting thresholds for admission (e.g., overcrowding in hospitals, placing a strain on available beds, healthcare personnel, and resources). In the context of our research conducted amid the COVID-19 pandemic, with a specific emphasis on febrile seizure-related hospitalizations, our hypothesis was that accounting for the Omicron/pre-Omicron period would offer a better understanding on the impacts of alterations in SARS-CoV-2 testing practices, public health interventions, and variations at the hospital or practice level, as opposed to considering only the influence of seasonality.

We wanted to adjust for any protective effect of COVID-19 vaccination that might have on febrile seizure-associated hospitalization, if sample size permitted. Also, we wanted to adjust for the impact of any recent vaccination on febrile seizure provided febrile seizure can occur as an adverse event following immunization with certain vaccines: 0-2 days after inactive vaccines, 5-13 days after live vaccines, and 0-3 days after COVID-19 vaccines (details in Appendix 3)[50-53.] We considered both as variables of interest: 'COVID-19 vaccination' and 'vaccination in the risk window for febrile seizure' for our descriptive analysis. It was presumed that we would not have sufficient samples to adjust these variables in the regression model as IMPACT collected vaccination information only for the prior 30 days of ED/hospital visits.

The potential impact of other public health measures on community-level transmission of infection, such as the requirement of wearing masks, were not considered in this study as mask-wearing was not mandatory throughout the study period. Furthermore, concurrent infection with acute SARS-CoV-2 infection could increase the

likelihood of hospitalization for febrile seizure. We did not consider it as a covariate to adjust for our definition 1 exposure variable, as detected infections were categorized in multiple levels in this variable. But we did adjust for concurrent infections when definition 2 exposure variable was used in the model. Lastly, in our study, we could not consider factors like family seizure history, breastfeeding, or vitamin deficiency status as their influence on febrile seizure-related hospitalization was not clear from the existing literature, and IMPACT did not collect these data.

3.6. Statistical analysis

3.6.1. Descriptive analysis

The descriptive analysis approaches were designed for Objective 1. Demographic and clinical characteristics were reported by hospitalization status (hospitalized vs non-hospitalized). We also described the characteristics of subjects with unknown SARS-CoV-2 testing status and compared it with known SARS-CoV-2 testing status group to assess the pattern of missing values (see Section 3.6.4 for details). Based on the pattern of missing values on infection status, we described the variation on testing and admission pattern across IMPACT sites during pre-Omicron and Omicron period.

Categorical variables were summarized using frequencies and proportions, while continuous variables were described with medians and interquartile ranges (IQR). The IQR representing the range between the first and third quartiles of the data, provided insight into the data spread and measures variability. To adhere to IMPACT policies, frequencies ranging from one and four were masked and reported as '<5' to preserve confidentiality and opposing cells were presented with a range to prevent back-calculation.

Difference between the characteristics of hospitalized versus non-hospitalized and SARS-CoV-2 known versus unknown status group were assessed using Chi square test or Fisher's exact test (when expected cell size was <5) for categorical variables. For continuous variables, Student's t-test, or Mann-Whitney U test (Wilcoxon rank-sum test) was used. A non-parametric test was chosen for skewed continuous variables, as indicated by histogram. The skewness indicated a departure from the assumption of normality.

3.6.2 Mixed-effects logistic regression analysis

The mixed effects logistic regression models were designed for objective 2 and 3.

3.6.2.1 Assessment of clustering effects

We hypothesized that there might be a random clustering effect associated with IMPACT site that may influence the relationship between exposure and outcome. We tested this by comparing the mixed-effects logistic regression model to a standard logistic model using Likelihood Ratio (LR) test and measuring the Intraclass Correlation Coefficient (ICC). In the LR test a p-value of <0.05 suggested a preference for the mixed-effects logistic regression model. The ICC measured the proportion of total variance in hospitalization for febrile seizure attributable to the variability between IMPACT sites. We also measured the Median Odds Ratio (MOR) to quantify the heterogeneity or variation between different IMPACT centers. The MOR served the purpose of quantifying the magnitude of the effect of IMPACT site in the context of employing a multilevel logistic regression model [82].

3.6.2.2 Multivariate regression model

Model with multi-level categorical exposure variable: At first, we assessed the relationship between hospitalization for febrile seizure and the first definition of exposure variable (multi-level categorical exposure variable 'acute infection type'). The reference category for OR measurement was the 'no detected infection' level of this exposure variable. Potential fixed effect confounding variables (e.g., age at presentation, health and immunodeficiency conditions, seizure history and Omicron period) and potential random-effect confounders (e.g., IMPACT center, Omicron period) were introduced individually, and if their inclusion resulted in a change greater than 10% in the exposure variable's regression coefficient, they were added to the final models. Subsequently, multiple mixed effect logistic regression was constructed, accounting for both fixed-effect confounders and random-effect confounders. To assess the potential effect modification, hypothesized interaction terms based on DAGs were introduced into the models (e.g., interaction terms between the exposure variable and potential confounders including acute infection type-age, acute infection type-IMPACT site, acute infection type- health and immunodeficiency conditions, and acute infection type-seizure history).

The measure of association, or the adjusted OR, estimated the odds of being hospitalized for febrile seizures among those with acute SARS-CoV-2 infection, influenza, RSV, or enterovirus/rhinovirus infection, as opposed to those being hospitalized without any detected infection, while adjusting for confounders. Adjusted OR, along with 95% CIs and p-values at a 5% level of significance, were reported.

Model with binary exposure variable: Here, we used the subsample of subjects who were tested for SARS-CoV-2. We estimated the relationship between hospitalization for febrile seizure and the second definition of the exposure variable (binary exposure

variable ‘acute SARS-CoV-2 infection’), using a mixed-effects logistic regression model, adjusting for the random effect of IMPACT sites. In this model, the reference category for OR measurement was those 'tested negative for acute SARS-CoV-2 infection with/without other infection’. Then, the covariates (e.g., age at presentation, health and immunodeficiency conditions, seizure history, and Omicron period) and relevant potential effect modifiers (e.g., acute SARS-CoV-2 infection-age, acute SARS-CoV-2 infection-IMPACT site, acute SARS-CoV-2 infection-health and immunodeficiency conditions, and acute SARS-CoV-2 infection -seizure history) were introduced into the model in the same manner for testing.

Model selection: For both model types (model with multi-level categorical exposure variable and model with binary exposure variable), we used model selection criteria, including Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), and Area under the Receiver Operating Curve (AUC). Lower AIC or BIC values indicate better model fitting, while an increased AUC shows improved model performance. Final models for both types were selected based on the lowest AIC and BIC values and the highest AUC.

3.6.3. Assessments of model assumptions

In assessing the assumptions of our mixed-effect logistic regression model, the requirement for a binary dependent variable was met by our outcome variable that had two levels: hospitalized and non-hospitalized. Secondly, we took precautions to identify the potential issue of multicollinearity among covariates using the Variance Inflation Factor (VIF). VIF values less than 2.5 were considered indicative of low multicollinearity [83].

We recognized that our data exhibited clustering effects associated with the IMPACT center variable. To address this, we used mixed effect logistic regression modeling to adjust the clustering effect of IMPACT sites. Also, the observations were not entirely independent due to the nature of repeated febrile seizure events within subjects. Febrile seizures recur in 30-50% of children following the first febrile seizure[44]. However, IMPACT recorded each ED/hospital presentation as a distinct event with a separate IMPACT ID, even if it pertained to the same individual. Consequently, there was no option to link or match those events for the same subject, making it impractical to differentiate the first event for a subject using IMPACT data. To assess the impact of this limitation, a sensitivity analysis was conducted, excluding subjects with reported seizure history of any kind (Scenario 4 in Appendix 2).

3.6.4. Missing data

Missing data is a common occurrence in clinical research. There are three different missing-data mechanisms. If the probability of a variable being missing for a given subject is independent from both observed and unobserved variables for that subject, then it is called “Missing completely at random”. Data are said to be “missing at random” if after accounting for all the observed variables, the probability of a variable being missing is independent from the unobserved data. Finally, if the probability of a variable being missing, even after accounting for all the observed variables, is dependent on the value of the missing variable, then it is called “missing not at random”. It is caused by systematic bias in the missing data[84].

In our study, there was a high level of missing values for laboratory test results related to acute SARS-CoV-2 infection, and missingness was strongly associated with the

outcome (missing not at random type). Patients who were hospitalized were much more likely to be tested. So, we decided not to drop or impute the missing values, and rather to understand the pattern of missingness and consider the impact in our analysis. In the descriptive analyses, we presented the differences between the SARS-CoV-2 known and unknown testing status groups in terms of hospitalization status, Omicron period, the type of febrile seizure, the average highest recorded temperature, and IMPACT site.

Based on these insights, we tested the random effect of IMPACT site and the Omicron period (both separately and combined) on the measured OR of acute-SARS-CoV-2 infection. Instead of excluding subjects with unknown infection status (not tested for SARS-CoV-2 and was not detected to have any other infection), we categorized them in a level (unknown infection) in our first definition of exposure variable (multi-level categorical exposure variable, 'acute infection type') and examined the OR for this category. If a positive association was found between the unknown testing status group and hospitalization for febrile seizure, further investigation would have been conducted on the patients' symptoms to propose possible explanations against the association. We also considered that incorporating missing values can distort the measured OR. Hence, we introduced the second definition of the exposure variable (binary exposure variable 'acute SARS-CoV-2 infection') and did separate modelling using only the subsample of subjects who were tested for SARS-CoV-2 (section 3.6.2.2).

3.6.5. Sensitivity Analysis

We conducted extensive sensitivity analyses to test the model performance and robustness under various scenarios. The analyses are given in Appendix 2.

3.7 Study power

Study power is a critical aspect of assessing the robustness and reliability of study findings. In our investigation, we performed a post-hoc calculation to assess the power of our study. We considered the SARS-CoV-2 infection status of subjects for the proportion calculations, disregarding other non-SARS-CoV-2 infection status of subjects. This decision was made because SARS-CoV-2 infection was our primary focus, and for other infections, we only had data on positive infection status (no and unknown infection status was blended)

Calculation 1 (Including all study subjects i.e., 3306): In this power analysis, we assessed the statistical power of a test comparing proportions between individuals with SARS-CoV-2 infection only (Group 1) and those without SARS-CoV-2 infection only (Group 2). The study design featured 11 clusters representing IMPACT sites, with an assumed intra-cluster correlation coefficient (ICC) of 0.1. Group 1 maintained a consistent cluster size of 10, while the cluster size in Group 2 varied from 20 to 300 in increments of 10. The probabilities of hospitalization were specified as 0.487 for those infected with SARS-CoV-2 only and 0.203 for those not infected with only SARS-CoV-2 (refer to Table 2). The power analysis used a two-sample proportion test utilizing a Pearson's chi-squared test accounting for the clustering structure at IMPACT sites. The power curves below provided an understanding of how varying cluster sizes impacted the ability to detect differences in proportions of being hospitalized between individuals infected with SARS-CoV-2 only and those not infected with only SARS-CoV-2 within a clustered study framework. The graph illustrated that as the cluster size increased, the statistical power also

increased, which was consistently greater than 80%, indicating a high likelihood of detecting true effect if that existed.

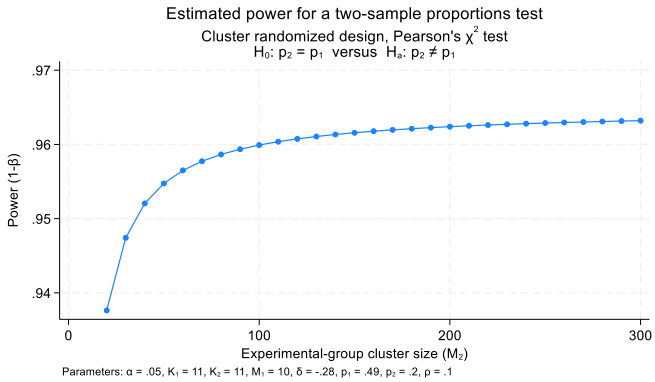

Figure 3: Power curve for calculation 1

The approach for the power calculation described above involved treating the multi-categorical exposure variable with 10 levels as 9 dummy variables. This is because most statistical software does not support a multilevel categorical exposure variable for power calculation. To validate the results of the power calculation, we conducted a simulation-based power calculation using R statistical software. The simulation involved repeatedly generating data 100 times from the mixed effect logistic regression model, with parameters set to the estimates obtained from the fitted model. Specifically, this included the proportions of participants belonging to each level of the multilevel categorical variable, the size of IMPACT centers (Table 2), estimated regression coefficients for all the dummy variables of the multilevel categorical covariate (calculated as the log of the adjusted OR), and the intra-class correlation coefficient (ICC) (Table 6). The power for testing SARS-CoV-2 infection only versus no detected infection, with a multilevel categorical exposure variable, was 99.00% (with a 95% confidence interval of 94.55% to 99.97%). It was in line with the result of the power calculation based on the approach presented earlier.

Calculation 2 (Including subsample of subjects who were tested for SARS-CoV-2, i.e., 1212): We assessed the statistical power of a test comparing proportions of being hospitalized between individuals with SARS-CoV-2 infection with or without other infection (Group 1) and those without SARS-CoV-2 infection with or without other infection (Group 2) after excluding those not tested for SARS-CoV-2 with or without other infection. The study design featured 11 clusters representing IMPACT sites, with an assumed intra-cluster correlation coefficient (ICC) of 0.1. Group 1 maintained a consistent cluster size of 10, while the cluster size in Group 2 varied from 20 to 300 in increments of 10. The probabilities of hospitalization were specified as 0.49 for those infected with SARS-CoV-2 and 0.42 for those without SARS-CoV-2. The power analysis used a two-sample proportion test utilizing a Pearson's chi-squared test and accounted for the clustering structure at IMPACT sites. The graph illustrated that as the cluster size increased, the statistical power also increased. The power consistently remained very low (<0.15), indicating a low likelihood of detecting a true effect if that existed.

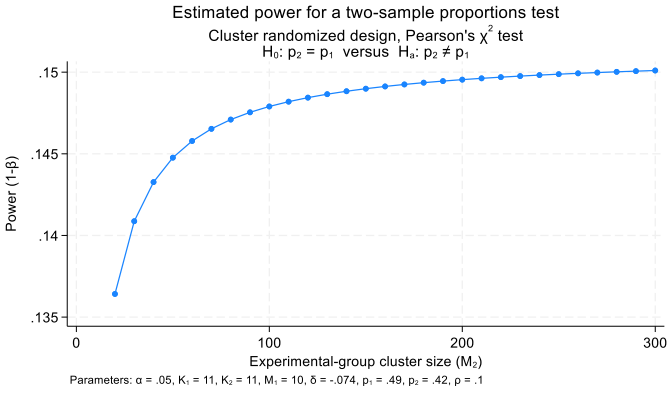

Figure 4: Power curve for calculation 2

Analyses were conducted using Stata 17.0 (College Station, Texas 77845, USA). Only for simulation-based power calculation statistical software R (version 4.2.3) was used.

CHAPTER 4: RESULTS

4.1 Result of descriptive analysis

The results of descriptive analyses addressed objective 1. Between August 1st, 2021, and December 31st, 2022, a total of 3556 febrile seizure patients initially met inclusion criteria (Figure 3). Subsequently, 250 patients were excluded due to the presence of chronic neurologic or nervous system conditions. Ultimately, 3,306 children met the inclusion criteria and did not meet exclusion criteria.

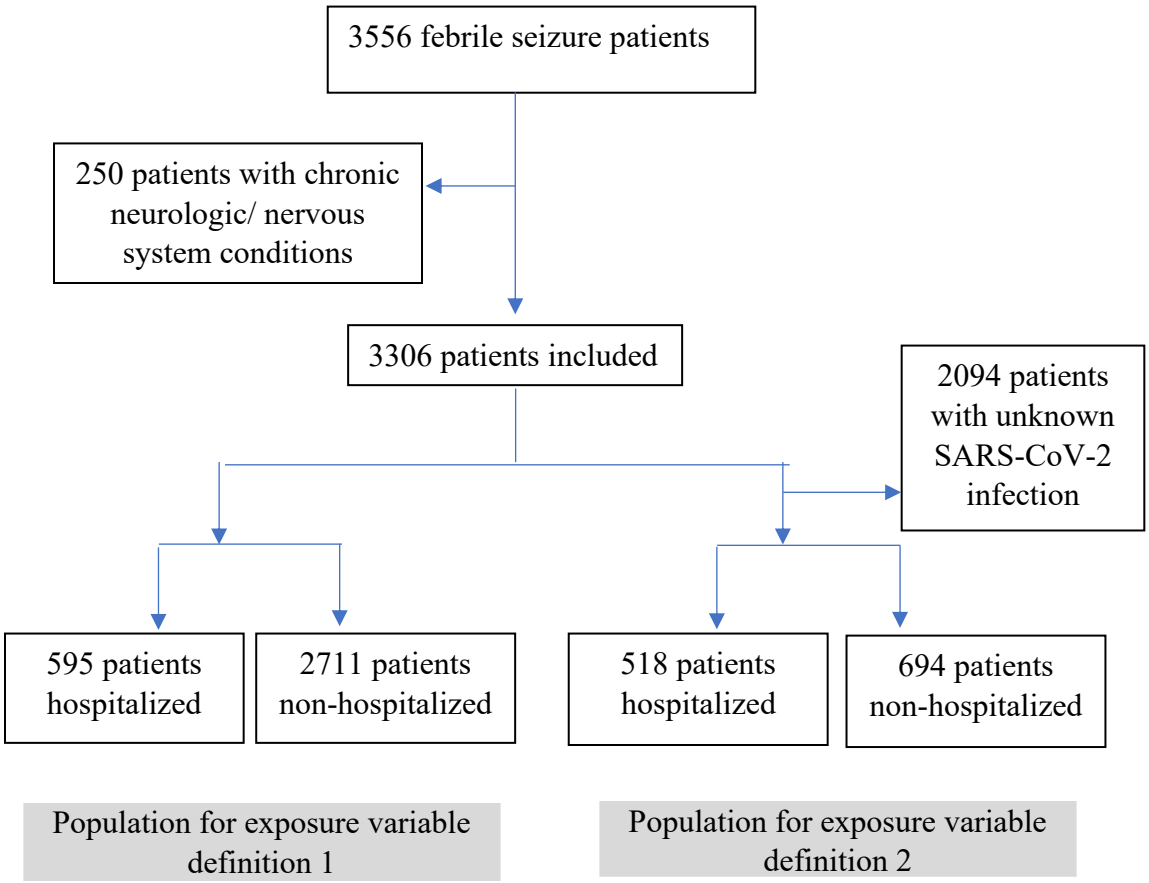

Figure 5: Flowchart of study subject selection

4.1.1. Comparison between hospitalized and non-hospitalized group

Overall, 18% (595 patients) were hospitalized, while 82% (2,711 individuals) were non-hospitalized (Table 2). Notably, 52% of the total study population fell within the age range of 6 to 23 months. The results revealed a significant difference in age distribution between hospitalized and non-hospitalized groups ($p < 0.001$). The proportion of infants aged 0-5 months was greater in the hospitalized group (32 patients, 5%) than in non-hospitalized group (22 patients, 1%), although this group contributed the lowest to the overall study population (2%). No significant association was found between sex and the hospitalization status ($p=0.1$). At the IMPACT site level, a significant distribution difference was observed between hospitalized and non-hospitalized groups ($p< 0.001$).

The hospitalized group had a higher proportion of confirmed acute SARS-CoV-2 (13%), influenza (6%), RSV (3%) & enterovirus/rhinovirus (9%) infected patients compared to non-hospitalized group (3% for acute SARS-CoV-2, 1.25% for influenza, 0.55 % for RSV and 0.66% for enterovirus/rhinovirus), $p<0.001$. However, this reflected testing, as only 10% of the hospitalized group had unknown infection status, compared to 73% for the non-hospitalized group. We investigated the study subjects with unknown infection status (62% of the total) in section 4.1.2.1 on missing values.

Complex seizures were more prevalent in the hospitalized group (81%), while simple seizures were more common in the non-hospitalized group (73%) ($p<0.001$). Recovery at discharge was observed in 99% of the study population. The proportion of subjects with persistent or new comorbidities was slightly higher in hospitalized group compared to non-hospitalized group (4.54% versus 0.18%, $p<0.001$). The average highest

recorded body temperature in the hospitalized group was slightly higher at 39.16°C, than the non-hospitalized group at 39.08°C (p=0.03). The median duration of seizure was 5 minutes for hospitalized patients (IQR: 2, 15) and 2 minutes for the non-hospitalized patients (IQR: 1, 5) (p<0.001). The median length of stay at the hospital was 2 days (IQR: 1, 3), with 13% of hospitalized patients requiring ICU care.

The frequency of ED visit or hospitalization for febrile seizure was 569 cases in the Pre-Omicron period (Aug-Nov 2021) and 2,737 cases in the Omicron period (Dec 2021-Dec 2022). It is important to note that the Omicron period (13 months) was longer than the pre-Omicron period (4 months). To facilitate a more direct comparison between the two periods, we measured the average cases/month during each period. During the Pre-Omicron period, the monthly average of hospitalized and non-hospitalized febrile seizure cases was 28 and 114, respectively. In the Omicron period, these averages were 37 and 173 respectively. The hospitalization rates for febrile seizures were similar in the pre-Omicron period (19.7%), and Omicron period (17.6%), p-value=0.2.

Most subjects, constituting more than 68% of the study population, had no previous history of seizures. The inpatient group had a slightly higher proportion of subjects with seizure history (32%) than the non-hospitalized group (29%, p=0.01). Most study subjects (97%) did not have a health condition considered risk factors for COVID-19 related hospitalization, as subjects with neurological/nervous system conditions were excluded. The hospitalized group had a slightly higher proportion (4%) of subjects with health conditions (considered risk factors for COVID-19 related hospitalization) compared to the non-hospitalized group (2%, p<0.001). Similarly, immunodeficiency was reported in fewer than 1% of cases for both groups (0.84% of hospitalized patients and 0.18% for non-

hospitalized group, p<0.001). Combining underlying health and immunodeficiency conditions, asthma was identified as the most common comorbidity, constituting 84% of all reported conditions. Apart from asthma, other chronic conditions (e.g., bronchomalacia, chronic lung disease, sickle cell disease) each occurred in fewer than five subjects, collectively representing the remaining 16% of reported health and immunodeficiency conditions.

Less than 1% of the sample consisted of COVID-19 vaccinated individuals (29/3306) and there was no significant difference in distribution between the hospitalized and non-hospitalized group (p=0.28), and we could not assess for any protective effect against febrile seizure hospitalization. Also, 8% of the study population (269/3306) received any vaccination (COVID-19 or other) within 30 days before their febrile seizures. The proportion of subjects experiencing febrile seizures during the risk period for vaccination was similar among hospitalized (5%) and non-hospitalized (3%) group, (p=0.15). So, we were unable to detect any significant increase in febrile seizure-associated hospitalizations during the vaccination risk window.

4.1.2 Missing values on exposure

We investigated 2,043 subjects (62% of the total) with an unknown infection status and observed strong differential ascertainment in the exposure variable by the outcome, as well as large variation between IMPACT sites and time (pre-Omicron and Omicron). Given that a substantial portion (63% of the total) of subjects lacked information on SARS-CoV-2 infection status, we examined factors influencing SARS-CoV-2 testing at IMPACT centers. Our analysis explored SARS-CoV-2 testing patterns among febrile seizure patients

both pre-Omicron and during Omicron across various IMPACT sites. Notable variations in testing rates between periods and testing sites were observed. This investigation enhanced the understanding of SARS-CoV-2 testing dynamics within our cohort, acknowledging potential implications for the observed association between SARS-CoV-2 infection and hospitalization for febrile seizure.

4.1.2.1 Comparison between known and unknown SARS-CoV-2 testing status groups

During ED or hospital visits for febrile seizures, SARS-CoV-2 testing status was known for 1,214 (37%) subjects and unknown for 2,092 (63%) subjects (Table 3). Among those 595 individuals hospitalized for febrile seizure, 520 (87%) had known SARS-CoV-2 testing status, while among those 2711 non-hospitalized individuals, only 694 (26%) had known SARS-CoV-2 testing status, $p<0.001$. During the pre-Omicron period, 384 (67%) individuals with febrile seizure were tested for SARS-CoV-2 at IMPACT centers, but after the arrival of Omicron, it dropped to 30% (830 individuals), $p<0.001$. Figure 4 presents a graphical representation of monthly percentages of known and unknown SARS-CoV-2 testing status, revealing the change in testing pattern during pre-Omicron and Omicron periods during the study period.

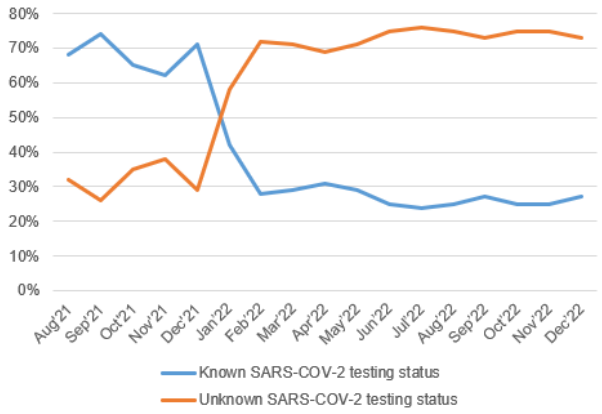

Figure 6: Monthly variation in SARS-CoV-2 testing pre and during Omicron

Among subjects with complex febrile seizure 51% had known SARS-CoV-2 testing status but for subjects with simple febrile seizure, only 28% had known SARS-CoV-2 testing status, ($p<0.001$). The average highest temperature for a known testing group was slightly higher at 39.15°C, than non-unknown testing group at 39.06°C ($p=0.0008$). Difference between the proportion of known & unknown SARS-CoV-2 testing status varied by IMPACT site ($p<0.001$).

4.1.2.2 Practice variation across IMPACT sites

To portray practice variations across IMPACT centers, Table 4 describes SARS-CoV-2 testing among febrile seizure cases and the prevalence of hospitalization by site during the Omicron and Pre-Omicron period.

The frequency of SARS-CoV-2 testing was much higher in hospitalized patients than non-hospitalized patients, aligning with typical hospital practices. However, this difference escalated further during the Omicron period, affecting the testing patterns disproportionately across IMPACT sites. For instance, in the pre-Omicron period, most sites tested 100% of their hospitalized febrile seizure patients, which dropped across most sites after the arrival of Omicron period. In contrast, site 09 consistently tested 67% of admitted febrile seizure patients in both Omicron and pre-Omicron periods. Site 02, on the other hand, maintained a high testing rate of 90% for non-hospitalized febrile seizure patients in the pre-Omicron period, and even after the arrival of Omicron, it sustained the highest testing rate among all sites at 77% for its non-hospitalized febrile seizure patients.

Although our data provided information on whether SARS-CoV-2 testing was conducted during ED visits or hospitalizations for febrile seizures, for other infections, IMPACT reported only when a subject tested positive during ED visits or hospitalizations. Consequently, data on the testing prevalence for influenza, RSV, enterovirus/rhinovirus, adenovirus, and other infections during the study period were unavailable. In Table 5, we presented frequencies of subjects testing positive for influenza/RSV panel or enterovirus/rhino/adenovirus panel (3 viruses tested together at some hospitals) in each center, providing an insight into the percentage of tested subjects among the total reported cases of febrile seizures per center. We identified that testing patterns varied across IMPACT centers and were impacted by the Omicron period.

It was notable that for the same event, febrile seizure, admission rates differed across IMPACT sites. For instance, site 04 admitted 44% of its febrile seizure patients, whereas site 10 only admitted 7%. These admission rates further varied when broken down into the percentages by Omicron and pre-Omicron periods. For example, site 04 admitted 61% of its febrile seizure patients in pre-Omicron period, which decreased to 41% in Omicron period. While hospitalization rates were reduced in most centers for febrile seizures during the Omicron period, some sites, such as site 5, maintained steady rates, and others, like site 2, even demonstrated an increase in hospitalizations. However, there was no significant difference overall (all sites combined) in proportion hospitalized in pre-Omicron vs Omicron period ($p=0.2$, Table 2).

4.2 Results of multivariable regression analyses

Our multivariable regression analyses aimed to address objective 2 and 3. In our analysis, at first, we examined the necessity of adjusting for the clustering structure at IMPACT centres, using likelihood ratio (LR) test and intraclass correlation coefficient (ICC). The LR test, comparing the mixed-effects logistic regression model to a standard logistic model, yielded a highly significant p-value of less than 0.001, and confirmed our preference for the mixed-effects logistic regression model. The estimated ICC for the IMPACT center level was approximately 0.11 (Table 6, Model 1). The ICC can be interpreted as: nearly 11% of the total variation in hospitalizations for febrile seizure was due to between IMPACT center differences in practice. Even with the introduction of Omicron as a random effect nested within IMPACT center level, the ICC remained consistent at 0.11 (Table 6, Model 2). The median odds ratio (MOR) was measured at 1.83, indicating that, in random pairwise comparisons, the median relative odds of hospitalization was 83% higher if presenting with febrile seizure to an IMPACT centre with a higher likelihood of hospitalizing patients for febrile seizures.

None of the hypothesized interaction terms (e.g., exposure variable definition 1-age, exposure variable definition 2-age, exposure variable definition 1-IMPACT centres, exposure variable definition 2-IMPACT centres) were significant at 5% level of significance and therefore were not reported.

Final model with multi-level categorical exposure variable: This multivariable regression model (Table 6, Model 3), incorporated the multi-level categorical exposure variable (definition-1) and adjusted for fixed-effect confounders (age at presentation, seizure history, and Omicron period), as well as random-effect confounder (IMPACT centers). The model (Model 3) was chosen over the other comparator models (Model 1 &

2) in Table 6 as it resulted in the lowest balance between the AIC value of 2028, BIC value of 2138, and highest AUC of 89%. It indicated that our final model outperformed other candidate models and represented the best-fitting model for our analysis.

While this model yielded a significant association between acute SARS-CoV-2 infection and hospitalization (adjusted OR 1.67, [p=0.01, 95% CI (1.12, 2.48)]), it was likely an artifact of differential misclassification bias in the exposure variable. The reference group consisted of subjects who tested negative for an acute SARS-CoV-2 infection and not reported or tested for other infection during ED or hospital visit for febrile seizures. Furthermore, the adjusted ORs for influenza, RSV and enterovirus/rhinovirus were 2.71 [p<0.001, 95% CI (1.55, 4.74)], 2.53 [p=0.02, 95% CI (1.13, 5.66)] and 6.22 [p<0.001, 95% CI (3.45, 11.19)], respectively. In addition, we found adjusted OR 8.89 [p<0.001, 95% CI (3.62, 21.86)] for adenovirus, 2.52 [p<0.001, 95% CI (1.53, 4.13)] for other infection, 2.05 [p=0.20, 95% CI (0.70, 6.01)] for acute SARS-CoV-2 with other infection, 3.88 [p<0.001, 95% CI (2.02, 7.45)] for >1 concurrent infection without SARS-CoV-2, and 0.06 [p<0.001, 95%CI (0.04, 0.08)] for unknown infection. This model was inconclusive in confirming or rejecting the hypothesis because of differential misclassification bias of exposure with the use of exposure variable definition 1.

Final model with binary exposure variable: Upon excluding individuals with unknown SARS-CoV-2 infection status to eliminate subjects with missing exposure data (Figure 5), our Model 4 comprised 1,212 participants, consisting of 518 hospitalized and 694 non-hospitalized individuals. Here, we experienced a greater loss of non-hospitalized patients compared to hospitalized patients, indicating a differential misclassification bias applied to the exposure variable definition 2, which also contributed to the reduced power

of this analysis. After adjusting for only random effect of IMPACT centers, the adjusted OR for the association of acute SARS-CoV-2 infection with or without other infection and febrile seizure hospitalization was 1.26, 95% CI (0.90, 1.78), p=0.183 (Table 6, Model 4). When we adjusted for both random effect of IMPACT centers and fixed effect of age, seizure history, Omicron period, other concurrent infection the adjusted OR was 1.33, 95% CI (0.91, 1.95), p=0.136 (Table 6, Model 5). Model 5 was chosen as final model with our binary exposure variable for lowest AIC/BIC and highest AUC. However, Model 5 could not detect a significant association between acute SARS-CoV-2 infection and hospitalization for febrile seizure. This model was highly underpowered to detect a true association if there was any.

Overall, both final models (Model 3 and Model 5) were inconclusive in addressing objectives 2 and 3.

CHAPTER 5: DISCUSSION

5.1. Interpretation and comparison with literature

In this 17-month prospective active surveillance study, we monitored children under 7 years presenting to IMPACT ED or hospital with febrile seizure. Descriptive analysis addressing our first objective revealed that among 3,306 study subjects, 595 (18%) required hospitalization. SARS-CoV-2 testing was conducted for 87% of hospitalized and 26% of non-hospitalized cases, highlighting a significant testing disparity based on hospitalization status. Variability in SARS-CoV-2 and other viral infection testing and hospitalization for febrile seizure was observed across IMPACT centers and over the COVID-19 pandemic. Some centers probably were not testing at all for influenza/RSV or enterovirus/rhinovirus/adenovirus or were testing less than expected. The decline in SARS-CoV-2 testing after Omicron emergence aligned with changes in government policy regarding children's eligibility for RT-PCR tests, particularly affecting non-hospitalized patients [39-43]. Testing variation across sites was confirmed through the IMPACT survey on respiratory virus testing practices [88-89]. We recognize testing status and its determinants as a crucial area for future research.

Our second and third objective to estimate whether acute SARS-CoV-2, influenza, RSV, or enterovirus/rhinovirus infection increased the likelihood of hospitalization was inconclusive. Although our model with a multilevel categorical exposure variable detected a statistically significant association between hospitalization for febrile seizure and acute SARS-CoV-2 infection, influenza, RSV, or enterovirus/rhinovirus, and was the best-fitted model statistically, data limitations hindered the confirmation or rejection of the

hypothesis. It is crucial to acknowledge that the detected association was influenced by the differential misclassification bias in the exposure variable, i.e., infection status. In other words, testing dynamics were significantly driven by hospitalization status, further impacted by the Omicron period and practice variation at IMPACT centers. Consequently, our ability to address the research question was compromised. If testing was proportional in both hospitalized and non-hospitalized groups across all centers and throughout the entire study period, we might not have detected a positive association between acute SARS-CoV-2 infection and hospitalization for febrile seizure. However, it is worth noting that the association between febrile seizure related hospitalization and the common non-SARS-CoV-2 viral infections are well established in the literature [44,49]. The adjusted OR of 0.06 ($p<0.001$, 95% CI: 0.04, 0.08) for the unknown infection group indicated that patients without any detected infection were less likely to be hospitalized for febrile seizure. Interestingly, all models and sensitivity analyses consistently identified this significant negative association. We interpreted that non-hospitalized patients were less likely to be tested for infection than hospitalized patients, and hence, that negative association was identified.

Upon excluding subjects not tested for SARS-CoV-2 infection and categorizing our exposure variable into two simple categories—acute SARS-CoV-2 with or without other infection and no acute SARS-CoV-2 with or without other infection—the adjusted OR was 1.33, $p=0.136$ [0.91, 1.95]. In this scenario, we did not observe a significant association between acute SARS-CoV-2 infection and hospitalization for febrile seizure. This model was highly underpowered, but the finding aligned with a recently published case-control study that found no significant association between febrile seizures and COVID-19

(OR=0.96, P=0.949 [95% CI 0.81, 1.14]), adjusting for age and race[58]. Although their outcome of interest was febrile seizure and ours was hospitalization for febrile seizure, the finding aligned with our interpretation i.e., there might not be a significant association between acute SARS-CoV-2 infection and febrile seizure.

Among those seeking care for complex febrile seizures, only 49% were not tested for acute SARS-CoV-2, while of those seeking care for simple febrile seizures, 72% were not tested. This difference may be attributed to a higher likelihood of hospitalization for individuals experiencing complex febrile seizures, leading to more extensive testing for various infections among hospitalized patients compared to those non-hospitalized. Again, this observation supported our conclusion that the positive association between infections and hospitalization for febrile seizure was detected because testing was significantly driven by hospitalization status.

In the future, to assess the association between hospitalization for febrile seizures and SARS-CoV-2 infection versus other infections in the pediatric population, we suggest adopting a systematic testing approach. For example, we recommend testing all patients presenting with febrile seizures at IMPACT centres for acute SARS-CoV-2 infection as well as other viruses i.e., influenza, RSV, enterovirus, rhinovirus. While a cohort study design remains optimal to compare hospitalization among patients with SARS-CoV-2 or other detected infection and patients who tested negative for all viruses, we acknowledge that testing all patients would escalate study costs and necessitate informed consent from participants. Additionally, we could modify the objective of this study slightly to reconsider the outcome variable (hospitalization for febrile seizure). This is because hospitalization is likely to be influenced by practice variation at sites and over time (e.g.,

variation during an outbreak or a particular season). To focus more on the clinical aspect in the pediatric population, we might choose 'anticonvulsant after febrile seizure' (indicating prescription at IMPACT ED/hospital for febrile seizure management) or 'complex febrile seizure' as the outcome variable.

Several additional findings from this study are notable. First, while hospitalization for febrile seizure was identified across all age groups, over 50% of cases belonged to the 6-23 months age range. This aligned with the established literature that the median age of the first febrile seizure to be between 17 and 23 months[49,55]. Children under six months of age were more likely to be hospitalized compared to other age groups. The higher likelihood of hospitalization was probably due to their young age. According to the National Institute of Neurological Disorders and Stroke, healthcare providers may recommend hospitalization for febrile seizure if the child is younger than 6 months of age [87]. They could have been hospitalized for symptom management, ensuring hydration, providing nutrition, supplemental oxygen, or mechanical ventilation.

Second, the percentage of hospitalization for febrile seizure varied across IMPACT sites and by Omicron period. We interpreted that this variation could be due to the differences in population, regional/geographical variations, difference in provincial public health measures (e.g., SARS-CoV-2 infection control protocols, intermittent closures and opening of daycare and schools and masking requirements at schools) as well as variations in health services facilities (e.g., difference in medical practices, criteria for hospital admission/admission threshold, ED capacity). We presumed that the arrival of the Omicron variant might have changed the healthcare-seeking behavior of parents and admission threshold at IMPACT sites. However, we found that the average hospitalizations per month

were similar in the Omicron and pre-Omicron periods overall. However, upon conducting a site-wise breakdown, we observed that some sites increased hospitalizations, some decreased, and some remained steady in terms of admission of febrile seizure patients after the arrival of Omicron.

The clinical characteristics of hospitalized febrile seizure patients paralleled existing literature. In our study, most hospitalized patients had complex febrile seizures whereas most non-hospitalized patients had simple febrile seizures. This aligned with the National Institute of Neurological Disorders and Stroke guideline that confirms that healthcare providers may recommend hospitalization for febrile seizure if the seizure is prolonged (prolonged event is one of the criteria for complex febrile seizure)[87]. In contrast to a US study on 0-5-year-old pediatric patients reporting a higher incidence of simple febrile seizures (68%) among those with COVID-19, our study found that a majority (52% of the 169 children with acute SARS-CoV-2 infection) experienced complex febrile seizures. This divergence was likely due to increased testing among hospitalized patients, where complex febrile seizure cases were more prevalent. Ninety nine percent of patients recovered at discharge which was aligned with the Korean study that described the clinical manifestations in children <5 years with concurrent COVID-19 and febrile seizures[59]. Our study showed that 13% of hospitalized patients required ICU care.

5.2 Strengths and limitations

The key strength of this study was the high-quality data on febrile seizures obtained through active surveillance, which reduced the chance of recall bias among patients and reporting bias among physicians. The inclusion of data from 11 sites across 8 provinces

increased generalizability. The utilization of a mixed-effect logistic regression model, accounting for clustering effects associated with IMPACT centers, was a strength of our analysis. The incorporation of both fixed-effect confounders (age at presentation, seizure history, and Omicron period) and random effects at IMPACT centers improved the precision of the estimated association. Despite challenges related to missing data on infection status, the study interpreted the results with transparency and acknowledged limitations. The consideration of factors such as unknown testing status and testing patterns added insights to the findings and underscored the cautions in conducting similar studies.

We listed the limitations of this study. First, as we explained in our interpretation, there was a differential misclassification bias to primary exposure of interest (SARS-CoV-2 infection), especially for the non-hospitalized group. The proportion of SARS-CoV-2 testing was not similar for the hospitalized and non-hospitalized patients. Moreover, according to our study protocol, IMPACT did not capture parent-reported SARS-CoV-2 infection information based on tests at home or other public health facilities. Hence, the non-hospitalized group had greater instances of unknown exposure status. This differential misclassification bias might have pushed the adjusted OR away from the null overestimating the association. This warranted further research with a more systematic approach to testing (i.e., testing everyone in ED with febrile seizure).

Similarly, there was differential misclassification bias to secondary exposures of interest (RSV or influenza or enterovirus/rhinovirus), especially for the non-hospitalized group. In our data, we could not distinguish non-infected and unknown infection status for RSV, influenza, enterovirus/rhinovirus, as IMPACT provided information only on the presence of infections, not on the absence of infections or untested individuals. The

subjects with no or unknown infection status were more prevalent in the non-hospitalized group. We assumed that hospitalized febrile seizure patients may have undergone additional respiratory tract infection tests after testing negative for SARS-CoV-2, given the pandemic context and variations in testing practices across centers. As a result, the missingness of infection status might be lower in the hospitalized group, potentially causing a differential misclassification bias that could lead to an overestimation of the adjusted OR.

Another limitation of IMPACT data was that it recorded each ED/hospital presentation as a distinct event, making it impossible to differentiate the first event for a subject. Logistic regression required observations to be independent, not from repeated measurements or matched data. In a sensitivity analysis excluding 974 individuals with any seizure history, the results were consistent with the primary and secondary analysis.

Our data had a limitation related to subjects with prior seizure history. The IMPACT data did not distinguish between febrile and afebrile seizures in the absence of a seizure disorder. So, we anticipated that we might have included subjects with afebrile seizure history, which were due to a neurological condition. To address this, we carefully reviewed health condition histories and excluded 250 cases at risk of repeated afebrile seizures based on a prior diagnosis of neurological conditions that would predispose to seizures. We included subjects with a history of afebrile or febrile seizures without diagnosed pre-existing neurological condition because a single afebrile or febrile seizure does not fall under the definition of epilepsy and a child can have such without any neurological condition further in life. We did this not to compromise the generalizability of the study. Also, as mentioned above, when we ran a sensitivity analysis excluding 974

people who reported seizure history of any kind and the result was similar with the primary analysis.

We were unable to adjust for any potential protective effect of COVID-19 vaccination on febrile seizure-associated hospitalization because we only had data on COVID-19 vaccination within the prior 30 days of IMPACT visit for febrile seizure and only 29 subjects (1%) had a history of COVID-19 vaccination. However, a protective effect (if it existed) was not likely to substantially shift the measured adjusted OR for acute SARS-CoV-2 infection towards null, based on public health data from Canada confirming a low uptake of COVID-19 vaccines among children during the study period. For instance, as of January 1, 2023, 52.7% of children aged 5-11 years and 9% of children aged 0-4 years (the majority of our study population) had received at least one dose of the COVID-19 vaccine [69].

Due to sample size limitation (only 8% of the study population had received recent vaccination), we were unable to assess the adverse effects of any vaccination on febrile seizure hospitalization. Our descriptive analysis showed no significant increase in febrile seizure-associated hospitalizations during the vaccination risk window. However, the lack of significance may be due to the limited sample size.

Being a tertiary care hospital network, IMPACT may have mostly captured severe events, as cases presenting to local ED/hospitals would be transferred to an IMPACT center only if deemed severe.

Lastly, a few risk factors of febrile seizure could not be measured in our analysis such as family history of seizure, low zinc and iron level in serum, maternal smoking,

stress[36] as well as certain unknown factors that we were not able to adjust for as IMPACT does not measure those.

5.3. Implications and contributions to knowledge

Limitations in the available data and the differential misclassification bias regarding exposure (limited testing done in non-hospitalized patients), prevented us from confirming or rejecting any association between acute SARS-CoV-2 infection or other viruses and febrile seizure hospitalization. To further explore the relationship between febrile seizures and SARS-CoV-2 infection, we recommend using a more systematic testing approach, for example, testing all patients presenting with febrile seizure. In the future, our study design and identified confounders can be utilized to design epidemiological studies to validate signals (if any) associated with febrile seizures and pediatric COVID-19 vaccines[85-86]

Most importantly, this study was valuable in identifying variations in testing and admission procedures across different IMPACT sites and over time (both in the Omicron and pre-Omicron periods). The high level of variation may indicate inconsistent practices or protocols among the various sites. Addressing these differences is crucial for achieving standardized and equitable care, as variations may arise from discrepancies in admission and testing criteria or disparities in resource allocation. Identifying and rectifying such variations presents opportunities for quality improvement, contributing to better patient care and more reliable data. Policymakers could take these variations into account in healthcare policy formulation to ensure consistent and equitable delivery. The observed variation may impact patient outcomes, emphasizing the need to address disparities in care and testing for optimal healthcare delivery.

Table 1. List of covariates for models and other descriptive characteristics

Variables	Description	Type of variable	Values
Age at presentation **Covariate**	This variable defined the age of a child at the start date of e.g., ED visit or hospitalization at an IMPACT center. It was calculated by the system from the date of birth of a child and the date when hospital admission or ED visit occurred	Categorical variable	1. 0-5 month 2. 6-23 month 3. 24-35 month 4. 36-59 month 5. 60-83 month
Sex **descriptive characteristic**	It was a child's assigned biological sex in electronic health record based on physical characteristics	Categorical variable	1. Male 2. Female
Omicron period **Covariate**	This variable defined whether the subject's ED/hospital visit for febrile seizure occurred during the Omicron period (December 1, 2021, to December 31, 2023) or the pre-Omicron period (August 1, 2021, to November 31, 2021).	Categorical variable	1. Yes 2. No
IMPACT centres	This variable identified the reporting hospital of the events of ED/hospital visit for febrile seizure. From the IMPACT ID in each CRF, the reporting sites were determined. We used numbers to musk the name of the reporting sites.	Categorical variable	1. Site 1 2. Site 2 3. Site 4 4. Site 5 5. Site 6 6. Site 8 7. Site 9 8. Site 10

Variables	Description	Type of variable	Values
Covariate			9. Site 11 10. Site 12 11. Site 13
Seizure history **Covariate**	This variable defined the subject who had history of febrile or afebrile seizure without any pre-existing neurological condition. They were not restricted because a single seizure or febrile seizure do not fall under the definition of epilepsy and a child can have such without any neurological condition further in life. IMPACT data had the limitation to differentiate prior febrile vs afebrile seizures. So, any of both history was captured under this variable.	Categorical variable	1.Yes 3. No 4. Unknown seizure history
Health conditions and immunodeficiency conditions	This variable determined if a subject had health or immunodeficiency conditions i.e., chronic comorbid conditions that might increase the risk of hospitalization due to COVID-19. Below was the list that we considered as Health and Immunodeficiency Condition- Asthma Bronchomalacia	Categorical variable	1.Yes 3. No 4. Unknown

Variables	Description	Type of variable	Values
Covariate	Chronic Lung Disease Chronic Neutropenia Pulmonary hypertension Pulmonary Bronchodysplasia/Bronchodyplasia Reactive Airway Disease Yao syndrome Partial IgA deficiency DiGeorge Syndrome Sickle cell disease High Risk Acute Lymphoblastic Leukemia Functional asplenia due to sickle cell disease		
COVID-19 vaccination **descriptive characteristic**	This variable defined subject who received COVID-19 vaccination 30 days prior to ED visit. This variable was applicable for ≥6 months to <7 years children as only they were eligible for COVID-19 vaccination during the study period	Categorical variable	1. Yes 2. No or unknown
Vaccination in risk window of febrile seizure	This variable indicated subjects' recent vaccination, occurring within 30 days before an ED/hospital visit for febrile seizures. The 'In risk period' category included individuals	Categorical variable	1. In risk period 2. Not in risk period 3. No vaccination

Variables	Description	Type of variable	Values
descriptive characteristic	visiting the ED/hospital for febrile seizures within specific timeframes: 0-2 days after receiving inactive vaccines, 5-13 days after LIVE vaccines, and 0-3 days after COVID-19 vaccines (details in Appendix-3). A 'risk window' is the period following vaccination when vaccine-related adverse events are biologically plausible. 'No vaccination' and 'Vaccination status unknown' levels referred to subjects with no vaccinations and unknown vaccination history in the 30 days prior to their febrile seizure-related ED/hospital visit.		4. Vaccination status unknown
Type of febrile seizure **descriptive characteristic**	We derived this variable from IMPACT data. >1 seizure episode in 24 hours or >15 mins duration per seizure or presence of focal motor manifestations was coded as a complex one. If none of these characteristics were present, the febrile seizure was defined as a simple one.	Categorical variable	1. Complex 2. Simple
Outcome at discharge	The outcome variable referred to a child's condition at discharge from ED/hospital. Patient those	Categorical variable	1.Recovered/Improving

Variables	Description	Type of variable	Values
descriptive characteristic	were fully recovered and or saw improvement while discharge was combined. Those who developed new comorbidity or had their comorbidity worsened were combined. Patient died for febrile seizure or for other reasons were combined.		2. Persistent or new comorbidity 3. Patient died
Highest measured temperature **descriptive characteristic**	It indicated the highest measured temperature (≥38.0°C) in Celcius in the healthcare setting. If the temperature was not measured in the healthcare setting (ED or admission) or if the temperature measured in the healthcare setting was normal, the temperature reported by the caregiver at home as indicated in the chart was recorded.	Continuous variable	
Duration of seizure **descriptive characteristic**	This variable represented the duration of the seizure or duration of the longest seizure in minutes when multiple seizures occurred within a 24-hour period	Continuous variable	
Duration of hospital stay	This variable referred to the total duration of stay at referring hospital and IMPACT hospital in days. If a child was not admitted to any hospital (ED visit only),	Continuous variable	

Variables	Description	Type of variable	Values
descriptive characteristic	the duration was recorded as zero days. So, this variable was applicable for hospitalized patients only.		
Intensive Care Unit (ICU) admission required **descriptive characteristic**	It referred to if hospitalized patients needed ICU admission during the stay	Categorical variable	1. Yes 2. No

Table 2: Demographic and clinical characteristics of hospitalized & non-hospitalized patients with febrile seizure (N=3306)

Variables	Subgroups	Hospitalized		Non-hospitalized [a]		Total		p-value
		n=595		n=2711				
		n (%)		n (%)				
Age at presentation	0-5 m	32	5.38%	22	0.81%	54	2%	<0.001
	6-23 m	352	59.16%	1354	49.94%	1706	52%	
	24-35 m	100	16.81%	669	24.68%	769	23%	
	36-59 m	88	14.79%	532	19.62%	620	19%	
	60-83 m	23	3.87%	134	4.94%	157	5%	
Sex	Male	326	54.79%	1584	58.43%	1910	58%	0.1
	Female	269	45.21%	1127	41.57%	1396	42%	
IMPACT centres	Site 01	24	4.03%	178	6.57%	202	6%	<0.001
	Site 02	122	20.50%	174	6.42%	296	9%	
	Site 04	77	12.94%	98	3.61%	175	5%	
	Site 05	51	8.57%	138	5.09%	189	6%	
	Site 06	47	7.90%	334	12.32%	381	12%	
	Site 08	16	2.69%	160	5.90%	176	5%	
	Site 09	15	2.52%	73	2.69%	88	3%	
	Site 10	41	6.89%	562	20.73%	603	18%	
	Site 11	108	18.15%	552	20.36%	660	20%	
	Site 12	38	6.39%	151	5.57%	189	6%	
	Site 13	56	9.41%	291	10.73%	347	10%	
Acute infection type	Acute SARS-COV-2	75	12.61%	79	2.91%	154	5%	<0.001
	Influenza	36	6.05%	34	1.25%	70	2%	

Variables	Subgroups	Hospitalized		Non-hospitalized [a]		Total		p-value
		n=595		n=2711				
		n (%)		n (%)				
	RSV	15	2.52%	15	0.55%	30	1%	
	Enterovirus/Rhinovirus	53	8.91%	18	0.66%	71	2%	
	Adenovirus	26	4.37%	7	0.26%	33	1%	
	Other infection	46	7.73%	38	1.40%	84	3%	
	>1 concurrent infections with SARS-CoV-2	8	1.34%	7	0.26%	15	0%	
	>1 concurrent infections without SARS-CoV-2	34	5.71%	16	0.59%	50	2%	
	No detected infection	242	40.67%	514	18.96%	756	23%	
	Unknown infection	60	10.08%	1983	73.15%	2043	62%	
Type of Febrile Seizure [b]	Complex	483	81.18%	739	27.26%	1222	37%	<0.001
	Simple	112	18.82%	1972	72.74%	2084	63%	
Outcome at discharge	Recovered/Improving	568	95.46%	2705	99.78%	3273	99%	<0.001
	Persistent or new comorbidity	27	4.54%	5	0.18%	32	1%	
	Patient died	0	0.00%	<5	0.18%	<5	NA	
Highest measured temperature (°Celsius; mean)		39.16	N/A	39.08	N/A	N/A	NA	0.03 (non-parametric)
Duration of seizure [c] (mins; median, IQR)		5, IQR:2, 15	N/A	2, IQR:1,5	N/A	N/A	NA	<0.001 (non-parametric)
Duration of hospital stay (days, median, IQR)		2, IQR: 1, 3	N/A	N/A	N/A	N/A	NA	N/A

Variables	Subgroups		Hospitalized n=595 n (%)		Non-hospitalized [a] n=2711 n (%)		Total		p-value
ICU admission required (hospitalized cases only)		Yes	80	13.45%	N/A	N/A	N/A	NA	<0.001
		No	515	86.55%	N/A	N/A	N/A	NA	
Omicron period [d]	Yes		482	81.01%	2255	83.18%	2737	83%	0.2
	No		113	18.99%	456	16.82%	569	17%	
Seizure history [e]	Yes		192	32.27%	780	28.77%	972	29%	0.01
	No		397-402	67-68%	1860	68.61%	2259	68%	
	Unknown seizure history		<5	<0.84%	71	2.62%	75	2%	
Health conditions [f]	Yes		23	3.87%	58	2.14%	81	2%	<0.001
	No		572	95.80%	2624	96.76%	3196	97%	
	Unknown		0	0.00%	29	1.07%	29	1%	
Immunodeficiency	Yes		<5	<0.84%	<5	<0.18%	7	<1%	<0.001
	No		590-595	99-99.33%	2671	98.52%	3262	99%	
	Unknown		0	0%	37	1.36%	37	1%	
COVID-19 vaccination [g]	Yes		<5	0.84%	24-29	0.96-1%	29	1%	0.28
	No or unknown		590-595	99-99.2%	2685	99.04%	3277	99%	
Febrile seizure in vaccination risk window	In risk period [h]		28	4.71%	88	3.25%	116	4%	0.15
	Not in risk period		31	5.21%	122	4.50%	153	5%	
	No vaccination		450	75.63%	1720	63.45%	2170	66%	
	Vaccination status unknown		86	14.45%	781	28.81%	867	26%	

[a] Discharged from ED/short stay/holding unit within 24h

[b] Simple febrile seizure is generalized, lasts <15 minutes, and does not recur within 24 h, while complex febrile seizures is defined by one or more of: focal features, duration ≥15 min, >1 seizure in 24 hours.

[c] Duration of longest seizure if >1 seizure in 24 hours

[d] Omicron period is when ED or hospital visit is between Dec'21-Dec'22 and Pre-Omicron period is between Aug'21-Nov'21

[e] Both febrile and afebrile seizure history

[f] Comorbidities that are risk factors for COVID-19 related hospitalization

[g] Eligible children aged ≥6 months to <7 years

[h] Post-vaccination ED visit or hospitalization for febrile seizure within 0-2 days for inactive vaccines, 5-13 days for live vaccines, and 0-3 days for COVID-19 vaccines

Table 3: Difference between the known and unknown microbiological testing status groups for acute SARS-CoV-2 infection

Variables	Subgroups	Known SARS-CoV-2 testing status [a]		Unknown SARS-CoV-2 testing status [a]		Total	p-value
		n=1214		n=2092			
		n (%)		n (%)			
Hospitalization status	Hospitalized	520	87%	75	13%	**595**	<0.001
	Non-hospitalized [b]	694	26%	2017	74%	**2711**	
Omicron period	Yes	830	30%	1907	70%	**2737**	<0.001
	No	384	67%	185	33%	**569**	
Type of Febrile Seizure [c]	Complex	629	51%	593	49%	**1222**	<0.001
	Simple	585	28%	1499	72%	**2084**	
Highest measured temperature (°Celsius)		39.15	N/A	39.06	N/A	**N/A**	0.0008
IMPACT sites	Site 01	61	30%	141	70%	**202**	<0.001
	Site 02	255	86%	41	14%	**296**	
	Site 04	88	50%	87	50%	**175**	
	Site 05	87	46%	102	54%	**189**	
	Site 06	97	25%	284	75%	**381**	
	Site 08	56	32%	120	68%	**176**	
	Site 09	26	30%	62	70%	**88**	
	Site 10	167	28%	436	72%	**603**	
	Site 11	202	31%	458	69%	**660**	
	Site 12	62	33%	127	67%	**189**	
	Site 13	113	33%	234	68%	**347**	

[a] SARS-CoV-2 infection status known if RT-PCR or rapid antigen testing done at IMPACT ED or during hospitalization for febrile seizure and unknown if done elsewhere or during a different period

[b] Discharged from ED/short stay/holding unit within 24h

[c] Simple febrile seizure is generalized, lasts <15 minutes, and does not recur within 24 h, while complex febrile seizures is defined by one or more of: focal features, duration ≥15 min, >1 seizure in 24 hours.

Table 4: Prevalence of hospitalization and SARS-CoV-2 testing of febrile seizure cases by Omicron/pre-Omicron period and IMPACT centers

IMPACT Centre	Febrile Seizure Cases	Overall	Hospitalized		Non-hospitalized	
		SARS-CoV-2 Tested (%)	Febrile Seizure Cases (%)	SARS-CoV-2 Tested (%)	Febrile Seizure Cases (%)	Tested (%)
Overall						
Site 01	**202**	61 (30%)	24 (12%)	19 (79%)	178 (88%)	42 (24%)
Site 02	**296**	255 (86%)	122 (41%)	115 (94%)	174 (59%)	140 (80%)
Site 04	**175**	88 (50%)	77 (44%)	71 (92%)	98 (56%)	17 (17%)
Site 05	**189**	87 (46%)	51 (27%)	47 (92%)	138 (73%)	40 (29%)
Site 06	**381**	97 (25%)	47 (12%)	45 (96%)	334 (88%)	52 (16%)
Site 08	**176**	56 (32%)	16 (9%)	14 (88%)	160 (91%)	42 (26%)
Site 09	**88**	26 (30%)	15 (17%)	10 (67%)	73 (83%)	16 (22%)
Site 10	**603**	167 (28%)	41 (7%)	40 (98%)	562 (93%)	127 (23%)
Site 11	**660**	202 (31%)	108 (16%)	88 (81%)	552 (84%)	114 (21%)
Site 12	**189**	62 (33%)	38 (20%)	29 (76%)	151 (80%)	33 (22%)
Site 13	**347**	113 (33%)	56 (16%)	42 (75%)	291 (84%)	71 (24%)
all sites	**3306**	**1214 (37%)**	**595 (18%)**	**520 (87%)**	**2711 (82%)**	**694 (26%)**
Pre-Omicron period						
Site 01	**28**	26 (93%)	5 (18%)	5 (100%)	23 (82%)	21 (91%)
Site 02	**76**	70 (92%)	28 (37%)	27 (96%)	48 (63%)	43 (90%)

IMPACT Centre	Febrile Seizure Cases	Overall	Hospitalized		Non-hospitalized	
		SARS-CoV-2 Tested (%)	Febrile Seizure Cases (%)	SARS-CoV-2 Tested (%)	Febrile Seizure Cases (%)	Tested (%)
Site 04	**23**	18 (78%)	14 (61%)	13 (93%)	9 (39%)	5 (56%)
Site 05	**22**	16 (73%)	6 (27%)	6 (100%)	16 (73%)	10 (63%)
Site 06	**57**	35 (61%)	8 (14%)	8 (100%)	49 (86%)	27 (55%)
Site 08	**23**	14 (61%)	1 (4%)	1 (100%)	22 (96%)	13 (59%)
Site 09	**16**	3 (19%)	3 (19%)	2 (67%)	13 (81%)	1 (8%)
Site 10	**138**	66 (48%)	6 (4%)	6 (100%)	132 (96%)	60 (45%)
Site 11	**124**	85 (69%)	27 (22%)	22 (81%)	97 (78%)	63 (65%)
Site 12	**17**	15 (88%)	5 (29%)	5 (100%)	12 (71%)	10 (83%)
Site 13	**45**	36 (80%)	10 (22%)	10 (100%)	35 (78%)	26 (74%)
all sites	**569**	**384 (67%)**	**113 (20%)**	**105 (93%)**	**456 (80%)**	**279 (61%)**
Omicron period						
Site 01	174	35 (20%)	19 (11%)	14 (74%)	155 (89%)	21 (14%)
Site 02	220	185 (84%)	94 (43%)	88 (94%)	126 (57%)	97 (77%)
Site 04	152	70 (46%)	63 (41%)	58 (92%)	89 (59%)	12 (13%)
Site 05	167	71 (43%)	45 (27%)	41 (91%)	122 (73%)	30 (25%)
Site 06	324	62 (19%)	39 (12%)	37 (95%)	285 (88%)	25 (9%)
Site 08	153	42 (27%)	15 (10%)	13 (87%)	138 (90%)	29 (21%)
Site 09	72	23 (32%)	12 (17%)	8 (67%)	60 (83%)	15 (25%)

IMPACT Centre	Febrile Seizure Cases	Overall		Hospitalized			Non-hospitalized	
		SARS-CoV-2 Tested (%)		Febrile Seizure Cases (%)	SARS-CoV-2 Tested (%)		Febrile Seizure Cases (%)	Tested (%)
Site 10	465	101 (22%)		35 (8%)	34 (97%)		430 (92%)	67 (16%)
Site 11	536	117 (22%)		81 (15%)	66 (81%)		455 (85%)	51 (11%)
Site 12	172	47 (27%)		33 (19%)	24 (73%)		139 (81%)	23 (17%)
Site 13	302	77 (25%)		46 (15%)	32 (70%)		256 (85%)	45 (18%)
all sites	**2737**	**830 (30%)**		**482 (18%)**	**415 (86%)**		**2255 (82%)**	**415 (18%)**

Table 5: IMPACT testing pattern for other infections in pre-Omicron and Omicron periods and among hospitalized and non-hospitalized patients

IMPACT centre	Total febrile seizure cases	Positive Influenza/RSV		Positive Enterovirus/Rhinovirus/Adenovirus	
		pre-Omicron	Omicron	pre-Omicron	Omicron
		Yes	yes	yes	yes
1	n=202	0	8 (4%)	0	0
2	n=296	5 (2%)	14 (5%)	7 (2%)	28 (9%)
4	n=175	0	11 (6%)	1 (1%)	4 (2%)
5	n=189	0	13 (7%)	1 (1%)	26 (14%)
6	n=381	1 (0.26%)	11 (3%)	7 (2%)	7 (2%)
8	n=176	1 (1%)	13 (7%)	2 (1%)	9 (5%)
9	n=88	0	6 (7%)	2 (2%)	9 (10%)
10	n=603	4 (1%)	21 (3%)	0	8 (1%)
11	n=660	1 (0.15%)	0	3 (0.45%)	22 (3%)
12	n=189	0	6 (3%)	2 (1%)	13 (7%)
13	n=347	2 (1%)	3 (1%)	4 (1%)	15 (4%)
IMPACT centre	**Total febrile seizure cases**	**Positive Influenza/RSV**		**Positive Enterovirus/Rhinovirus/Adenovirus**	
		Hospitalized	**Non-hospitalized**	**Hospitalized**	**Non-hospitalized**
1	n=202	6 (3%)	2 (1%)	0 (0%)	0 (0%)
2	n=296	18 (6%)	1 (0%)	32 (11%)	3 (1%)
4	n=175	10 (6%)	1 (1%)	5 (3%)	0 (0%)
5	n=189	9 (5%)	4 (2%)	23 (12%)	4 (2%)
6	n=381	4 (1%)	8 (2%)	7 (2%)	7 (2%)
8	n=176	5 (3%)	9 (5%)	5 (3%)	6 (3%)
9	n=88	2 (2%)	4 (5%)	7 (8%)	4 (5%)
10	n=603	3 (0%)	22 (4%)	5 (1%)	3 (0%)
11	n=660	7 (1%)	4 (1%)	20 (3%)	5 (1%)
12	n=189	3 (2%)	3 (2%)	11 (6%)	4 (2%)
13	n=347	2 (1%)	3 (1%)	9 (3%)	10 (3%)

Table 6: Mixed-effect logistic regression models incorporating definition 1 and definition 2 of exposure variable, along with covariates

No.	Model type	Variables adjusted for	adjusted OR [P value (95% CI)]		Reference category	AIC/ BIC[a]	AUC[b]
Model 1	Model selection: Incorporating multilevel categorical exposure variable, (n=3306)	Random effect of IMPACT centres (ICC[c]=0.11)	Acute SARS-CoV-2	2.09, [p <0.001, (1.44, 3.04)]	who tested negative for an acute SARS-CoV-2 infection and were not detected or tested for other infection during ED/hospital visit	2087/ 2154	88%
			Influenza	3.07, [p <0.001, (1.80, 5.22)]			
			RSV	2.43, [p =0.03, (1.12, 5.32)]			
			Enterovirus/Rhino virus	6.74, [p<0.001, (3.79, 12.00)]			
			Adenovirus	9.76, [p<0.001, (4.06, 23.42)]			
			Other infection	3.10, [p<0.001, (1.91, 5.03)]			
			SARS-CoV-2 with other infection	2.66, [p=0.07, (0.91, 7.76)]			
			>1 infection without SARS-CoV-2	4.68, [p<0.001, (2.46, 8.89)]			
			Unknown infection	0.07, [p<0.001, (0.05, 0.10)]			
Model 2	Model selection: Incorporating multilevel categorical exposure	Random effect of IMPACT centres & Omicron	Acute SARS-CoV-2	2.01, [p<0.001, (1.36, 2.98)]	who tested negative for an acute SARS-CoV-2 infection and were not detected or tested for other	2088/ 2161	88%
			Influenza	2.98, [p<0.001, (1.73, 5.13)]			
			RSV	2.40, [p =0.03, (1.10, 5.26)]			
			Enterovirus/Rhino virus	6.66, [p<0.001, (3.73, 11.89)]			

No.	Model type	Variables adjusted for	adjusted OR [P value (95% CI)]		Reference category	AIC/ BIC[a]	AUC[b]
	variable, (n=3306)	period (ICC: 0.11)	Adenovirus only	9.54, [p<0.001, (3.96, 23.02)]	infection during ED/hospital visit		
			Other infection	3.06, [p<0.001, (1.88, 4.98)]			
			SARS-CoV-2 with other infection	2.54, [p=0.09, (0.86, 7.51)]			
			>1 infection without SARS-CoV-2	4.64, [p<0.001, (2.44, 8.85)]			
			Unknown infection	0.07, [p<0.001, (0.05, 0.10)]			
Model 3	Final Model: Incorporating multilevel categorical exposure variable, (n=3306)	Fixed-effect of age at presentation, seizure history, Omicron period, as well as random-effect of IMPACT centers (ICC=0.11)	Acute SARS-CoV-2	1.67, [p=0.01, (1.12, 2.48)]	who tested negative for an acute SARS-CoV-2 infection and were not detected or tested for other infection during ED/hospital visit	2028/ 2138	89%
			Influenza	2.71, [p <0.001, (1.55, 4.74)]			
			RSV	2.53, [p=0.02, (1.13, 5.66)]			
			Enterovirus/Rhinovirus	6.22, [p<0.001, (3.45, 11.19)]			
			Adenovirus	8.89, [p<0.001, (3.62, 21.86)]			
			Other infection	2.52, [p<0.001, (1.53, 4.13)]			
			SARS-CoV-2 with other infection	2.05, [p=0.20, (0.70, 6.01)]			
			>1 infection without SARS-CoV-2	3.88, [p<0.001, (2.02, 7.45)]			

No.	Model type	Variables adjusted for	adjusted OR [P value (95% CI)]		Reference category	AIC/ BIC[a]	AUC[b]
			Unknown infection	0.06, [p<0.001, (0.04, 0.08)]			
Model 4	Model selection: Incorporating binary exposure variable, (n=1212)	Random effect of IMPACT centres (ICC=0.11)	Acute SARS-CoV-2 with/without other infection	1.26, [p=0.183, (0.90, 1.78)]	Subjects who tested negative for an acute SARS-CoV-2 infection with or without other infections	1596/ 1611	65%
Model 5	Final Model: Incorporating binary exposure variable, (n=1212)	Fixed-effect of age at presentation, seizure history, Omicron period, other concurrent infection as well as random-effect of IMPACT centers (ICC=0.12)	Acute SARS-CoV-2 with/without other infection	1.33, [p=0.136, (0.91, 1.95)]	Subjects who tested negative for an acute SARS-CoV-2 infection with or without other infections	1429/ 1495	76%

[a] Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC)
[b] Area under receiver operating curve (AUC)
[c] Intraclass Correlation Coefficient (ICC)

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89. IMPACT metadata on testing 2021 & 2022

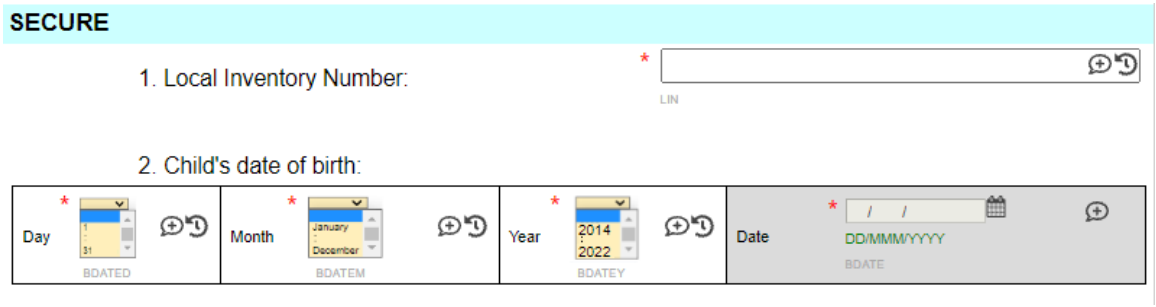
APPENDIX-1

eCRF_Febrile_Seizures_V2_March_18_2022

CANADIAN IMMUNIZATION MONITORING PROGRAM, ACTIVE

STUDY CODE: IMPACT FEBRILE SEIZURES

Note: The data on the SECURE form will not be released from the Data Centre.



ADMINISTRATION AND DEMOGRAPHICS

Event Identifier (IMPACT Number):

IMPACT

Sex: *

- ○ Male
- ○ Female

SEX

Ethnicity (tick all that apply):

- ☐ Caucasian (North American, European, Australian)
- ☐ Asian

Specify Asian group (tick all that apply):

- ☐ Chinese
- ☐ Filipino
- ☐ Japanese
- ☐ Korean
- ☐ Southeast Asian (eg. Vietnamese, Cambodian, Laotian)
- ☐ South Asian (eg. East Indian, Pakistani, Sri Lankan)
- ☐ West Asian (eg. Iranian, Afghan)
- ☐ Unknown

- ☐ Middle Eastern/Arabic
- ☐ Black (African, African-American)
- ☐ Latin, Central and South American
- ☐ Aboriginal

Specify Aboriginal group (tick all that apply):

- ☐ First Nations
- ☐ Métis
- ☐ Inuit
- ☐ Unknown

- ☐ Other, specify

Specify other ethnicity:

ETHNIC_DESC

- ☐ Unknown

Highest level of health care required: *

- ○ Inpatient care
- ○ Outpatient care
- ○ Short stay/holding unit only
- ○ Emergency department only
- ○ Autopsy only

CARELEVEL

Date of event (start date of Highest Level of Care above):

Day * (1 - 31) ADATED | Month * (January - December) ADATEM | Year * (2021 - 2022) AVAL1 BY | Date * __/___/____ DD/MMM/YYYY ADATE

Age:

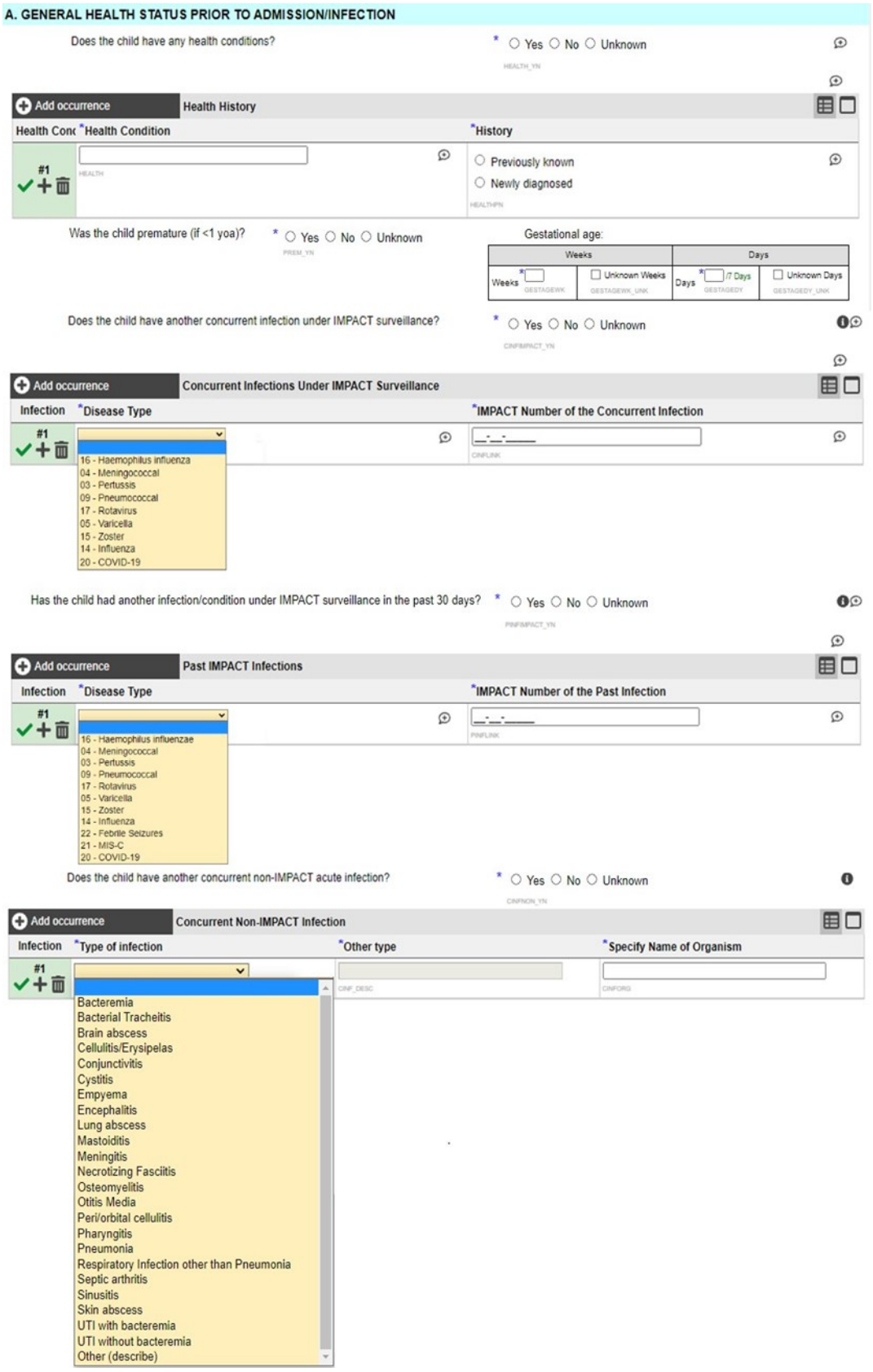
Years AGEY | Months AGEM

FSA: *

FSA

Reporting Province:

PROVINCE


A. GENERAL HEALTH STATUS PRIOR TO ADMISSION/INFECTION
Does the child have any health conditions?
* Yes No Unknown
HEALTH_YN
Add occurrence
Health History
Health Cond
*Health Condition
*History
#1
HEALTH
Previously known
Newly diagnosed
HEALTHPN
Was the child premature (if <1 yoa)?
* Yes No Unknown
PREM_YN
Gestational age:
Weeks
Days
*Weeks
GESTAGEWK
Unknown Weeks
GESTAGEWK_UNK
*Days
/7 Days
GESTAGEDY
Unknown Days
GESTAGEDY_UNK
Does the child have another concurrent infection under IMPACT surveillance?
* Yes No Unknown
CINFIMPACT_YN
Add occurrence
Concurrent Infections Under IMPACT Surveillance
Infection
*Disease Type
*IMPACT Number of the Concurrent Infection
#1
__-__-____
CINFLINK
16 - Haemophilus influenza
04 - Meningococcal
03 - Pertussis
09 - Pneumococcal
17 - Rotavirus
05 - Varicella
15 - Zoster
14 - Influenza
20 - COVID-19
Has the child had another infection/condition under IMPACT surveillance in the past 30 days?
* Yes No Unknown
PINFIMPACT_YN
Add occurrence
Past IMPACT Infections
Infection
*Disease Type
*IMPACT Number of the Past Infection
#1
__-__-____
PINFLINK
16 - Haemophilus influenzae
04 - Meningococcal
03 - Pertussis
09 - Pneumococcal
17 - Rotavirus
05 - Varicella
15 - Zoster
14 - Influenza
22 - Febrile Seizures
21 - MIS-C
20 - COVID-19
Does the child have another concurrent non-IMPACT acute infection?
* Yes No Unknown
CINFNON_YN
Add occurrence
Concurrent Non-IMPACT Infection
Infection
*Type of infection
*Other type
*Specify Name of Organism
#1
CINF_DESC
CINFORG
Bacteremia
Bacterial Tracheitis
Brain abscess
Cellulitis/Erysipelas
Conjunctivitis
Cystitis
Empyema
Encephalitis
Lung abscess
Mastoiditis
Meningitis
Necrotizing Fasciitis
Osteomyelitis
Otitis Media
Peri/orbital cellulitis
Pharyngitis
Pneumonia
Respiratory Infection other than Pneumonia
Septic arthritis
Sinusitis
Skin abscess
UTI with bacteremia
UTI without bacteremia
Other (describe)

B. RISK FACTORS

At the time of the infection does the child have an immunodeficiency disorder? * ○ Yes ○ No ○ Unknown

IMUNDEF_YN

Add occurrence | Immunodeficiencies

Immune Co	*Specify the Immunodeficiency Disorder	*Diagnosis/Description	*History
#1		IMUNDEF_DESC	○ Previously known ○ Newly diagnosed (IMUNDEFPN)

Primary (congenital)
- Severe combined immunodeficiency (SCID)
- B-cell (Hypogammaglobulinemia)
- T-cell
- Asplenia from birth (syndromic)
- Complement deficiency
- Neutrophil deficiency

Secondary (acquired)
- HIV/AIDS
- Neoplasm with treatment
- Neoplasm without treatment
- Stem cell transplant
- Asplenia from surgical removal, or functional
- Solid organ transplant on chronic anti-rejection meds
- Neutrophil deficiency (acquired)

Other
- Other

Has the child had a history of previous seizure? * ○ Yes ○ No ○ Unknown

SEIZURE_YN

If yes, check all that apply:

☐ Afebrile Seizure

☐ Febrile Seizure

Indicate the date of the last known Febrile Seizure:

Day * (1 : 31 Unknown) FEBSZDATED | Month * (January : December Unknown) FEBSZDATEM | Year * (2014 : 2022 Unknown) FEBSZDATEY | Date * __/__/____ DD/MMM/YYYY FEBSZDATE

C. EVIDENCE FOR DIAGNOSIS

Did the child have a previous positive test for COVID-19 infection prior to this admission or ED visit? * ○ Yes ○ No ○ Unknown

COVID_PRIOR_YN

Date of prior positive test:

Day * (1 : 31) TESTDATED_PRIOR | Month * (January : December) TESTDATEM_PRIOR | Year * (2021 : 2022) TESTDATEY_PRIOR | Date * __/__/____ DD/MMM/YYYY TESTDATE_PRIOR

Did the child have a test for COVID-19 infection during this admission or ED visit? * ○ Yes ○ No ○ Unknown

COVID_YN

Type of test (tick all that apply):

☐ Molecular testing (PCR, NAT, RVP)

☐ Rapid antigen test

☐ Other, specify:

Date of test:

Day * (1 : 31) TESTDATED | Month * (January : December) TESTDATEM | Year * (2021 : 2022) TESTDATEY | Date * __/__/____ DD/MMM/YYYY TESTDATE

Result of Test? * ○ Positive ○ Negative ○ Indeterminate

TESTRESULT

D. MANIFESTATION OF CONDITION

Manifestations of current condition (tick all that apply):

☐ Fever

Highest measured temperature: * [ ] °C
HIGHTEMP

☐ Seizure

Duration of seizure: * [ ] minutes
SZDUR

Number of seizure within 24 hours: * [ ] # of seizures
SZNUM

Was there a witnessed sudden loss of consciousness? * ○ Yes ○ No ○ Unknown
WITLOSSCON_YN

Did the child experience loss of consciousness? * ○ Yes ○ No ○ Unknown
LOSSCON_YN

Motor manifestations? * ○ Yes ○ No
MOTOR_YN

If yes, please indicate: * ○ Focal motor manifestations
○ Generalized motor manifestations
FOCALGENER

Select the type of motor manifestation: * [ ]
- Tonic
- Clonic
- Tonic-clonic
- Atonic
- Myoclonic
- Other, specify
- Unknown

D. MANIFESTATION OF INFECTION

Were there any suspected secondary bacterial infections? * ○ Yes ○ No
INF_YN

Add occurrence | Bacterial infections

Infection	*Type of infection	*Other type	*Lab confirmed	*Name of organism	*Other organism	*Specimen source	*Location
#1	INF	INF_DESC	☐ Yes ☐ No INFCONF_YN	INFORG	INFORG_DESC	INFSPEC	INFLOC

Type of infection:
- Bacteremia
- Bacterial Tracheitis
- Cellulitis/Erysipelas
- Conjunctivitis
- Empyema
- Mastoiditis
- Meningitis
- Necrotizing Fasciitis
- Osteomyelitis
- Otitis Media
- Peri/orbital cellulitis
- Pneumonia
- Septic arthritis
- Sinusitis
- UTI with bacteremia
- Other (describe)

Name of organism:
- Bordetella Pertussis
- Coagulase negative staphylococcus/staphylococcus epidermidus
- Enterobacter spp
- Enterococcus spp
- Escherichia coli
- Group A Streptococcus
- Haemophilus influenzae
- Klebsiella pneumoniae
- Methicillin Resistant Staphylococcus aureus
- Methicillin Susceptible Staphylococcus aureus
- Neisseria meningitidis
- Pseudomonas aeruginosa
- Streptococcus pneumoniae
- Other

E. LEVEL OF CARE REQUIRED FOR THIS INFECTION

Duration of referring hospital stay: * ☐ days REFERDUR ☐ Unknown REFERDUR_UNK ☐ N/A REFERDUR_NA

Duration of IMPACT hospital stay: * ☐ days IMPACTDUR ☐ Unknown IMPACTDUR_UNK ☐ N/A IMPACTDUR_NA

Was child admitted to the referring or IMPACT hospital's ICU? * ○ Yes ○ No ○ Unknown ○ N/A ICU_YN

Duration of stay in ICU at referring hospital: * ☐ days REFICUDUR ☐ Unknown REFICUDUR_UNK

Duration of stay in ICU at IMPACT hospital: * ☐ days IMPACTICUDUR ☐ Unknown IMPACTICUDUR_UNK

Was ventilatory support required? * ○ Yes ○ No ○ Unknown ○ N/A VENT_YN

Highest level required: *
- ○ Mechanical ventilation
- ○ ECMO
- ○ CPAP
- ○ Low and high flow O2

VENTLEVEL

Duration of ventilation: * ☐ days VENTDUR

F. OUTCOME

Outcome: * ○ Patient survived this condition episode ○ Patient died

OUTCOME

Select condition at outcome: *
- ○ Patient recovered, or improving with no residual morbidity expected (including underlying co-morbidity that is not worse)
- ○ Patient has persistent new morbidity after condition
- ○ Patient has persistent worsening of pre-existing morbidity after condition
- ○ Died of reported condition or morbidity
- ○ Died of other cause
- ○ Unknown

OUTCOND

Select disposition at outcome: *

Discharged
Patient was transferred
Treated as outpatient only
Remained in hospital

Outcome date:

Day	Month	Year	Date *	Tick box if admitted and discharged the same day:
1 : 31 Unknown	January : December Unknown	2021 : 2022 Unknown	__-___-____ DD-MMM-YYYY	☐
ODATED	ODATEM	ODATEY	ODATE	SAMEDAY_TICK

Sequelae/morbidity at outcome (tick all that apply):

☐ Major neurological sequelae

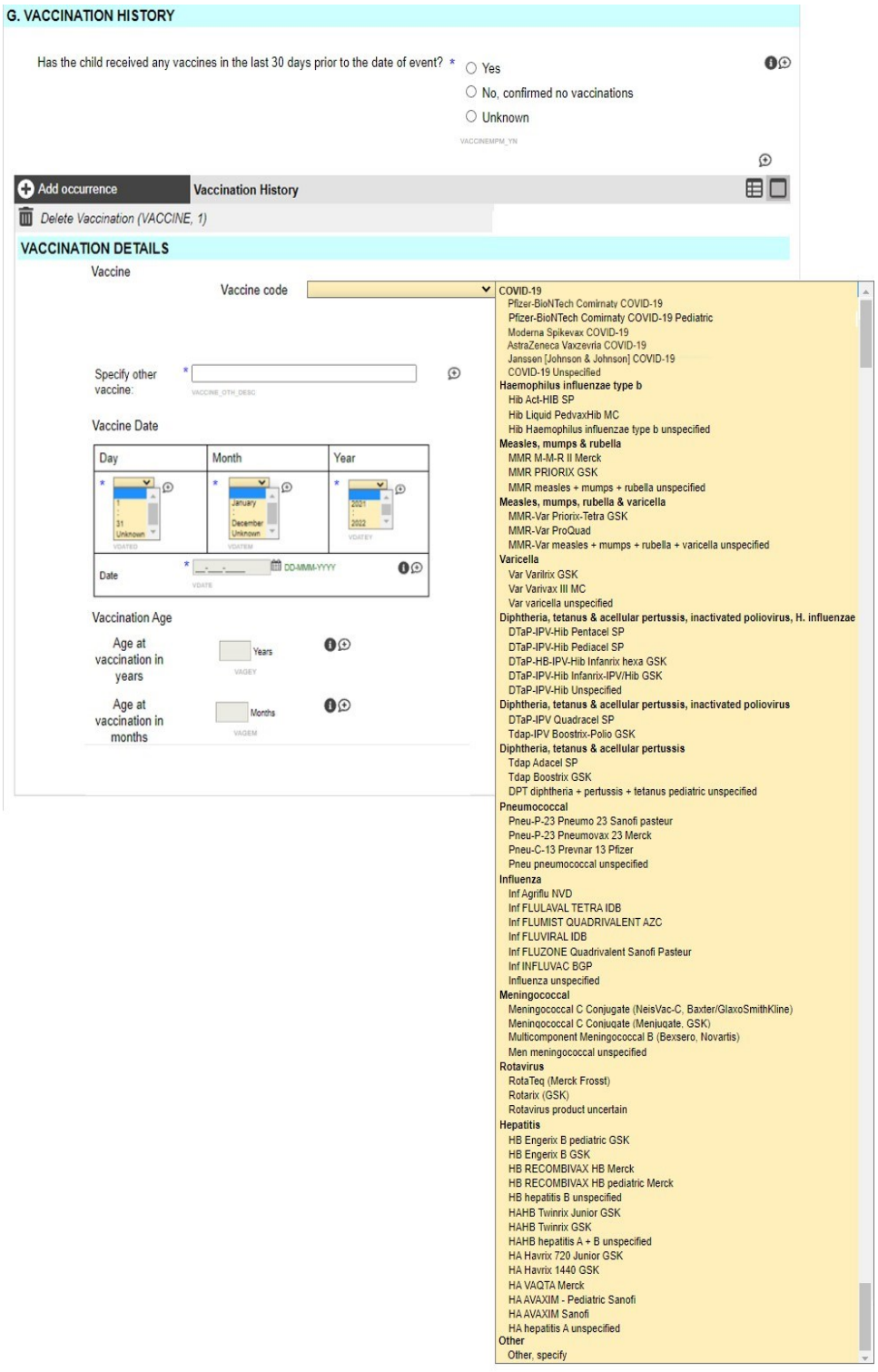
Specify: *

SEQ_NEURO_DESC

☐ Seizures

On discharge, is child on anticonvulsants? * ○ Yes ○ No ○ Unknown

SZMEDS_YN


G. VACCINATION HISTORY
Has the child received any vaccines in the last 30 days prior to the date of event? *
Yes
No, confirmed no vaccinations
Unknown
VACCINEMPM_YN
Add occurrence
Vaccination History
Delete Vaccination (VACCINE, 1)
VACCINATION DETAILS
Vaccine
Vaccine code
Specify other vaccine:
VACCINE_OTH_DESC
Vaccine Date
Day
Month
Year
Unknown
January
December
Unknown
2021
2022
VDATED
VDATEM
VDATEY
Date
DD-MMM-YYYY
VDATE
Vaccination Age
Age at vaccination in years
Years
VAGEY
Age at vaccination in months
Months
VAGEM
COVID-19
Pfizer-BioNTech Comirnaty COVID-19
Pfizer-BioNTech Comirnaty COVID-19 Pediatric
Moderna Spikevax COVID-19
AstraZeneca Vaxzevria COVID-19
Janssen [Johnson & Johnson] COVID-19
COVID-19 Unspecified
Haemophilus influenzae type b
Hib Act-HIB SP
Hib Liquid PedvaxHib MC
Hib Haemophilus influenzae type b unspecified
Measles, mumps & rubella
MMR M-M-R II Merck
MMR PRIORIX GSK
MMR measles + mumps + rubella unspecified
Measles, mumps, rubella & varicella
MMR-Var Priorix-Tetra GSK
MMR-Var ProQuad
MMR-Var measles + mumps + rubella + varicella unspecified
Varicella
Var Varilrix GSK
Var Varivax III MC
Var varicella unspecified
Diphtheria, tetanus & acellular pertussis, inactivated poliovirus, H. influenzae
DTaP-IPV-Hib Pentacel SP
DTaP-IPV-Hib Pediacel SP
DTaP-HB-IPV-Hib Infanrix hexa GSK
DTaP-IPV-Hib Infanrix-IPV/Hib GSK
DTaP-IPV-Hib Unspecified
Diphtheria, tetanus & acellular pertussis, inactivated poliovirus
DTaP-IPV Quadracel SP
Tdap-IPV Boostrix-Polio GSK
Diphtheria, tetanus & acellular pertussis
Tdap Adacel SP
Tdap Boostrix GSK
DPT diphtheria + pertussis + tetanus pediatric unspecified
Pneumococcal
Pneu-P-23 Pneumo 23 Sanofi pasteur
Pneu-P-23 Pneumovax 23 Merck
Pneu-C-13 Prevnar 13 Pfizer
Pneu pneumococcal unspecified
Influenza
Inf Agriflu NVD
Inf FLULAVAL TETRA IDB
Inf FLUMIST QUADRIVALENT AZC
Inf FLUVIRAL IDB
Inf FLUZONE Quadrivalent Sanofi Pasteur
Inf INFLUVAC BGP
Influenza unspecified
Meningococcal
Meningococcal C Conjugate (NeisVac-C, Baxter/GlaxoSmithKline)
Meningococcal C Conjugate (Menjugate, GSK)
Multicomponent Meningococcal B (Bexsero, Novartis)
Men meningococcal unspecified
Rotavirus
RotaTeq (Merck Frosst)
Rotarix (GSK)
Rotavirus product uncertain
Hepatitis
HB Engerix B pediatric GSK
HB Engerix B GSK
HB RECOMBIVAX HB Merck
HB RECOMBIVAX HB pediatric Merck
HB hepatitis B unspecified
HAHB Twinrix Junior GSK
HAHB Twinrix GSK
HAHB hepatitis A + B unspecified
HA Havrix 720 Junior GSK
HA Havrix 1440 GSK
HA VAQTA Merck
HA AVAXIM - Pediatric Sanofi
HA AVAXIM Sanofi
HA hepatitis A unspecified
Other
Other, specify

APPENDIX 2

Table 7: Sensitivity Analyses Models of Acute Infection Type on Hospitalization

No.	Scenarios	adjusted OR [P value (95% CI)]			Reference category	AIC/ BIC[a]	AUC[b]
Scenario 1	Excluding unknown infection status subjects (n=1263)	Acute SARS-CoV-2		1.56, [p =0.03, (1.05, 2.32)]	Subjects who tested negative for an acute SARS-CoV-2 infection and had no other detected infection during ED/hospital visit	1515/ 1602	75%
		Influenza		2.53, [p <0.001, (1.45, 4.73)]			
		RSV		2.30, [p =0.04, (1.03, 5.17)]			
		Enterovirus/Rhinovirus		6.47, [p<0.001, (3.58, 11.71)]			
		Adenovirus		8.51, [p<0.001, (3.48, 20.82)]			
		Other infection		2.37, [p<0.001, (1.45, 3.90)]			
		>1 infection withSARS-CoV-2		1.90, [p=0.20, (0.65, 5.60)]			
		>1 infection without SARS-CoV-2		3.96, [p<0.001, (2.05, 7.68)]			
Scenario 2	Creating two sperate categorical exposure variables for acute SARS-CoV-2 infection and other concurrent	Acute SARS-CoV-2 infection with/without other infection	Yes	1.52, [p =0.03, (1.04, 2.22)]	Subjects who tested negative for an acute SARS-CoV-2 infection with or without other infections	2008/ 2155	89%
			Unknown	0.07, [p<0.001, (0 .05, 0.09)]			
		Other infection with/without	Influenza or RSV	3.18, [p <0.001, (2.04, 5.33)]	Subjects who tested negative or were		

No.	Scenarios	adjusted OR [P value (95% CI)]			Reference category	AIC/ BIC[a]	AUC[b]
	infections (n=3306)	acute SARS-CoV-2	Adenovirus/ Rhinovirus/Enterovirus or any other single infection	7.49, [p <0.001, (5.97, 11.04)]	unknown for other infection with or without an acute SARS-CoV-2 infection		
			>1 infection	4.26, [p <0.001, (2.26, 8.03)]			
Scenario 3	Including subjects who sought care in Omicron period only, (n=2737)	Acute SARS-CoV-2		1.51, [p =0.05, (1.00, 2.28)]	Subjects who tested negative for an acute SARS-CoV-2 infection and had no other detected infection during ED/hospital visit	1590/ 1690	90%
		Influenza		2.33, [p <0.001, (1.33, 4.10)]			
		RSV		1.25, [p =0.66, (0.46, 3.43)]			
		Enterovirus/Rhinovirus		6.18, [p<0.001, (3.03, 12.60)]			
		Adenovirus		8.94, [p<0.001, (3.24, 24.67)]			
		Other infection		1.68, [p=0.07, (0.95, 2.95)]			
		>1 infection with SARS-CoV-2		1.84, [p=0.27, (0.62, 5.38)]			
		>1 infection without SARS-CoV-2		3.05, [p<0.001, (1,49, 6.23)]			
		Unknown infection		0.05, [p<0.001, (0 .03, 0.07)]			
Scenario 4	Excluding subjects who had previous seizure history, (n=2332)	Acute SARS-CoV-2		1.61, [p =0.04, (1.01, 2.54)]	Subjects who tested negative for an acute SARS-CoV-2 infection and had no other detected infection during ED/hospital visit	1389/ 1487	89%
		Influenza		2.85, [p <0.003, (1.43, 5.66)]			
		RSV		2.80, [p =0.03, (1.09, 7.21)]			
		Enterovirus/Rhinovirus		6.98, [p<0.001, (3.36, 14.50)]			
		Adenovirus		8.07, [p<0.001, (3.01, 21.62)]			
		Other infection		2.64, [p<0.002, (1.43, 4.88)]			
		>1 infection with SARS-CoV-2		3.18, [p=0.09, (0.83, 12.18)]			
		>1 infection without SARS-CoV-2		4.39, [p<0.001, (2.03, 9.50)]			

No.	Scenarios	adjusted OR [P value (95% CI)]		Reference category	AIC/ BIC[a]	AUC[b]
		Unknown infection	0.05, [p<0.001, (0 .03, 0.08)]			
Scenario 5	Fixed-effect of age at presentation, seizure history, seasonality as well as random-effect of IMPACT centers, (n=3306)	Acute SARS-CoV-2	1.60, [p =0.02, (1.07, 2.40)]	Subjects who tested negative for an acute SARS-CoV-2 infection and had no other detected infection during ED/hospital visit	1984/ 2112	90%
		Influenza	4.67, [p <0.001, (2.52, 8.62)]			
		RSV	3.25, [p <0.004, (1.46, 7.22)]			
		Enterovirus/Rhino virus	5.23, [p<0.001, (2.88, 9.50)]			
		Adenovirus	9.46, [p<0.001, (3.79, 23.58)]			
		Other infection	2.78, [p<0.001, (1.68, 4.60)]			
		>1 infection without SARS-CoV-2	2.38, [p=0.12, (0.80, 7.12)]			
		>1 infection without SARS-CoV-2	4.64, [p<0.001, (2.37, 9.09)]			
		Unknown infection status	0.05, [p<0.001, (0 .04, 0.07)]			
Scenario 6	Combining unknown infection group with other single infection group, (n=3306)	Acute SARS-CoV-2	1.74, [p=0.006, (1.17, 2.59)]	Subjects who tested negative for an acute SARS-CoV-2 infection and had no other detected infection during ED/hospital visit	2201/ 2305	89%
		Influenza	2.86, [p<0.001, (1.63, 5.00)]			
		RSV	2.49, [p=0.025, (1.12, 5.53)]			
		Enterovirus/Rhinovirus	6.14, [p<0.001, (3.42, 11.03)]			
		Adenovirus	9.14, [p<0.001, (3.73, 22.39)]			
		Unknown or any other single infection	0.11, [p<0.001, (0.08, 0.14)]			
		>1 infection with SARS-CoV-2	2.15, [p=0.162, (0.73, 6.29)]			
		>1 infection without SARS-CoV-2	3.80, [p<0.001, (1.98, 7.30)]			

No.	Scenarios	adjusted OR [P value (95% CI)]		Reference category	AIC/ BIC[a]	AUC[b]
Scenario 7	Excluding subjects who were not tested for acute SARS-CoV-2 infection, (n=1212)	Acute SARS-CoV-2 with/without other infection	1.26, [p=0.183, (0.90, 1.78)]	Subjects who tested negative for an acute SARS-CoV-2 infection with or without other infections	1596/ 1611	65%

[a] Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC)

[b] Area under receiver operating curve (AUC)

While interpreting the results of the sensitivity analyses, we kept in mind that the misclassification bias to exposure and data limitation were still present.

Scenario 1: We measured the adjusted OR excluding subjects with 'unknown infection status' during their ED visit/hospital stay for febrile seizure. These were subjects who were not tested for an acute SARS-CoV-2 infection and had no other detected infection during ED visit/hospitalization. The results were similar to one of our final models (Table 6, Model 3)- adjusted OR 1.56 ($p=0.01$, 95% CI 1.05, 2.32) for acute SARS-CoV-2 infection, 2.30 ($p<0.04$, 95% CI 1.03, 5.17) for RSV, 2.53 ($p<0.001$, 95% CI 1.45, 4.73) for influenza, and 6.47 ($p<0.001$, 95% CI 3.58, 11.71) for enterovirus/rhinovirus infection, although for acute SARS-CoV-2 infection and RSV the 95% CI contained 1 (approximately).

Scenario 2: We also checked if the adjusted OR for primary (acute SARS-CoV-2 infection) and secondary focus (influenza or RSV or enterovirus/rhinovirus infection) varied if we divided our exposure variable (acute infection type) into two separate exposure variables. So, we generated one exposure variable as 'acute SARS-CoV-2 infection' which consisted of 3 levels- acute SARS-CoV-2 infection with or without other concurrent infection, no acute SARS-CoV-2 infection with or without other concurrent infection (reference category), unknown acute SARS-CoV-2 infection with or without other concurrent infection. Another exposure variable was developed called 'other concurrent infection' that had 4 levels: influenza or RSV with or without acute SARS-CoV-2 infection, any other single infection with or without acute SARS-CoV-2 infection, >1 infections with or without acute SARS-CoV-2 infection, unknown or no infection with or without acute SARS-CoV-2 infection. The no infection and unknown infection subjects were together as IMPACT data did not provide a distinction between individuals who were

not tested and those who were tested but had negative results. Then we measured for the adjusted ORs for both exposure variables 'acute SARS-CoV-2 infection' and 'other concurrent infections' adjusting for the confounders. When we used two separate exposure variables in regression model, it generated the adjusted OR 1.52 (p=0.03, 95% CI: 1.04, 2.22) for acute SARS-CoV-2 infection and a significant negative association between unknown SARS-CoV-2 infection and the outcome. The reference category was subjects without acute SARS-CoV-2 infection (with or without other concurrent infection). For other concurrent infections, the adjusted OR was 3.18, [p <0.001, 95% CI (2.04, 5.33)] for influenza and RSV (combined) and 7.49, [p <0.001, 95% CI (5.97, 11.04)] for enterovirus/rhinovirus and adenovirus (combined). The reference category was subjects without any detected concurrent infection (with or without acute SARS-CoV-2 infection). Again, the results were similar to those of our final models (Table 6, Model 3 and 5). We presented the descriptive analysis as well in Table 8.

Table 8: Acute Infections of hospitalized and non-hospitalized patients with febrile seizure (N=3306)

Variables		Subgroups	Hospitalized		Non-hospitalized [a]		Total		p-value
			n=595		n=2711				
			n (%)		n (%)				
Acute infection type	Acute SARS-COV-2 infection[a]	Yes	83	13.95%	86	3.17%	169	5%	<0.001
		No	435	73.11%	608	22.43%	1043	32%	
		Unknown	77	12.94%	2017	74.40%	2094	63%	
	Other concurrent infection[b]	Influenza or RSV	52	8.74%	51	1.88%	103	3%	<0.001
		Any other single infection	130	21.85%	64	2.36%	194	6%	
		>1 infections	36	6.05%	20	0.74%	56	2%	
		Unknown or no infection	377	63.36%	2576	95.02%	2953	89%	

[a] Acute SARS-CoV-2 infection includes cases with or without other concurrent infections identified at the ED or during hospitalization

[b] Other concurrent infections cover cases with or without acute SARS-CoV-2 infection identified at the ED or during hospitalization

Scenario 3: Considering the change in SARS-CoV-2 testing pattern in Omicron period (e.g., reduce access to RT-PCR testing), we found that non-hospitalized patients might had greater instances of unknown exposure status and likelihood of false-negative result than the hospitalized group if they presented to ED/hospital for febrile seizure during Omicron period. So, we checked whether the adjusted OR for acute-SARS-CoV-2 infection level in exposure variable 'acute infection type' changed if we ran separate models with Omicron period data versus Pre-Omicron period data. With 2717 subjects who sought care in Omicron period only, we found the adjusted OR as 1.51, [p =0.05, 95% CI (1.00, 2.28)] for acute SARS-CoV-2 infection and 1.25, [p <0.66, (0.46, 3.43)] for RSV, adjusting for confounders. No significant association was observed between hospitalization for febrile seizure and acute SARS-CoV-2 infection as well RSV as the 95% CI contained 1. Regression was not possible for Pre-Omicron period patients due to the small sample size.

Scenario 4: As IMPACT data did not distinguish between febrile and afebrile seizures in absence of a seizure disorder, we checked if the adjusted OR for exposure variable changed if we excluded individuals who had previous seizure history of any kind (i.e., including only first febrile seizure events in analysis). This sensitivity analysis also informed the change in adjusted OR due to the potential violation of an assumption in logistic regression, i.e., observations to be independent of each other (see section 3.6.3). Excluding 974 people who reported seizure history of any kind, we estimated the adjusted OR as 1.61, [p=0.04 & 95% CI (1.01, 2.54)] for acute SARS-CoV-2 infection and 2.80, [p=0.03, 95% (1.09, 7.21)] for RSV, adjusting for confounders (except seizure history). The results were similar to those of one of our final models (Table 6, Model 3).

Scenario 5: We checked if the adjusted ORs for 'acute infection type' varied if we adjusted for the seasonal impact on hospitalization for febrile seizure (if any), instead of Omicron/Pre-Omicron period. This is because certain seasons might increase the rate of certain infections which could increase the incidence of febrile seizure in the pediatric population impacting hospitalization for febrile seizure[44,49]. To adjust for the seasonal impact we generated a variable named 'seasonality' based on the date of ED/hospital visit and consisting of 5 levels i.e., Fall 2021 (Aug-Oct'2021), Winter 2021 (Nov 2021-Feb 2022), Spring 2022 (Mar-Jun'2022), Fall 2022 (Jul-Oct'2022) and Winter 2022 (Nov-Dec'2022). In this model, we adjusted for seasonality instead of Omicron period in addition to other confounders. The results were similar to Table 6, Model 3.

Scenario 6: We checked if all subjects with unknown infection status were tested and detected with an infection of any type what consequence it could have on the adjusted ORs. We envisioned the scenario, considering that febrile seizures are usually triggered by fever from an infection in children. To conduct this sensitivity analysis, in the exposure variable 'all acute infection', we combined the subgroup of subjects with unknown infection status and the subgroup of subjects with any other single infections. The results were similar to Table 6, Model 3. Reference category consisted of subjects who tested negative for an acute SARS-CoV-2 infection and had no other detected infection during ED/hospital visit

APPENDIX-3

Table 9: Categories of vaccines based on post-vaccination febrile seizure risk window

Live vaccines (febrile seizure in 5-13 days)	Inactivated vaccines (Febrile seizure S in 0-2 days)	COVID-19 vaccines (Febrile seizure in 0-3 days)
Var varicella unspecified	Inf FLUZONE Quadrivalent Sanofi Pasteur	COVID-19 Unspecified
Rotavirus product uncertain	Inf FLULAVAL TETRA IDB	Moderna Spikevax COVID-19
MMR-Var ProQuad	Meningococcal C Conjugate (Menjugate, GSK)	Pfizer-BioNTech Comirnaty COVID-19 Pediatric
MMR-Var measles + mumps + rubella + varicella unspecified	Pneu pneumococcal unspecified	
MMR measles + mumps + rubella unspecified	HB hepatitis B unspecified	
MMR M-M-R II Merck	Men meningococcal unspecified	
MMR PRIORIX GSK	Influenza unspecified	
MMR-Var Priorix-Tetra GSK	HA Havrix 720 Junior GSK	
Rotarix (GSK)	HA VAQTA Merck	
RotaTeq (Merck Frosst)	HB Engerix B pediatric GSK	
Var Varilrix GSK	HAHB Twinrix Junior GSK	
Var Varivax III MC	HAHB Twinrix GSK	
Flu mist	Meningococcal C Conjugate (NeisVac-C, Baxter/GlaxoSmithKline)	
	Tdap Boostrix GSK	
	DTaP-HB-IPV-Hib Infanrix hexa GSK	
	DTaP-IPV Quadracel SP	
	Tdap-IPV Boostrix-Polio GSK	
	DTaP-IPV-Hib Infanrix-IPV/Hib GSK	
	DTaP-IPV-Hib Pentacel SP	
	DTaP-IPV-Hib Pediacel SP	