LymphoSign Journal

The journal of inherited immune disorders

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TABLE OF CONTENTS

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LymphoSign Journal publishes novel clinical, translational, and basic research in the fields of immunology, gastroenterology, neurology, dermatology, rheumatology, hematology, and infectious disease. The aim of the journal is to provide a forum for clinicians and scientists to discuss clinical observations, therapies, and insights into underlying disease mechanisms in immune disorders including immune deficiencies, auto-inflammatory disorders, allergy, bone marrow failure, and lymphoid malignancies. Special consideration will be given, but is not limited to, articles exploring adaptive and innate immunity, mucosal immunity, signal transduction, lymphocyte development and cell death, genomic medicine, gene regulation, DNA repair, and cell cytoskeletal networks. *LymphoSign Journal* publishes peer-reviewed original research articles, reviews, clinical trials, case reports, novel mutations, and imaging, as well as practice guidelines, algorithms, and protocols.

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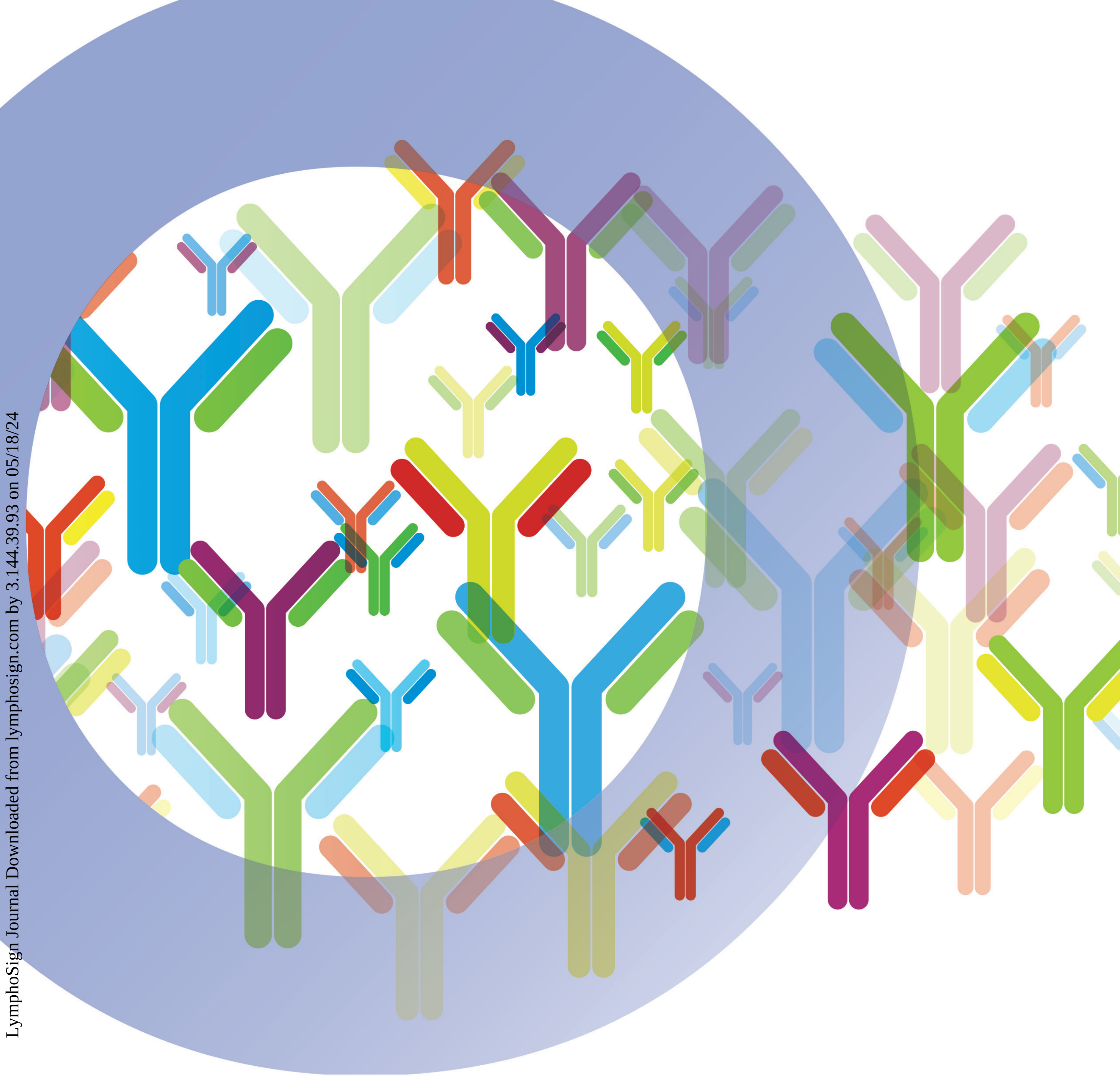

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COVID-19 vaccination for patients with primary immunodeficiency

Chaim M. Roifman, CM, MD, FRCPC, FCACB[a,b*] and Linda Vong, PhD[a]

Introduction

The worldwide tally of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections, causing novel coronavirus disease 2019 (COVID-19), currently approaches 149.7 million (as of 30 April 2021) (Government of Canada 2021*a*). Canada's cases amount to 1 211 083 confirmed infections and 24 169 deaths (Government of Canada 2021*b*). In the midst of the pandemic and a third wave of infections, programs aimed at widespread vaccination against COVID-19 remain an essential stop-gap to slow the spread of infection and help achieve protective herd immunity (Fontanet and Cauchemez 2020). Patients with primary immunodeficiency (PID) have impaired immune responses and may be at greater risk of severe illness due to COVID-19, thus, are strongly recommended to avoid interactions with those outside of their immediate household "bubble", practice hand hygiene, and wear masks when spending time outside or in enclosed spaces where close contact with other people cannot be avoided (Roifman 2020).

With the ongoing rollout of COVID-19 vaccinations, we provide here recommendations for patients with PID. It is important to note that individuals who are immunocompromised should always consult their immunologist for additional considerations/contraindications when reviewing their suitability for vaccination.

Vaccination and the immune response

Vaccination (or immunization) is a safe and effective way to protect against infection from foreign agents such as viruses or bacteria (Plotkin 2013; Siegrist 2018). Vaccines train the immune system by activating the 2 arms of the adaptive immune system—humoral immunity and cellular immunity (Pulendran 2014). Humoral immunity utilizes macromolecules secreted in body fluids to clear extracellular pathogens. Antibodies produced by B cells are the predominate effectors—these specifically recognize and bind to the pathogen or toxin, thereby neutralizing and preventing entry into host cells. Complement proteins participate by "marking" pathogens for clearance by phagocytic cells. In contrast, mobilization of cellular immunity relies on T cell responses. CD8+ T cells kill infected cells and produce antiviral cytokines, while CD4+ T helper (T_H) cell subsets secrete cytokines and provide co-stimulatory signals that are needed to orchestrate the clearance of intracellular and extracellular pathogens, regulate immune tolerance, and maintain protection at mucosal surfaces. Bi-directional interactions between T cells and B cells/antibodies are necessary to ensure robust and long-lasting protective vaccine responses (Igietseme et al. 2004; Crotty 2015).

The introduction of a foreign agent during vaccination leads to the rapid recruitment of immature

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dendritic cells, monocytes, and neutrophils. These cells routinely surveil the body and are equipped with pattern recognition receptors to recognize potential pathogens (Palm and Medzhitov 2009). Detection of vaccine microbial antigens triggers cell activation and the production of inflammatory cytokines and chemokines, resulting in the recruitment of further monocytes, granulocytes, and natural killer cells. Within this proinflammatory milieu, dendritic cells mature and become activated, take up/present small pieces of the antigen on their cell surface (a process dependent on major histocompatibility (MHC) class I or II molecules) (Joffre et al. 2012), and finally migrate to the draining lymph nodes where they encounter and activate resident naïve B and T cells (Randolph et al. 2005; Iwasaki and Medzhitov 2010). Microbial antigens can also reach draining lymph nodes by passive diffusion.

Within the lymph nodes (or other secondary lymphoid organs), naïve B cells that survey the B cell follicle microenvironment bind, internalize, and process the foreign antigen into small segments and present them on cell surface MHC class II molecules. They then migrate towards the B cell-T cell border and engage antigen-specific T_H cells (primed by activated dendritic cells), which provides activating signals needed to elicit B cell differentiation into antibody-secreting plasma cells (Goodnow et al. 2010). During this process, known as the extrafollicular reaction (MacLennan et al. 2003), immunoglobulin (Ig) class-switch recombination occurs (from IgM to IgG, IgA or IgE), producing short-lived, low affinity antibodies within a few days after vaccination (Goodnow et al. 2010).

If sufficient co-stimulatory signals are present, follicular dendritic cells (which trap and retain antigens) and follicular T_H (T_{FH}) cells direct antigen-specific B cells to undergo clonal proliferation in specialized structures known as germinal centers (Goodnow et al. 2010; De Silva and Klein 2015). Here, 2 key steps take place: (*i*) Ig class-switch recombination, and (*ii*) maturation of B cell affinity for specific antigens—a process involving somatic hypermutation of Ig genes. Together, the germinal center reaction selects and enriches for the survival and proliferation of B cells with highest affinity antigen-specific binding (Goodnow et al. 2010). Within this microenvironment, germinal center B cells are provided the necessary cues to support differentiation into large numbers of specific antibody-secreting plasma cells, a process which produces peak IgG vaccine antigen antibodies 4–6 weeks after initial vaccination. Some plasma cells migrate to distinct niches within the bone marrow, allowing them to survive and produce antibodies for years (Good-Jacobson and Shlomchik 2010).

The germinal center reaction also gives rise to long lasting (decade-long) memory B cells (Kurosaki et al. 2015). These cells, when reactivated by an antigen (for example, during the second dose vaccination or exposure to natural boosters), undergo rapid proliferation and differentiation into antibody-secreting plasma cells. The antibodies produced by memory B cells have a higher affinity for vaccine antigens than those produced by naïve B cells and are present in much higher levels (Good-Jacobson and Shlomchik 2010).

T cell-dependent responses play an important role in controlling and clearing pathogens. T cells are produced in the thymus, circulate in the periphery, and are activated in secondary lymphoid organs. Depending on the antigens encountered, naïve T cells differentiate into effector T cells, either (*i*) CD8+ cytotoxic T cells which can kill infected cells through release of lytic enzymes (direct) and antimicrobial cytokines (indirect), or (*ii*) CD4+ T_H cells (Kapsenberg 2003). CD4+ T_H1 cells support cytotoxic CD8+ T cell function and secrete pro-inflammatory interferon (IFN)-γ, interleukin (IL)-2, and tumor necrosis factor (TNF)-β, while CD4+ T_H2 cells secrete IL-4, IL-5, IL-10, and IL-13 (O'Garra and Robinson 2004; Stetson et al. 2004). CD4+ T_{FH} are essential for the development of germinal centers and memory B cell development (Vinuesa et al. 2005).

While the majority of effector T cells die by apoptosis, a small proportion retain their antigen specificity and survive to become long lasting memory T cells (Sallusto and Lanzavecchia 2000). Central memory CD8+ and CD4+ T cells reside in the lymph nodes and can rapidly proliferate in response to re-exposure to specific antigens. In contrast, effector memory T cells surveil the peripheral tissues, ready to generate immediate cytotoxic functions if a specific antigen is detected.

Overall, vaccination primes the immune system against infection to allow rapid detection and re-mobilization of protective responses, without the risk of serious complications that may occur if exposed to the actual pathogen. Importantly, for individuals who cannot produce antibodies, especially those with PID, T cell dependent responses can still provide a level of protection.

COVID-19 vaccines

Vaccine efficacy, the ability to elicit high affinity antibodies and immune memory, is directly related to the type of vaccine administered: whether it is live (attenuated), killed (inactivated), or contains a subunit of the pathogen. Other determinants include the dose of antigen (Ahman et al. 1999) and whether there are adjuvants present (Spreafico et al. 2010). In general, live vaccines are the most immunogenic, and are extremely efficient at triggering T and B cell activation (Zabel et al. 2013). Nevertheless, most vaccines (aside from those that are capsular polysaccharide-based) have been developed to induce protective T and B cell responses.

It is important to note that vaccines using a live (attenuated) form of the pathogen, such as the measles vaccine, *should not* be administered to individuals who are immunocompromised given the inherent risk of disseminated infection.

The race to produce effective vaccines against SARS-CoV-2 (Zhu et al. 2020), causing COVID-19, that could be (*i*) rapidly developed and (*ii*) deployed on a large-scale has hastened the introduction of novel gene-based vaccines (Pushparajah et al. 2021). These vaccines, utilizing mRNA or vectors containing genetic code, rely on the host immune cell's protein synthesis machinery to produce a key surface protein found on the SARS-CoV-2 virus. The spike protein, a trimeric glycoprotein expressed on SARS-CoV-2, is essential for uptake of the virus into host cells (Letko et al. 2020). Upon entry into the cell, the virus releases its RNA and hijacks the host system to replicate, producing viral copies that can then infect surrounding cells (Fehr and Perlman 2015). The spike protein is therefore an ideal target for the COVID-19 vaccine since neutralizing antibodies would recognize and bind the surface epitopes, preventing SARS-CoV-2 virus entry into cells (Baden et al. 2021).

COVID-19 vaccines based on the mRNA platform (Pardi et al. 2018) contain instructions for cells to make a stabilized version of the SARS-CoV-2 spike protein. The instructions, in the form of mRNA, are encapsulated in lipid nanoparticles to protect and enable them to traverse the cellular membrane of dendritic cells which are recruited to the site of injection. Ribosomes in the host cell cytoplasm translate the mRNA, produce the spike protein, and small fragments are then presented to the cell surface. Together, this triggers both B cell-dependent humoral responses and T cell-dependent cellular responses.

COVID-19 vaccines utilizing non-replicating viral vectors (Pushparajah et al. 2021) are designed to deliver the DNA instructions for the SARS-CoV-2 spike protein into the host immune cell nucleus. The vector, a harmless version of a virus, has been modified to provide only instructions for the spike protein, but cannot replicate to produce copies of itself. Thus, 1 viral vector can only infect 1 host cell. The spike protein is produced by the host immune cell's protein synthesis machinery, processed into fragments and then presented to the cell surface to elicit humoral and cellular responses.

There are currently 4 COVID-19 vaccines authorized for use in Canada. The Pfizer-BioNTech (BNT162b2) and Moderna (mRNA-1273) COVID-19 vaccines use mRNA-based platforms, while the vaccines produced by AstraZeneca (ChAdOx1-S, also manufactured by Verity Pharmaceuticals/Serum Institute of India) and Janssen (Ad26.COV2.S, Johnson & Johnson) are non-replicating viral vector-based. In clinical trials, all have been shown to induce humoral and cellular immune responses (Sahin et al. 2020; Baden et al. 2021).

The Pfizer-BioNTech, Moderna, and AstraZeneca COVID-19 vaccines follow a 2-dose schedule. In clinical trials, the first dose of the mRNA-based vaccines resulted in a relatively weak immune response, while the second dose produced a stronger immune response (efficacy against symptomatic COVID-19 after 2nd dose: Pfizer-BioNTech = 94.6%, Moderna = 94.1%) (Polack et al. 2020; Baden et al. 2021). In contrast, AstraZeneca's viral vector vaccine provides comparable immune responses following the first and second dose response, albeit lower than the mRNA vaccines (efficacy against symptomatic COVID-19 after 2nd dose: AstraZeneca = 62.5%) (Voysey et al. 2021). For vaccines requiring 2 doses, there is no evidence to suggest that 1 dose is sufficient to provide long-term protection against COVID-19. Full protection is only achieved 2 weeks after the second dose (CDC 2021). This is important in the context of newly emerging SARS-CoV-2 variants (Mascola et al. 2021), including the B.1.1.7 variant (UK), B.1.617.2 (Delta) variant (India), B.1.351 variant (South Africa), and P.1 variant (Brazil), as a single dose of the Pfizer-BioNTech COVID-19 vaccine was shown to provide only partial protection against the B.1.1.7 variant (Reynolds et al. 2021).

The Janssen COVID-19 vaccine, approved for use in March 2021, requires only a single-dose to be protective against COVID-19 (efficacy against moderate to severe/ critical COVID-19 after 14 days = 66.9%) (Sadoff et al. 2021).

At present, the Moderna COVID-19 vaccine is approved for people who are 18+ years of age. On 5 May 2021 Canada became the first country to authorize use of the Pfizer-BioNTech COVID-19 vaccine in adolescents aged 12+, expanding on Health Canada's previous approval for its use in those aged 16+ years of age. The mRNA vaccines are preferentially recommended over the non-replicating viral vector vaccine types (NACI 2021). Canada's National Advisory Committee on Immunization recently revised their recommendations for the use of AstraZeneca and Janssen's non-viral vector COVID-19 vaccines due to reports of a number of rare cases of serious blood clots known as vaccine-induced immune thrombotic thrombocytopenia (Mahase 2021; Pai et al. 2021). Both the AstraZeneca and Janssen COVID-19 vaccines may be offered those who are 30+ years of age and who prefer to not wait for the mRNA vaccines. All 4 vaccines are planned or currently being trialed in pediatric cohorts.

COVID-19 vaccination of patients with PID

Reports of COVID-19 in patients with PID have highlighted more severe clinical course in those with defects in type I IFN signaling (Bastard et al. 2020; van der Made et al. 2020; Zhang et al. 2020) and greater risk of ICU admission in younger age groups compared to the general population (Meyts et al. 2021). In the United Kingdom, the case-fatality ratio of PID patients with COVID-19 was significantly higher compared to the general population, demonstrating greater morbidity and mortality (Shields et al. 2021). However, in other geographic areas, such as Israel, there is data to suggest that symptoms in some PID patients may be milder (Quinti et al. 2020; Marcus et al. 2021; Meyts et al. 2021), perhaps due to the innate inability to mount appropriate inflammatory responses. Dysregulated or hyperimmune reactions underlie some of the more severe sequalae of COVID-19.

Clinical trials for COVID-19 vaccines have so far involved only a limited number of people who are immunocompromised or have autoimmunity, and no data is available on those who are immunosuppressed or receiving immunosuppressive therapy. By extension, it is not known whether patients with PID will be able to mount the same humoral and cellular immune responses as the general population, or have a diminished protective response, to the vaccine.

The currently available mRNA-based COVID-19 vaccines are considered on par with inactivated vaccines and thus do not present a greater risk to immunocompromised individuals than what would normally be encountered.

Recommendations

1. Given the favorable safety, tolerability, and efficacy data from the COVID-19 vaccine trials, all patients with PID should be vaccinated against COVID-19, especially those who have known biological risk factors and (or) social factors that predispose to severe COVID-19 illness. Experience with other vaccines suggests that patients with PID may have a less robust immune response to the COVID-19 vaccine, and the vaccine may not be as effective. Regardless, the possibility of mild protection against COVID-19 is advantageous compared no protection at all.
2. mRNA-based COVID-19 vaccines are recommended for use in patients with PID (NACI 2021).
3. Caregivers and close contacts should also be vaccinated to limit the risk of exposure to the virus. This is particularly important for caregivers of paediatric (<12 years) PID patients for which COVID-19 vaccines have not yet been approved.
4. Patients with PID who have previously had COVID-19 should still get the vaccine. Vaccination can be delayed until 90 days after the initial infection, since there are few reports of re-infection during this interval.
5. It is important to note that all patients should consult their immunologist or PID physician for specific advice regarding their suitability for the COVID-19 vaccine, including contraindications or allergies to any components of the vaccines.

Common questions

Can immunoglobulin replacement therapy protect PID patients from COVID-19?

Immunoglobulin replacement products contain gammaglobulin (IgG) pooled from the plasma of many donors and provides protection against a wide range of infections. We know that robust levels of neutralizing antibodies against the SARS-CoV-2 virus are produced after infection (Lau et al. 2021). Vaccination also produces protective levels of neutralizing antibodies. Therefore, it is possible that antibody titers against the virus will appear in immunoglobulin replacement products as the number of people who are (*i*) infected by SARS-CoV-2 or (*ii*) vaccinated against COVID-19 increases. However, at present, there is not enough data to guarantee protective antibody levels against the virus in immunoglobulin replacement products.

Will the COVID-19 vaccine benefit PID patients who do not produce measurable antibody titers to other vaccines?

Yes, patients with defects in antibody production may still develop some level of protection against SARS-CoV-2 through T cell dependent responses. Vaccines activate 2 arms of the immune system—humoral immunity (involving antibodies) and cellular immunity (involving T cells). Antibodies, produced by B cells, prevent or reduce infections by blocking their entry into cells. T cells, on the other hand, do not prevent infection but help to control and clear the pathogen.

Will delays in receiving the second dose, beyond the manufacturers' suggested 3–4 week schedule, affect protection against COVID-19?

In clinical trials, the 2-dose COVID-19 vaccines approved for use followed a 21- or 28-day dosing interval. This is the duration between the first and second dose. In practice, delays in the availability of COVID-19 vaccines have meant that most people will be unable to receive a second dose within this time frame. The first dose provides some protection. Preliminary data from the United Kingdom suggests that protection after the first vaccine dose may last 10 weeks (Pritchard et al. 2021; Wei et al. 2021), although it is unclear when protective antibody levels start to wane. Nevertheless, delays in administration of the second dose beyond the range approved by FDA licensing studies are not based on scientific evidence. For this reason, it remains ideal to receive the second dose on time or, if impossible, as soon as it is available.

Can children with PID receive the COVID-19 vaccine?

Children 12 years of age and over are eligible to receive the Pfizer-BioNTech COVID-19 vaccine. Clinical trials for younger pediatric cohorts are in progress or planned. To reduce the risk of infection to younger children, immediate family members and close contacts should be vaccinated, and all members of the household should be vigilant in practicing social distancing measures, regular hand washing, and masking.

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Diversity and inclusion in science

Amarilla B. Mandola[a,b,c*]

Perspective on women in science

"Personally, I find immunology to be an intellectually stimulating science. The complexity of most primary immunodeficiencies together with the increasingly complicated treatment regimens require a working partnership between the patient, their families, and the medical team. As a woman in medicine in general, and in clinical immunology and allergy in particular, I find it very important to work as a respected, valued, and equal part of a team, with a dedicated contribution to improve patient care and partake in research. Equally important is having the safety of a balanced family life and physical/mental health and wellness. I find myself very lucky at this point in my life, being part of a great physician team and having full support from my spouse.

I am forever grateful to those who came before me, as it was their commitment to improving the system that has led to so many more opportunities for women in science today. To reach where I am now has still been very challenging, demanding, and lonely at times. I had to work hard to attain the training I desired most, however, I was fortunate to be mentored with utmost respect by both men and women who were role models – they showed me the way forward, to identify with the scientific community, and provided me freedom and creativity in my clinical and research work during my training.

My advice is to believe in yourself and believe that what you do matters. You may question your abilities at each failure, but you are not alone! Don't forget, great scientists are made, not born, and even they faced on-the-job doubts along the way.

Strive to create your networks, find your peer support and mentoring that you need. Be open and outspoken about the challenges that you face as a woman. With many tasks demanded by society, eventually, you have to decide what your ultimate goals are and what things are worth fighting for.

Be there for others; over time, even the strongest women might be affected and discouraged from staying in academia. Make a point to acknowledge the work and achievements of other women.

And finally, always keep in your mind that this is your life. It is a one-time opportunity – make it as happy, satisfying and balanced as you can, until you reach your dreams."

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Biography

Dr. Amarilla Mandola was born in Budapest, Hungary, and completed her general medicine training at the Semmelweis University, Budapest. She improved her English over the years, and worked as a teacher and tutor for pre-med foreign students in medical English and medical terminology. She started her pediatric residency at the 1st Department of Pediatrics, Semmelweis University, and subsequently moved to Israel with her husband where she completed her pediatrics residency at the Soroka University Medical Center, Ben-Gurion University of the Negev, Israel. She was awarded resident of the year in 2012. Between 2013 and 2017, Dr. Mandola worked as a staff Pediatrician at the Pediatric Department A, Soroka University Medical Center, and took on an active role in the Pediatric Immunology and Allergy Clinic treating primary immunodeficient children from across southern Israel. Due to the diverse population in this geographic region (Jewish origin, immigrants from around the world, refugees from Africa, and the vast majority of the Arabic speaking Bedouin population in Israel), Dr. Mandola gained exposure to various immunological pathologies and conditions, some of which are unique and rare. To attain a more thorough and in-depth understanding of disease mechanisms and treatments pertinent to immunology and allergy, she pursued and completed a 3-year combined fellowship in Clinical Immunology and Allergy at the Hospital for Sick Children and University of Toronto, Toronto, Ontario, Canada. To gain skills in wet lab research, she also took part in investigations unravelling the causes of multiple novel primary immunodeficiencies, under the supervision of Prof. Chaim Roifman. Currently, Dr. Mandola is a staff physician in the Pediatric Department A, Soroka University Medical Center, and undertakes a leading role in the team of the Pediatric Primary Immune Deficiency Clinic and the Pediatric Allergy Clinic, together with Prof. Amit Nahum and Prof. Arnon Broides, serving patients in Southern Israel from Beer Sheva to Eilat. By combining her expertise in Pediatrics and Immunology, Dr. Mandola advocates for immunodeficient patients and their families, to promote early diagnosis and ensure optimal access to medical care, secure patients' interest, and improve patient's medical care by adopting new treatments and follow-up strategies. With a strong foundation in clinical research, she has the opportunity to integrate research into her clinical work in order to better understand the mechanisms of the disease in her patients. Dr. Mandola is also involved education in the postgraduate and undergraduate education of residents and medical students in the field of Clinical Immunology, Allergy and Pediatrics.

The spectrum of multisystem inflammatory syndrome (MIS-C) in children infected with severe acute respiratory syndrome coronavirus 2

Ahmad Amer[a*], Adi Ovadia[a], Gila Meirson[a], Diana Tasher[a,b], and Ilan Dalal[a,b]

ABSTRACT

Introduction: The impact of SARS-CoV-2 infections in children has generally been described as relatively benign. However, since April 2020, there have been reports of a new multisystem inflammatory illness affecting children and related to COVID-19 termed multisystem inflammatory syndrome in children (MIS-C).

Aim: To describe 3 cases of children diagnosed with MIS-C and discuss the disease spectrum.

Methods: We collected and reviewed data from 3 cases diagnosed with MIS-C admitted to our pediatric ward between October 2020 and January 2021.

Discussion: MIS-C is a newly described disease that spans a spectrum of phenotypes and severity, and while it shares clinical similarities with Kawasaki disease, it has a unique set of epidemiological, laboratory, and prognostic characteristics. In this review, we hope to add to the understanding of this new entity.

Statement of Novelty: This report discusses 3 cases of MIS-C and elaborates on the spectrum and immunology of this entity. Our cases are unique in their relatively wide spectrum and variability. We hope our own experience with MIS-C adds to the accumulating knowledge and understanding of this emerging disease.

Background

Coronavirus disease 2019 (COVID-19) is a rapidly spreading pandemic caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Since its origins in Wuhan, Hubei Province of China, in December 2019, the disease has affected more than 100 million people worldwide, and with over 2 million deaths as of February 2021 according to the World Health Organization (WHO) COVID-19 Dashboard (Organisation 2021). As of March 2021, COVID-19 has resulted in over 6,000 deaths in Israel, while more than 500 patients are currently in a severe condition, including 250 whom are mechanically ventilated (Datadashboard.health.gov.il n.d).

The impact of SARS-CoV-2 infections in children has generally been described as relatively benign (Castagnoli et al. 2020). However, since April 2020, there have been reports of a new multisystem inflammatory illness affecting children, related to COVID-19, termed multisystem inflammatory syndrome in children (MIS-C), sometimes also described as "pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2" (PIMS) (Ng et al. 2020; Riphagen et al. 2020; Verdoni et al. 2020; Viner and Whittaker 2020). Patients with MIS-C exhibit similar symptoms to those found in Kawasaki disease (KD), and streptococcal and staphylococcal toxic shock syndromes (TSS); however, there are several key clinical, epidemiological, and importantly immunological fea-

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tures that are unique to this syndrome (RCPCH n.d; Arad et al. 2011; Abrams et al. 2020; Ahmed et al. 2020; Consiglio et al. 2020). Here, we describe 3 cases of this newly described disease and discuss its spectrum.

Case presentations

All patients described below were admitted to our pediatric ward between October 2020 and January 2021. All clinical and laboratory manifestations are summarized in Table 1.

Patient 1, an 11-year-old male, presented in the pediatric emergency room with a medical history of 5 days fever and vomiting, new onset of drowsiness, diffuse maculopapular rash, non–purulent conjunctivitis, and cracked red lips. On physical examination, he was found to have meningeal irritation and nuchal rigidity. He was treated with a fluid bolus and antibiotics on a working diagnosis of bacterial meningitis, but within an hour he deteriorated and developed hemodynamic instability leading to cardiovascular shock that required vasopressor support.

Patient 2, a 9-year-old female, presented with abdominal pain. She was suspected to have appendicitis based on the clinical picture, and was hospitalized for further investigation. While hospitalized, she developed KD-like symptoms including fever, rash, cracked lips, and non-purulent conjunctivitis. In addition, she also developed low blood pressure that responded well to fluid treatment.

Patient 3, a 1-year-old female, presented with a 3 day fever, and later developed a rash, mild conjunctival injection, and cracked lips.

All patients had strikingly elevated inflammation markers. Other hematological abnormalities are presented in Table 1. Two patients (1 and 3), had a transient acute kidney injury, while all 3 had a mild elevation of liver enzymes that later resolved. Patient 1 had a mildly elevated troponin I, while patient 2 had sterile pyuria on admission. Chest X-ray was performed for all patients with no significant findings. Patient 2 had low amounts of free abdominal fluid on ultrasonography; patients 3 had hydrops of the gallbladder. Electrocardiography was normal except for sinus tachycardia in all patients. Echocardiography demonstrated prominent coronary arteries for all patients, cardiac function was normal; patient 1 had a mild pericardial effusion.

All patients underwent extensive microbiological investigation (Table 1).

SARS-CoV-2 polymerase chain reaction (PCR) testing was positive for patient 1, while Anti-SARS-CoV-2 immunoglobulin G (IgG) was positive for patients 1 and 3. The serology testing was performed using the DiaSorin (Saluggia VC, Italy) Liaison SARS-CoV-2 S1/S2 IgG assay, which detects antibodies specific to the SARS-CoV-2 spike (S) proteins. All other infectious investigations were negative.

All patients were treated with a fluid bolus and intravenous immunoglobulin (IVIG). Treatment with wide spectrum antibiotics was initiated in all 3 patients until negative results of blood and urine cultures. Patients 1 and 2 were also treated with glucocorticoids. Patient 1 was admitted to the pediatric intensive care unit (PICU) for vasopressor (adrenaline) and respiratory support (nasal cannula) for 1 day, the patient responded well to treatment with a rapid clinical improvement and was discharged from the PICU to the pediatric ward after 2 days. All 3 patients were discharged home after 5 days.

Discussion

Accumulating evidence that an inflammatory syndrome may follow SARS-CoV-2 infection in some children is in contrast to the general impression that COVID-19 is mostly asymptomatic in children but may present with mild respiratory or gastrointestinal symptoms (Castagnoli et al. 2020).

The Royal College of Pediatrics and Child Health (RCPCH), center for disease control (CDC) and WHO have fairly similar but not identical criteria for the emerging condition of MIS-C. All 3 cite inflammation, and single or multi organ dysfunction, although the RCPCH does not require virological evidence, while the WHO and the CDC criteria include viral positive PCR or serology, or close contact to a known COVID-19 patient (RCPCH n.d.; www.who.int n.d.; Centers for Disease Control and Prevention 2021; Riphagen et al. 2020). All 3 cases in our report fulfilled the RCPCH case definition, while patients 1 and 3 also satisfy the CDC and WHO criteria. Patient 2 had no virological evidence

Table 1: **Clinical and laboratory characteristics of three patients with MIS-C following SARS-CoV-2.**

Characteristic	Patient 1	Patient 2	Patient 3
Age (years)	11.4	9	1.2
Ethnicity	Jewish	Jewish	Jewish
Sex	Male	Female	Female
Medical history	Previously healthy	Previously healthy	Previously healthy
Clinical features	5 d fever, drowsiness, vomiting, rash, non-purulent conjunctivitis, reduced alertness, hemodynamic instability.	5 d fever, abdominal pain, 3 d erythematous rash, dry and lacerated lips, non purulent conjunctivitis, hemodynamic instability	3 d fever, diffuse maculopapular rash, mild conjunctival injection, dry and lacerated lips.
Exposure to COVID-19 positive patient	No	Not known	Father
Duration of symptoms up to admission (days)	5	5	3 d
Laboratory evaluation			
Inflammation markers			
C-reactive protein (highest)	14.1 mg/dl	8.3 mg/dl	20 mg/dl
Erythrocyte sedimentation rate (highest)	Not performed	Not performed	62
Complete blood count (at admission)			
White blood cells (1/μl)	12,700 (Neutrophils 86%)	3,200 (Lymphocytes 300)	16,400 (Neutrophils 70.9%)
Platelets (1/μl)	88,000	197,000	232,000
Hemoglobin (g/dl)	9.7	10.1	11.4
Renal function	Urea 49 mg/dl, Creatinine 1.17 mg/dl	Urea 28 mg/dl, Creatinine 0.36 mg/dl	Urea 67 mg/dl, Creatinine 0.85 mg/dl
Urine Dip stick	Not performed	+1 leukocytes, +3 erythrocytes	+1 leukocytes
Liver enzymes	AST 54 (U/l), ALT 54 (U/l)	AST 59 (U/l), ALT 48 (U/l)	AST 60 (U/l), ALT 44 (U/l)
Cardiac markers	Troponin 32 (ng/dl), CPK 106 (U/l)	Troponin 3 (ng/dl), CPK 42 (U/l)	Troponin 9 (ng/dl), CPK 81 (U/l)
Imaging	C-xr – normal. Abdominal US – normal. Head CT – normal.	C-xr – Not performed, Abdominal US – mild free abdominal fluid, otherwise normal.	C-xr – peribronchial thickening, normal heart shadow. Abdominal US – gallbladder hydrops, otherwise normal.
ECG	Sinus tachycardia, otherwise normal.	Sinus tachycardia, otherwise normal.	Sinus tachycardia, otherwise normal.
Echocardiography	Mild accentuation of coronary arteries, otherwise normal.	Accentuation of both coronary arteries by 2-3mm, otherwise normal.	Accentuation of both coronary arteries by 1mm, otherwise normal.
Microbiology	Negative blood cultures	Negative blood and urine cultures	Negative blood and urine cultures
COVID-19 PCR	Positive	Negative	Negative
COVID-19 serology	Positive S1/S2 IgG	Negative	Positive S1/S2 IgG
Treatment			
Highest respiratory support	Nasal cannula	None	None
Fluid bolus	60 ml/kg	20 ml/kg	40 ml/kg
Inotropic support	Adrenalin (maximal 1 mcg/kg/min)	None	None
Antibiotics	Ceftriaxone	Cefuroxime (3 d)	Cefuroxime (3 d)
Glucocorticoids	Dexamethasone 15mg	Methylprednisolone 2mg/kg (3 d)	None
IVIG	2 g/kg	2 g/kg	2 g/kg
Other	No	Aspirin 80 mg/kg (4 d)	Aspirin 80 mg/kg (4 d)
Length of stay at PICU	2 d	None	None
Total hospital length of stay	5 d	5 d	5 d

AST, aspartate aminotransferase; ALT, alanine aminotransferase; CPK, creatine phosphokinase; C-xr, chest X-ray; IVIG, intravenous immune globulin; US, ultrasound of infection; however, the presentation is highly suggestive of the disease (RCPCH n.d; www.who.int n.d.; Centers for Disease Control and Prevention 2021).

MIS-C appears to span a spectrum; this is reflected in the patients we described. Most patients in case series and reviews were above 5 years of age (Ahmed et al. 2020; Ramcharan et al. 2020; Bustos et al. 2021); however, infants with the disease have also been described (Bautista-Rodriguez et al. 2021). The disease has also been reported across different races, ethnicities, and countries (Ahmed et al. 2020; Ramcharan et al. 2020; Bustos et al. 2021; Bautista-Rodriguez et al. 2021). It should be mentioned that some studies found a higher prevalence in children of African origin (Riphagen et al. 2020; Toubiana et al. 2020). Studies from Europe and the United States report a range of clinical presentations, with most patients having prolonged fever and elevation of inflammatory markers, while some present with abdominal pain or cardiovascular shock (Ng et al. 2020; Riphagen et al. 2020; Viner and Whittaker 2020; Verdoni et al. 2020). Symptoms can encompass multiple organs including skin, neurological, gastrointestinal, and cardiovascular manifestations (Ahmed et al. 2020; Bustos et al. 2021; Ramcharan et al. 2020). Outcomes can range in severity; earlier reports described a high rate of PICU admittance with most patients requiring respiratory and vasopressor support (Riphagen et al. 2020; Viner and Whittaker 2020). Later case series describe a less severe disease progression, with lower numbers of PICU hospitalizations and fewer patients needing intensive support of any kind (Dufort et al. 2020; Feldstein et al. 2020; Whittaker et al. 2020). This might be attributed to improved understanding as well as earlier diagnosis of this disease with increasing experience. All 3 of our patients had an excellent prognosis.

All our patients had coronary changes when diagnosed, and while coronary findings are well described in MIS-C (Alsaied et al. 2021), the incidence varies significantly among reports; larger series have reported coronary abnormalities in 8-24% of cases (Valverde et al. 2021).

Mortality rates vary between reports, with most studies reporting death rates close to 2% (Dufort et al. 2020; Feldstein et al. 2020; Whittaker et al. 2020).

Although similar, KD and MIS-C have important differences in phenotype and laboratory profile. MIS-C tends to manifest in older children. Patients have more gastrointestinal involvement and are more prone to severe hemodynamic involvement including shock. While KD is known to cause thrombocytosis, MIS-C patients have variable platelet counts, other laboratory findings specific to MIS-C include lymphopenia and elevated ferritin (Chen et al. 2021). Another important difference is disease prognosis; while up to 5% of adequately treated patients with KD might still have significant coronary changes, the prognosis for MIS-C seems to be excellent (Eleftheriou et al. 2013; Valverde et al. 2021).

The association between MIS-C and SARS-CoV-2 infection was suggested by the temporal relation and clustering of cases with the rise of the pandemic (European Centre for Disease Prevention and Control 2020). An increasing number of studies reported high rates of serologic positivity to SARS-CoV-2: a UK case series found 85% IgG positivity (European Centre for Disease Prevention and Control 2020); a study from Italy describing ten patients found similar IgG positivity rates (Verdoni et al. 2020); finally a French study reported that 90% of their 21 patients had anti-SARS-CoV-2 IgG (Toubiana et al. 2020). This might suggest a causative and perhaps immunologically mediated relation between SARS-CoV-2 infection and seroconversion in this syndrome.

Studies attempting to explain this relationship have shed light on the basic molecular biology processes that might explain this syndrome. One study by Rivas et al. (2021) noted that the SARS-CoV-2 spike protein encodes a high-affinity SAg-like sequence motif near the S1/S2 cleavage site of the spike protein, which exhibits a high affinity for T-cell receptors (Noval Rivsa et al. 2020). Interestingly, the region is very similar in sequence and structure to a fragment of the superantigenic Staphylococcal Enterotoxin B (SEB) that is known to cause the cytokine storm typical of TSS (Arad et al. 2011; Cheng et al. 2020).

A study by Consiglio et al., published in the journal Cell in November 2020, described multiple aspects of the hyper-inflammatory response in children with MIS-C. The study revealed some similarities with KD but also demonstrated important differences, of which one such example is the lack of interleukin-17 (IL-17) mediated hyper-inflammation in MIS-C. Other differences might include changes in T cell populations suggesting immune dysregulation (Consiglio et al. 2020).

These observations might explain the good response of these patients to IVIG and glucocorticoid treatment (Dufort et al. 2020; Feldstein et al. 2020; Whittaker et al. 2020), as these treatments inhibit the pathological immune response, both humoral and cellular (Consiglio et al. 2020).

This syndrome is one of the multiple long term effects of SARS-CoV-2. Other long term effects have been described by a number of studies from different countries, the largest being from China (Xiong et al. 2021), and the US (Taquet et al. 2020). A systematic review of studies from many countries with follow-up of up to 110 days found that 80% of patients had one or more symptoms on long term follow-up, with the most common being fatigue, headache, attention disorder, and dyspnea. These studies included only adult patients (Lopez-Leon et al. 2021). Data for the pediatric population is scarce (Ludvigsson 2021).

In summary, we present 3 cases of MIS-C and discuss its spectrum. The short term and long-term effects of this entity require further investigations.

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Point-Of-Care clinical evaluation of the Clungene® SARS-CoV-2 virus IgG/IgM 15-minute rapid test cassette with the Cobas® Roche RT-PCR platform in patients with or without Covid-19

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ABSTRACT

Background: Coronavirus disease 2019 (Covid-19) remains a pandemic with multiple challenges to confirm patient infectivity: lack of sufficient tests, accurate results, validated quality, and timeliness of results. We hypothesize that a rapid 15-minute Point-Of-Care serological test to evaluate past infection complements diagnostic testing for Covid-19 and significantly enhances testing availability.

Method: A three arm observational study at Sharp Healthcare, San Diego, California was conducted using the Clungene® lateral flow immunoassay (LFI) and compared with the Cobas® Roche real-time polymerase chain reaction (RT-PCR) results. Arm 1: Thirty-five (35) subjects with confirmed Covid-19 using RT-PCR were tested twice: prior to 14 days following symptom onset and once between 12 and 70 days. Arm 2: Thirty (30) subjects with confirmed Covid-19 using RT-PCR were tested 12-70 days post symptom onset. Arm 3: Thirty (30) subjects with a negative RT-PCR for Covid-19 were tested 1–10 days following the RT-PCR test date.

Results: Specificity of confirmed negative Covid-19 by RT-PCR was 100% (95% CI, 88.4%–100.0%); meaning there was 100% negative positive agreement between the RT-PCR and the Clungene® serological test results. Covid-19 subjects tested prior to day 7 of symptom onset were antibody negative. In subjects 7–12 days following symptom onset with a confirmed positive Covid-19 by RT-PCR, the combined sensitivity of IgM and IgG was 58.6% (95% CI, 38.9%–76.5%). In subjects 13–70 days following symptom onset with a confirmed positive Covid-19 by RT-PCR, the combined sensitivity of IgM and IgG was 90.5% (95% CI, 80.4%–96.4%).

Conclusion: The Clungene® lateral flow immunoassay (LFI) is a useful tool to confirm individuals with an adaptive immune response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) indicating past infection. Providing Point-Of-Care results within 15 minutes without any laboratory instrumentation or specialized software has an added value of increasing test availability to patients who have been symptomatic for more than 1 week to confirm past infection. Performance characteristics are optimal after 13 days with a sensitivity and specificity of 90% and 100%, respectively.

Statement of novelty: Formal controlled clinical studies of Covid-19 antibody tests have been limited. This study demonstrates the utility of the 15 minute rapid Clungene® test and the potential for expanded use where Covid-19 RT-PCR testing and vaccination is limited.

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Introduction

On 31 January, 2020, the U.S. Department of Health & Human Services (HHS) Secretary declared a public health emergency related to the virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), that causes coronavirus disease 2019 (Covid-19) and was followed by the World Health Organization's (WHO) declaration of a global Covid-19 pandemic. Up to 29 March, 2021, SARS-CoV2 has infected over 125 million people worldwide and claimed more than 2.8 million human lives (Lamb 2020; Lewis 2020; US Department of Health Human Services 2020; WHO 2020; Our World in Data 2021).

Following HHS' announcement, the US Food and Drug Administration (FDA) issued immediate, in effect guidance on 29 February, 2020, related to the development of in vitro diagnostic tests during this public health emergency. Shortly after FDA's announcement, hundreds of manufacturers developed a variety of diagnostic and serological in vitro tests and began providing these kits to health care providers and laboratories. Testing for SARS-CoV-2 can be performed by either testing for the virus (diagnostic) or testing for past infection by assessing antibody response produced by the host (serological) (CDC 2020*a*). Since the Covid-19 pandemic started, reverse-transcriptase polymerase chain reaction (RT-PCR) tests have been the mainstay of diagnosis. However, the supply and demand for these diagnostic tests has been variable depending on waves of infections occurring at different locations. The Covid-19 RT-PCR usually involves upper or lower respiratory specimens as test samples. Upper respiratory specimens include nasopharyngeal, oropharyngeal, or nares swabs. Results can be expected within a few hours to days depending on laboratory capacity and volume of samples to be tested. The sensitivity of RT-PCR testing varies depending on timing of specimen collection in relation to days following symptoms, viral inoculum, sample collection site, and the assay's limit of detection. Studies have documented a sensitivity range between 50%–80% (Basu et al. 2020; Bhimraj et al. 2020; Guo et al. 2020; Hanson et al. 2020; Vabret 2020; Zhao et al. 2020).

In the United States, the current shortage of reagents to run tests has resulted in longer turnaround times and test rationing. Fifteen (15) minute rapid lateral flow immunoassay (LFI) antibody tests can be used to quickly assess past infection as described in prior studies (CDC 2020*c*, Yang et al. 2020). Anti-SARS-CoV-2 antibodies typically become detectable starting approximately 1 week after onset of symptoms, with IgM antibodies detectable around day 5–10 after onset of symptoms and IgG antibody levels following the IgM response soon thereafter (Deeks et al. 2020; Guo et al. 2020). Serologic tests, therefore, are not useful early in the course of illness for diagnosing Covid-19. Furthermore, not all patients with SARS-CoV-2 infection develop an antibody response, and so a negative serologic result does not exclude past infection. The aim of this study was to understand seroconversion in patients diagnosed with Covid-19 in relation to their symptoms using a rapid 15-minute Point-Of-Care test (Clungene® lateral flow immunoassay (LFI), and the positive or negative percentage agreement with RT-PCR testing.

Study design

This was a formal IRB approved clinical study conducted within Sharp Healthcare, a not-for-profit multi-center regional health care group located in San Diego, California. Subjects were included if hospitalized or recently discharged following a SARS-CoV-2 RT-PCR nares test. The Cobas® Roche platform was used for detection of SARS-CoV-2 RNA and performed at the Sharp Healthcare Laboratory. A study protocol and an informed consent were initiated and approved by the Sharp Institutional Review Board. Subjects were included if >18 years of age and understood the study and its requirements. Patients who had impairment of cognition or decision-making capacity were excluded. Subjects were screened by research coordinators to determine if they had a nares SARS-CoV-2 RT-PCR test result and then consent was requested to enroll in the study.

There were three (3) groups of subjects. Arm 1: Subjects with positive a SARS-CoV-2 RT-PCR positive test result were serologically tested twice with the Clungene® immunoassay; the first test was performed up to 14 days following self-reported onset of symptoms and the second between day 12 and 70. After discharge, subjects were contacted by phone and requested to come into the clinic to have a finger prick for the blood collection. If patients were unable to come to the clinic, study staff took blood samples at home. Arm 2: A second cohort of hospitalized patients with a positive SARS-CoV-2 RT-PCR test result were serologically tested once with the Clungene® immunoassay

12–70 days after symptoms onset. Arm 3: Patients with a negative SARS-CoV-2 RT-PCR test result were serologically tested once between 1 and 10 days with the Clungene® immunoassay following a negative RT-PCR test result.

Methods

The Clungene® Point-Of-Care test was run according to manufacturer's instructions. The test was performed by three (3) independent study coordinators who confirmed that the test was properly working and simple to operate. The test result was read after 15 minutes. Leftover blood was used in the inpatient setting; whole blood finger prick samples were used at the outpatient clinic after discharge.

The Clungene® SARS-COV-2 virus (Covid-19) IgG/IgM rapid test cassette is a qualitative membrane strip-based immunoassay for the detection of antibodies (IgG and IgM) to SARS-CoV-2 in human whole blood, serum, or plasma. The test cassette consists of a burgundy-colored conjugate pad containing SARS-CoV-2 virus recombinant envelope antigens conjugated with colloid gold (SARS-CoV-2 conjugates). It also has a nitrocellulose membrane strip containing 2 test lines (IgG and IgM lines) and a control line (C line). The IgM line is pre-coated with the Mouse anti-Human IgM antibody, IgG line is coated with Mouse anti-Human IgG antibody. The test principle is based on the receptor-binding domain (RBD) of the spike and nucleocapsid proteins. The serum level of RBD-binding antibodies correlates with SARS-CoV-2 neutralization. Previous studies have shown that the Clungene® performs well when evaluating convalescent plasma donors and patients presenting at physicians' offices (Osher et al. 2020; Ransegnola et al. 2020).

When an adequate volume of test specimen is dispensed into the sample well of the test cassette, the specimen migrates by capillary action across the cassette. IgM anti-SARS-CoV-2, if present in the specimen, will bind to the SARS-CoV-2 conjugates (Our World in Data 2021). The immunocomplex is then captured by the reagent pre-coated on the IgM band, forming a burgundy-colored IgM line, indicating a SARS-CoV-2 IgM positive test result. IgG anti-SARS-CoV-2 if present in the specimen will bind to the SARS-CoV-2 conjugates. The immunocomplex is then captured by the reagent coated on the IgG line, forming a burgundy-colored IgG line, indicating a SARS-CoV-2 IgG positive test result. Absence of any T lines (IgG and IgM) suggests a negative result. To serve as a procedural control, a colored line will always appear at the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred. See Figure 1.

Days from symptom onset were captured from the electronic medical record (EMR) which documented self-reported data from patients on the number of days they had been sick at the time of study enrollment. Symptoms of Covid-19 included fever, weakness, cough, shortness of breath, tiredness, anosmia, and loss of taste. Patient characteristics were extracted from the EMR by clinical research coordinators who documented age, gender, body mass index (BMI), comorbid conditions, maximum temperature, C-reactive protein (CRP), and ferritin levels at time of enrollment.

Statistical analysis

Categorical variables were compared using the chi-squared or Fisher exact test, and continuous variables were compared using the Student *t*-test or Mann-Whitney U test, as appropriate. All tests were 2 tailed, and $P < 0.05$ was considered statistically significant. SPSS Statistics, IBM SPSS software, version 27.0 (SPSS, Inc., Chicago, IL) was used for all calculations.

Results

Between May 2020 and August 2020, 97 subjects were enrolled, consented, and tested. Two potential subjects who tested negative for SARS-CoV2 RT-PCR tests were excluded by the principal investigator. After enrollment, it was learned that 1 patient had a pre-hospital positive SARS-CoV-2 RT-PCR test. The second patient was excluded due to 30 days having lapsed between the negative RT-PCR test and the antibody test in a high-risk hospital environment. Figure 2 displays a schematic of patients included in the final study sample size.

A protocol amendment allowed for patients to be tested in the outpatient setting post discharge after 14 days from symptom onset. An analysis was run on 95 patients who completed the study. Thirty patients who had negative Covid-19 RT-PCR were tested and found antibody negative using the Clungene® test. Three (3) RT-PCR positive Covid-19 subjects tested prior to day 7 symptom onset were antibody negative.

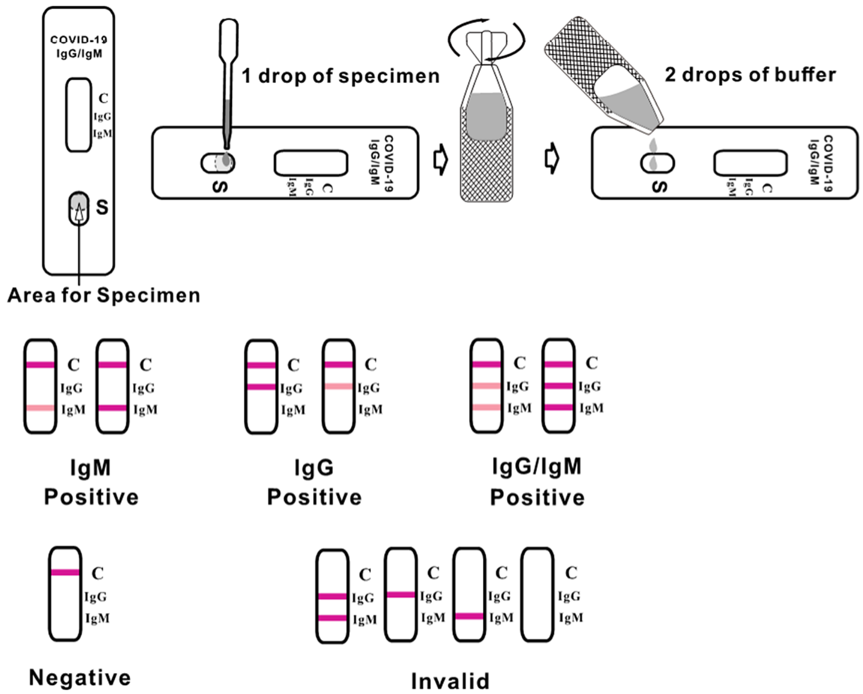

Figure 1: Instructions and example results of Clungene® tests.

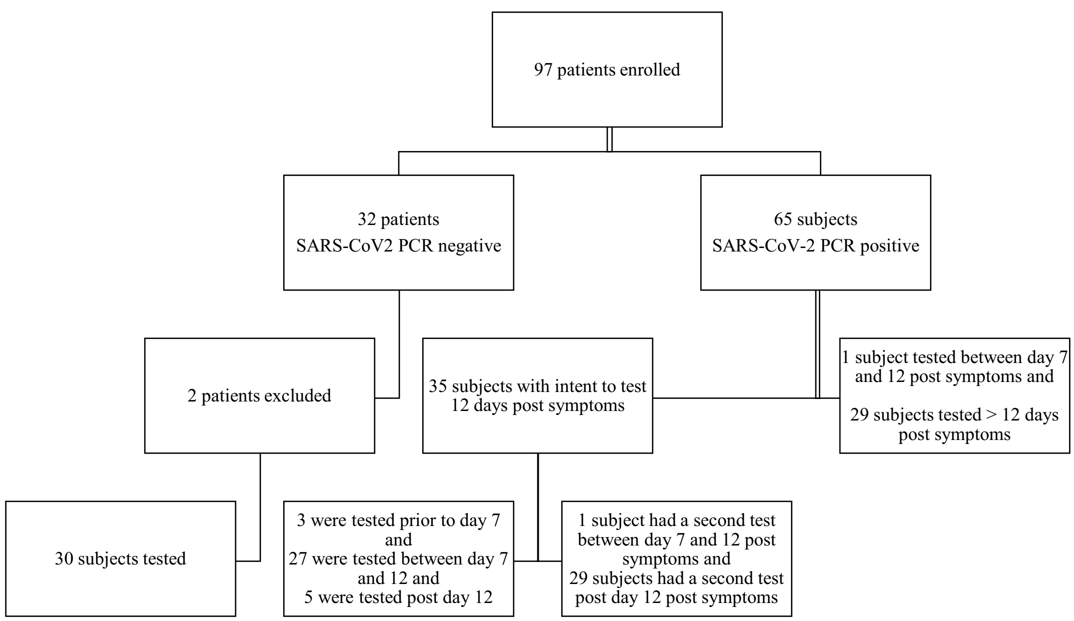

Figure 2: Schematic flowchart of patients in the study.

29 subjects were tested between day 7 and 12 from symptoms and 63 were tested after day 12 from symptom onset. In patients with confirmed Covid-19 with RT-PCR, the combined sensitivity of IgM and IgG was 58.6% (95% CI, 38.9%–76.5%) between day 7 and day 12 from symptom onset and 90.5% (95% CI, 80.4%–96.4%) after day 12. The specificity was 100% (95% CI, 88.4-100.0%), meaning there was 100% agreement between a negative RT-PCR test and 100% negative antibody result. These results are displayed in Table 2.

Overall, the median (IQR) days to RT-PCR testing from symptom onset was 7.5 days (IQR, 4-10 days). Compared to subjects with negative Covid-19 RT-PCR tests ($n = 30$),

Table 1: **Demographics and laboratory values in Covid-19 negative and positive patients.**

Characteristic	Confirmed Covid-19 Negative ($n = 30$)	Confirmed Covid-19 Positive ($n = 65$)	P-Value
Male (N; %)	18 (60%)	40 (61.5%)	0.89
Age (years) (Mean)	60.6	51.1	0.02
Diabetes (N; %)	4 (13.3%)	23 (35.4%)	0.03
Hypertension (N; %)	16 (53.3%)	29 (44.6%)	0.54
Smoker (N; %)	11 (36.7%)	11 (16.9%)	0.04
BMI (kg/m2) (Mean)	30.2	32.1	0.24
CRP (mg/L) (Mean)	27.8	144.9	0.001
Tmax (°C) (Mean)	37.1	37.8	0.000
Ferritin (µg/L) (Mean)	180.2	814.9	0.003

Table 2: **IgG, IgM, and IgG + IgM results based on days from onset of symptoms.**

Days from onset of symptoms	IgG positive	IgG/IgM positive	IgM positive	Negative	Grand total
0 – 6 days	—	—	—	3	3
7 – 12 days	2	13	2	12	29
13 to 70	27	28	2	6	63
Total	29	41	4	21	95

patients with Covid-19 ($n = 65$) were younger (51.1 years vs. 60.6 years, $P = 0.02$), and had higher CRP (144.9 mg/L vs. 27.8 mg/L; $P = 0.001$), ferritin (814.9 µg/L vs. 180.2 µg/L; $P = 0.003$), and Tmax (37.8 °C vs. 37.1 °C; $P < 0.001$) values at time of enrollment (Table 1).

There was a statistical difference in sampling time to the onset of symptoms and true positive results (11.4 days vs. 22.3 days; $P < 0.001$), with samples collected after a longer period upon symptom onset associated with higher sensitivity. In the 15 patients with false negative results prior to 14 days from symptom onset, 13 patients seroconverted in a median 11 days (7.5–36.0 days) (8 IgG, 1 IgM, 4 IgG + IgM) post median 9 days (7.5–10.5 days). Results of the test according to the number of days from the onset of symptoms are presented in Figure 3.

Smoking was significantly less prevalent in the positive group as presented in Table 1. This was an unexpected result since tobacco smokers are thought to be more vulnerable to contracting Covid-19, as the act of smoking involves contact of fingers (and possibly contaminated cigarettes) with the lips, which increases the possibility of transmission of viruses from hand to mouth. However, we are not aware of any peer-reviewed studies that have evaluated the risk of SARS-CoV-2 infection associated with smoking. Given the small sample size, this may be an anomaly and should be investigated further.

Discussion

In this study, the performance characteristics of Clungene® were evaluated and showed a specificity of 100% and a sensitivity of 90.5% for samples collected more than 12 days after the onset of symptoms. These results are consistent with previously reported results (Flower et al. 2020; Nicol et al. 2020; Wu et al. 2020; Zhu et al. 2020). Since March 2020, testing for Covid-19 in the United States has undergone multiple phases. From the U.S. Centers for Disease Control and Prevention (CDC) to U.S. individual state health departments, the availability for testing has remained constrained. There are certain limitations that make RT-PCR testing less readily available: shortage of RT-PCR kits, swabs, and chemical reagents. The requirement for trained laboratory personnel and the lack of protective personal equipment to collect RT-PCR samples also plays a factor. Additionally, the number of available public labs (784 in the U.S.), and their centralization, has led to RT-PCR results taking days and even weeks (CDC 2020*b*).

Antibody testing can provide a useful aid for diagnosis. Recently, the Infectious Disease Society of America (IDSA) updated their serology Covid-19 recommendations to include testing in certain limited circumstances (CDC 2020*a*). In addition to the use of antibody testing in epidemiologic prevalence studies, the CDC recommends antibody testing for persons

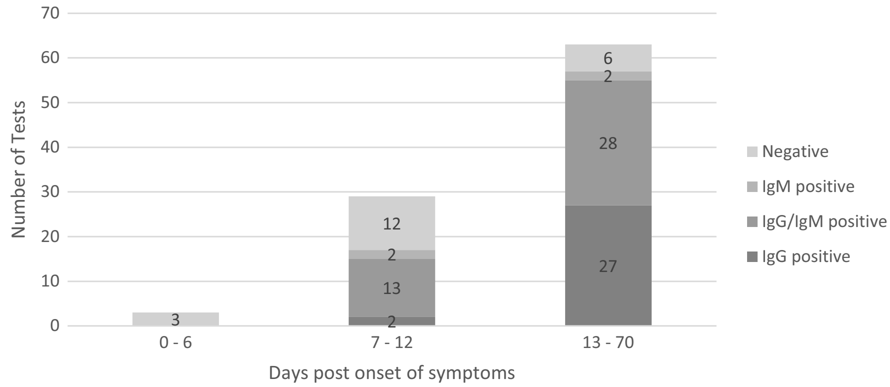

Figure 3: Number of Clungene® tests displaying IgM alone, IgG alone, or both IgM and IgG according to the number of days from the onset of symptoms.

suspected of having a post-infectious syndrome caused by Covid-19 (e.g., Multisystem Inflammatory Syndrome in Children) (CDC 2020*a*). Sharp Healthcare recommends antibody testing in the latter situation and if 2 consecutive RT-PCR tests are thought to be false negatives. This guidance discourages clinicians from using antibody testing for diagnosis and a recommendation was made to use these tests as complimentary to RT-PCR testing. We see the potential for a much broader use and recommend a combined approach that adapts using both RT-PCR and serological testing especially after the first week of illness. The advantage of the Clungene® Point-Of-Care antibody test is its simplicity since there is no need for laboratory personnel to perform and interpret results. The low rate of false positivity makes this test ideal to rule in disease and eliminate the need for further RT-PCR testing if seroconversion occurs.

Preliminary studies proposed that IgM antibodies against SARS-CoV-2 may appear earlier than IgG, and that measuring both IgM and IgG concomitantly would improve the diagnosis of SARS-Cov-2 infection (Basu et al. 2020; Guo et al. 2020; Vabret 2020; Zhao et al. 2020). A recent Cochrane Review examining the diagnostic accuracy of antibody tests in 57 publications determined combination of IgG/IgM had a sensitivity of 30.1% (95% CI, 21.4–40.7%) for 1 to 7 days, 72.2% (95% CI, 63.5–79.5%) for 8 to 14 days, 91.4% (95% CI, 87.0–94.4%) for 15 to 21 days. They also concluded there is insufficient data to estimate the sensitivity of serology 35 days or more post-symptom onset (Hanson et al. 2020). Our data supports the Cochrane review, and we see no diminution of antibody positive subjects post 35 days (100% positive in 10 subjects).

Additionally, Zhao et al found that in 173 patients with SARS-CoV-2 infection, confirmed by RT-PCR in the early phase of illness (within 7-day since onset), RT-PCR had the highest sensitivity of 66.7%, whereas the antibody assays had a positive rate of 38.3% (CDC 2020*c*). However, the presence of antibodies increased to 100% (Ab), 94.3% (IgM) and 79.8% (IgG) 15 days after the onset of symptoms. Combining RT-PCR and antibody tests significantly improved the sensitivity of the diagnosis for Covid-19 ($P < 0.001$), even in early phase of 1-week since onset ($P = 0.007$). These findings suggest that serological testing can be a critical addition to RNA detection during the illness course.

Antibody testing for diagnosis in the first twelve (12) days of illness is not recommended since most patients develop antibodies later in the course of disease. However, if RT-PCR testing is not available and patients have symptoms for more than 12 days, then the Clungene® antibody test can diagnose most infected Covid-19 patients tested. If the test is negative a recommendation should be made to have a follow up RT-PCR test. New findings from a Michigan Medicine study confirm that antibody testing is predictive of prior COVID-19 infection, and rapid screening methods – even from finger pricks – are effective testing tools (Michigan 2021).

In our study, 1 patient who had a negative RT-PCR test was enrolled and had IgM and IgG bands on our test. Upon reviewing the medical record, the patient was on Remdesivir and had bilateral infiltrates consistent with Covid-19 on chest imaging. Upon further investigation, the patient had a positive pre-hospital SARS-CoV-2 RT-PCR test. Combining PCR and antibody tests at Point-Of-Care can dramatically increase Covid-19 detection (University of Cambridge 2020).

In addition, the ability of the Clungene® antibody test to detect antibodies to the coronavirus's spike protein's receptor binding domain means it has the potential to assess the efficacy of most vaccines in development as well as convalescent plasma therapy. Recently international airports and American Blood Banks have been providing Covid-19 antibody testing services to determine whether a person has developed immunity to Covid-19 through vaccination or through contracting the virus previously (McGlynn 2021; The Blood Connection 2021). Limited evaluation of the Clungene antibody test has confirmed positive antibody test results following patients who have been vaccinated; additional study is needed to confirm the reliability of these results.

Limitations

Limitations of the study include a small sample size in 1 geographic area. The study also did not include special groups such as pregnant women or children. The subjectivity of symptom reporting by patients can be a confounding factor in determining the duration of illness. Some patients may have been symptomatic for a different time period than they recalled. To reduce patients' burden and discomfort, we opted to use leftover blood from venipuncture in inpatient setting and finger pricks in outpatient setting to collect blood. We do not know if using 1 universal method of collecting blood would have made a difference in results.

The positive predictive value of any test is dependent on the prevalence of disease in the community. If a test for a disease has 90.5% sensitivity and 100% specificity, and the disease prevalence is 10%, the positive predictive value (PPV) is 100% and the negative predictive value (NPV) is 98.95% (in a population of 10 000 people, 905 tests will be positive, and 95 of those will be false). If the disease prevalence is 50%, the PPV will be 100% and the NPV will be 91.3% (in a population of 10 000 people, 4525 tests will be positive, and 47 550 of those will be false). In areas where there is little Covid-19 community spread, the test may be suboptimal and result in a false sense of security regarding the level of immunity in the population. We understand that our findings may not be replicated in settings where seroprevalence of disease may be less than 50% as we expect in hospitalized patients. The positivity rate for the nares SARS-CoV-2 RT-PCR tests ranged between 3.5% to 10% in the Sharpe healthcare system.

Conclusion

In this pandemic crisis with significant economic and health implications, this study confirms our hypothesis that serological testing for Covid-19 can have significant utility in terms of disease diagnosis and rapid test availability. Tests such as the 15-minute Clungene® immunoassay can aid clinicians in both inpatient and Point-Of-Care settings regardless of the time and place of patient care. The guidance that a negative test does not rule out disease should always be followed. With these caveats, clinicians should be encouraged to use these serological tests with the usual caution that diagnosis should always be examined in the eye of the interpreter.

Disclosures

Christopher C. Lamb, PhD, has worked with the manufacturers of SARS-CoV-2 tests for Emergency Use Authorization submissions to the US FDA.

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Chronic mucocutaneous Candidiasis caused by a novel *STAT1* mutation: a report of 4 patients

Jenny Garkaby* and Ori Scott

ABSTRACT

Background: Chronic mucocutaneous Candidiasis (CMCC) is characterized by recurrent or persistent fungal infections of the skin, nails, and oral and genital mucosae. There are several underlying genetic causes for CMCC, with mutations in Signal Transducer and Activator of Transcription-1 (STAT1) accounting for the majority of cases.

Aim: To broaden the genotypic spectrum of CMCC caused by *STAT1* mutations.

Methods: We evaluated a young patient and her family with CMCC. Immune workup and targeted gene sequencing were performed.

Results: The proband presented at 7 years of age with persistent oral thrush. Immune evaluation revealed her cellular and humoral immunity to be within normal range. Given that her family history was significant for oral lesions in father, siblings, and paternal family members, *STAT1* gene sequencing was performed. A novel heterozygous missense c.G799A, predicting a p. Ala267Thr amino acid change within the coiled-coil domain, was identified in our patient and 3 of her family members.

Conclusion: Gain-of-function mutations in *STAT1* have been associated with a variety of phenotypes, ranging from isolated CMCC to severe fatal combined immunodeficiency, mycobacterial infections, autoimmune disorders, as well as malignancy and aneurysms. Here, we describe a novel *STAT1* mutation, c.G799A, resulting in a very mild phenotype of isolated CMCC in 4 members of one kindred.

Statement of novelty: We describe 4 patients with a mild phenotype of CMCC caused by a novel *STAT1* heterozygous mutation.

Introduction

Chronic mucocutaneous Candidiasis (CMCC) is a group of disorders characterized by susceptibility to Candidal infection of the skin, nails, and mucous membranes. The range of genetic etiologies underlying CMCC is broad, including defects in Autoimmune Regulator (*AIRE*), IL-17 pathway members (*IL17RA*, *IL17RC*, *IL17F*), Dectin-1, Caspase Recruitment Domain Family Member 9 (*CARD9*), Signal Transducer and Activator of Transcription (*STAT1*), and *STAT3* (Tangye et al. 2020).

STAT1 is a key transcription factor mediating signaling of various cytokines, notably interferons (IFN), playing roles in cell homeostasis, stress response, and defense against intracellular pathogens. Its activation is dependent upon initial phosphorylation in the cytoplasm by tyrosine kinases, of the Janus-kinase (JAK) family, formation of dimerization, and translocation to the nucleus, where it binds to promoters to impact transcription (Zheng et al. 2015). In response to IFN-γ stimulation, STAT1 forms homodimers (known as gamma-activating factor, GAF) or heterodimers with STAT3 that bind to gamma-activating sequence (GAS)

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in gene promoters. In response to IFN-α or IFN-β stimulation, STAT1 forms a heterotrimer with STAT2 and IFN-regulatory factor 9 (IRF9), also known as IFN-stimulated gene factor 3 (ISGF3) which binds to interferon-stimulated response element (ISRE) in gene promoters (Gough et al. 2008).

The clinical spectrum associated with *STAT1* GOF is broad, ranging from mild infections to life-threatening bacterial, viral and opportunistic infections, CMCC, endocrinopathies, variable autoimmune manifestations and gradually declining lymphocyte number and function (van de Veerdonk et al. 2011; Sharfe et al. 2014; Toubiana et al. 2016). Complications such as bronchiectasis (Toubiana et al. 2016; Breuer et al. 2017), intracranial aneurysms (Toubiana et al. 2016) and squamous cell carcinoma (Koo et al. 2017) have also been reported.

Herein, we report on 4 family members with a novel *STAT1* mutation, resulting in a mild phenotype of isolated CMCC.

Case presentation

Proband

A 7-year-old female was referred to the Immunology clinic for persistent oral thrush involving her tongue, buccal mucosa, and hard palate, starting at 4 years of age. She was diagnosed with CMCC confirmed by positive swabs and oral biopsy. She did not experience dysphagia or odynophagia, nor involvement of the nails, skin, or vaginal mucosa. There was no history of invasive fungal infections or other systemic infections, and no features suggestive of endocrinopathy. Review of past medical history demonstrated that she had been born at term to nonconsanguineous parents of English descent following a normal pregnancy and uncomplicated delivery. She had undergone an eye surgery for correction of strabismus at early childhood. She had been otherwise well and developed normally. Family history was significant for similar oral lesions in her 2 younger male twin siblings and father, all of whom were healthy apart from CMCC. Other paternal family members (great grandfather and great aunts) also had a history of oral fungal infections (Figure 1).

Investigations

A full immunological laboratory assessment, including complete blood-count and differential,

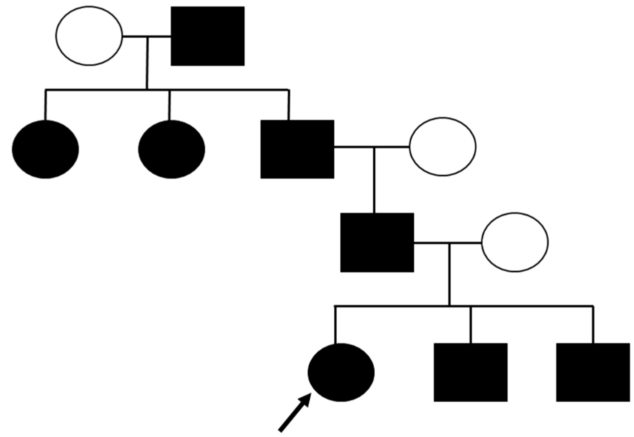

Figure 1: Pedigree of patient and family members with a phenotype of CMCC. Affected family members designated in black.

lymphocyte subsets, total immunoglobulins, and T-cell stimulation to mitogen, were all within normal range, while specific antibody responses to measles, mumps, and varicella were all non-reactive (Table 1). Thyroid and parathyroid functions normal as well. Given a history of possibly autosomal dominant CMCC, targeted gene sequencing was carried out, revealing a novel heterozygous *STAT1* gene mutation in all 4 clinically affected family members. The mutation, c.G799A, results in the amino acid change Ala267Thr affecting the coiled-coil domain (Figure 2). To our knowledge, this variant has not been previously reported in large population databases, nor in previous reports of STAT1 GOF.

Outcome

The proband (currently 14 years old), her 11-year-old twin siblings, and 48-year-old father, have continued to be well over the last 7 years, with the exception of ongoing oral CMCC requiring prolonged topical anti-fungal treatment.

Discussion

STAT1 GOF was first described in 2011 by 2 groups, with initial disease manifestations reported CMCC and autoimmunity (in particular, hypothyroidism) (Liu et al. 2011; van de Veerdonk et al. 2011). Over the next few years, the disease spectrum was expanded to include a wide host of infectious susceptibilities, autoimmunity, intracranial aneurysms, and malignancy (Sampaio et al. 2013; Sharfe et al. 2014; Toubiana et al. 2016; Koo et al. 2017).

Table 1: **Laboratory evaluation of proband at 7 years of age.**

	Value	Reference range
WBC (× 10^9/L)	8.9	4.3–11
Hemoglobin (g/L)	133	107–134
Platelets (× 10^9/L)	326	150–370
Neutrophils (× 10^9/L)	5.14	1.5–8
Lymphocytes (× 10^9/L)	2.84	1.5–7
Eosinophils (× 10^9/L)	0.15	0.02–0.05
Monocytes (× 10^9/L)	0.69	0.05–0.08
Basophils (× 10^9/L)	0.10	0.00–0.02
CD3+ (cells/μL)	2099	700–4200
CD3+/CD4+ (cells/μL)	1122	300–2000
CD3+/CD8+ (cells/μL)	786	300–1800
CD19+ (cells/μL)	424	200–1600
NK (cells/μL)	370	120–480
PHA stimulation index	390	>50% of control or >300
IgG (g/L)	11	5.4–13.6
IgM (g/L)	1	0.4–1.5
IgA (g/L)	2.6	0.3–1.5
Anti-tetanus Ab (IU/mL)	0.57	>0.1
Anti-Measles, Mumps, Varicella specific IgG	All non-reactive	—
Anti-rubella specific IgG	Reactive	—
TSH (mIU/L)	1.57	0.73–4.34
Free T4 (pmol/L)	12.5	11.4–17.6
PTH (ng/L)	53	16–63

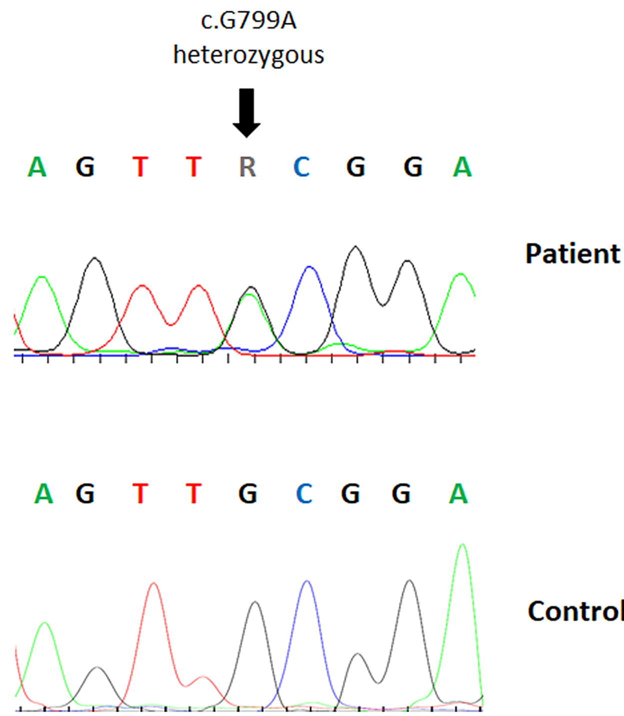

Figure 2: Electropherogram demonstrating the c.G799A missense variant in *STAT1*. The heterozygous variant was identified in the patient (upper panel), as well as her siblings and father, using targeted gene sequencing. The control sequence is shown in the lower panel.

We herein report on multiple members of one kindred, found to have a heterozygous *STAT1* gene mutation causing chronic oral Candidiasis. In this family, the onset of CMCC was in early childhood, and all affected members presented with a mild disease phenotype. The mutation, c.G799A, predicts an amino acid change A267T in the coiled-coil domain of *STAT1*, a domain involved in protein-protein interactions which plays a key role in the dimerization of STAT1 and nuclear STAT1 dephosphorylation (Levy and Darnell 2002). It is the most commonly affected domain implicated in STAT1 GOF, with the 267 residue (and in particular the A267V mutation) being the most common mutation identified in large STAT1 GOF cohorts (Toubiana et al. 2016). Clinical manifestations seen in patients with the A267V mutation have included CMCC, bacterial and viral infections, atopy, thyroid dysfunction, bronchiectasis, aneurysms, and squamous cell carcinoma. Notably, some of the more severe disease manifestations, such as malignancy, typically developed in adulthood between the third and fifth decade of life (Toubiana et al. 2016).

The current report expands the genotypic spectrum of STAT1 GOF, and supports the notion of genetic testing for an underlying immunodeficiency in patients and families with CMCC. While the patients in the current report have displayed mild disease to date, we suggest that regular and life-long follow up should be performed in all cases of STAT1 GOF, screening periodically not only for changes in immune function, but also for late-onset disease manifestations, such as malignancies. Further studies looking into establishing genotype-phenotype correlation for STAT1 GOF, are warranted and may help determine which mutations predispose patients to severe or life-threatening complications.

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Primary Immunodeficiency

There are more than 400 genetic defects and disorders of the immune system that are recognized as Primary Immunodeficiency. Approximately 29,000 Canadians suffer from forms ranging widely in severity and symptoms. Over 70% are undiagnosed.

Red Flags for **Primary Immunodeficiency**

- Repeated invasive infection (two or more pneumonias, recurrent septicemia, abscesses, meningitis).[1]
- Infections with unusual or opportunistic pathogens (PJP).[1]
- Poor response to prolonged or multiple antibiotic therapies.[1]
- Chronic diarrhea with or without evidence of colitis.[1]
- Chronic failure to gain weight and grow.[2]
- Persistent (or recurrent) unusual (atypical) or resistant to treatment oral lesions (thrush) or skin rash (erythroderma, telangiectasias, recurrent pustules/nodules/plaques).[1]
- Structurally abnormal hair (kinky, silvery) nails (dystrophic) or teeth (pointy).[2]
- Low serum IgG, chronic lymphopenia, neutropenia or thrombocytopenia.[1]
- Absent lymph nodes and tonsils or chronic enlargement of lymphoid tissues.[1]
- A family history of Primary Immunodeficiency, autoimmunity or leukemia/lymphoma. [1]

References:
[1] All age groups
[2] Infancy and childhood

Written and approved by the Scientific Director and the Medical Advisory Board

Early diagnosis and treatment are vital in saving lives. Treatment can improve or prevent long term organ damage. Each Red Flag alone should alert healthcare providers to the possibility of Primary Immunodeficiency and require further testing and investigation. Two or more Red Flags should trigger an urgent referral to an Immunologist.

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