LymphoSign Journal

The journal of inherited immune disorders

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Created in 1999, Immunodeficiency Canada is a national organization working closely with diagnostic and treatment centres across Canada. It plays a vital role in being one of the first organizations in Canada to finance and encourage research in the field of primary immunodeficiency, as well as, providing education for patients and medical professionals. Programs and services are provided through the support of generous donors.

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TABLE OF CONTENTS

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Scope

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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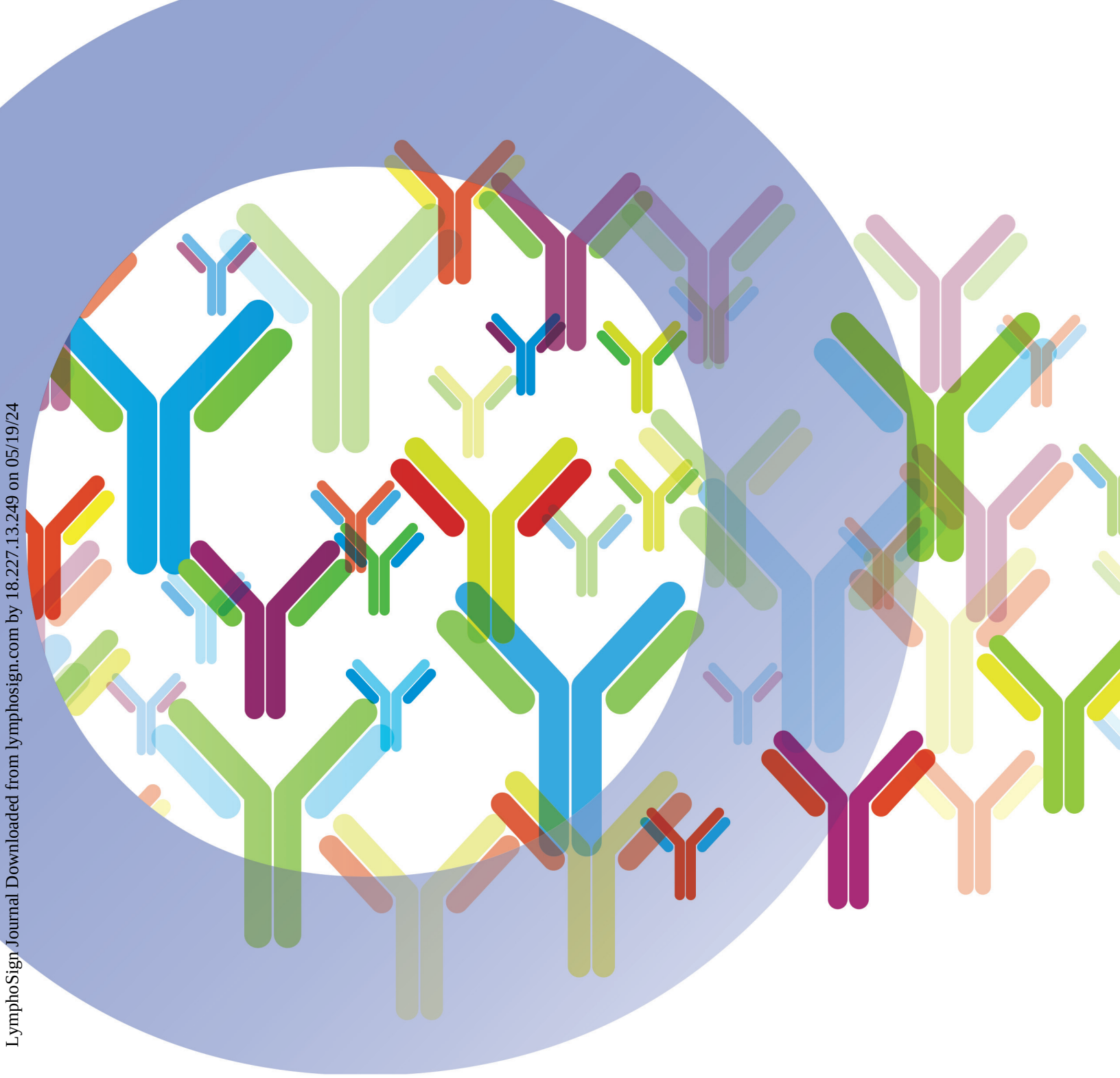

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COVID-19 post-vaccination recommendations for primary immunodeficiency

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

This has been a very difficult and challenging time for humanity to combat the coronavirus disease 2019 (COVID-19) pandemic (Zhu et al. 2020). Science stood up to the challenge in the most admirable manner by producing an unprecedented vaccine against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Polack et al. 2020; Sahin et al. 2020; Baden et al. 2021; Sadoff et al. 2021). This highly effective vaccine was also recommended and administered to individuals with inborn errors of immunity that lead to primary immunodeficiency (PID) (Roifman and Vong 2021). While multiple studies have confirmed the efficacy of the vaccine in preventing significant disease in the general public (Dagan et al. 2021; Haas et al. 2021; Lopez Bernal et al. 2021), this protective effect has not been thoroughly evaluated in immune compromised hosts (Barda et al. 2021). In addition to the inherents fault in their immune system, patients with immune disorders were also sheltered from exposure to the virus, making vaccine efficacy evaluation difficult (Meyts et al. 2021; Shields et al. 2021; Quinti et al. 2020; Marcus et al. 2021).

PID encompasses a growing number of patients with a common set of manifestations including recurrent and (or) severe microbial infections, autoimmune features, and increased association or predisposition to cancer (Bousfiha et al. 2020). PID is a highly heterogeneous group of disorders caused by genetic variation in more than 450 different immune-system related genes. Clinical presentation and immune lesions frequently vary widely among different gene defects, different variants in the same gene, and even cases within the same family bearing the same mutation.

While the various components of the immune system normally function interdependently, the humoral and cellular components play a critical role in the response to vaccines (Pulendran 2014). Most immune disorders can be classified as adversely affecting mostly immunoglobulin and antibody production (humoral), predominately T cell deficiency (cellular defect) or a combination of both (combined immunodeficiency) (Roifman et al. 2012). Upon exposure to a pathogen (virus, bacteria), both arms of the immune system co-operate to produce adequate antibodies as well as propagating some T cell populations critical in battling infection in response to microbial exposure or vaccines (Igietseme et al. 2004; Crotty 2015).

The lack of response to vaccination in some extreme cases of profound T cell deficiency, such as severe combined immunodeficiency (SCID), can be predicted. But, in most other immune defects the degree of response and resulting level of protection can be highly variable and difficult to predict, hence, the recommendation by experts to offer vaccination to all these patients (Bonilla 2018).

Evaluation of vaccine efficacy is routinely done by measuring titer levels of specific antibodies and less frequently by studying in vitro T cell responses

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(Paris 2020). In the case of COVID-19, these assays have either not yet been standardized (antibody levels) or are not available for clinical use (T cell responses). This limits further the ability to discern the effect of COVID-19 vaccination in patients with PID.

Many patients with PID are treated with immunoglobulins (IgG) for antibody deficiency (Roifman et al. 2008; Betschel et al. 2019). This form of passive immunization encompasses protein fractions extracted from multiple blood donors. Immunoglobulin replacement products (IVIG, SCIG) available in Canada are almost invariably obtained from US donors. Given the widespread infection as well as vaccination rates in the US it is expected that IgG products would contain a significant level of anti-SARS-CoV-2 antibodies (Romero et al. 2021). Consequently, recommendations for PID patients may differ from the relaxing measures offered to the general public.

Precise data on anti-SARS-CoV-2 antibody levels and possible lot-to-lot variations of these products remain scarce, and may be complicated by false-positivity associated with cross-reactive antibodies (Dalakas et al. 2021). While this passive immunization against COVID-19 is a welcome benefit to those patients, it may be dampened by the fact that administration of exogenous IgG can suppress endogenous production of antibodies (Tacke et al. 2013) in response to active immunization with the COVID-19 vaccine.

All these complex issues require individual evaluation of each PID patient by their physician in order to apply the most appropriate course of testing, treatment, or protections measures. In general, it is recommended that until more credible information (scientific evidence) becomes available, patients with PID, in particular those with combined immunodeficiency, should exercise extra precautions as long as COVID-19 and its variants continue to spread in the community (Table 1). This includes proper distancing measures and wearing effective masks (medical grade) in enclosed and crowded spaces (Roifman 2020).

Booster dose of the COVID-19 vaccine

For PID patients, many of whom develop only partial responses to vaccination, a booster dose would provide enhanced protection against COVID-19. On 12 August 2021, the U.S. Food and Drug Administration authorized the use of an additional (third) dose of the Pfizer-BioNTech and Moderna COVID-19 vaccines in people who are immunocompromised (FDA 2021). This group includes patients with PID, individuals on immunosuppressant medications, and the elderly (age >65 y). The third dose can be administered at least 28 d following the second dose of the COVID-19 vaccine. Similarly, on 18 August 2021, Ontario announced plans to authorize third doses of the Pfizer-BioNTech or Moderna vaccines in individuals who are severely immunocompromised (including transplant recipients, those treated with anti-CD20 agents, or undergoing treatment for malignant hematologic disorders), and elderly residents in long-term care homes or high-risk group settings. PID patients should discuss this option with their physician or specialist.

Antibody testing after COVID-19 vaccination

Vaccination against COVID-19 activates the humoral and cellular components of the host immune system, in the same manner as natural exposure to the SARS-CoV-2 virus, leading to the production of B cell-dependent antibodies and mobilization of T cell-dependent pathogen clearance and protective mechanisms (Roifman and Vong 2021). Since the COVID-19 vaccination program began in Canada, over 71% of the population (as of 12 August 2021, https://covid19tracker.ca/vaccinationtracker.html) have received at least 1 dose of an authorized COVID-19 vaccine. It is noteworthy that initial vaccine trials did not include patients with PID, thus, it is not known whether the level of protection seen in the general population would be reflected in this cohort. Nevertheless, COVID-19 vaccination is broadly recommended for those with PID (in consultation with an immunologist) as some measure of protection may still be in place, even if antibody responses remain low/limited.

Specific IgG antibodies against SARS-CoV-2 are generally detectable 14 d after natural exposure, or for the SARS-CoV-2 vaccine, 14 d after the second vaccine dose (Iacobucci 2021; Lou et al. 2020). Antibody (serological) assays which measure specific antibody titer levels can help identify whether an individual is able to mount an immune response following vaccination.

Table 1: **COVID-19 post-vaccination recommendations.**

Protective response to COVID-19 vaccination in PID patients with humoral or cellular defects may be variable or incomplete. Thus:

- Evaluation of the efficacy of the vaccine in PID is desirable and should be pursued once appropriate standardized testing becomes available, or, following discussion with your physician.
- PID patients are advised to continue to exercise precautions, including hand hygiene, social distancing and masking, especially in indoor settings but also in outdoor crowded spaces.
- A boost (third dose) of the mRNA-based COVID-19 vaccine is recommended for immunocompromised individuals, especially PID patients and individuals treated with immunosuppressant drugs.
- Continue to monitor/self-monitor for signs of COVID-19 infection and seek virus testing.
- If tested positive, contact your physician and specialist for implementation of a proper management plan.

It is important to note that, at present, **it is unclear what levels of antibodies are needed to provide protection against COVID-19**. This lack of a defined correlate of protection (a threshold value to serve as a standard) makes it difficult to determine the amount of protection PID patients have against COVID-19, even if specific antibodies are present. Further, antibody titers are just one (of several) indicators of protection. For example, measurement of lymphocyte proliferation in response to antigen stimulation, a process dependent on antigen-specific T cells that develop during initial (prior) exposure, may provide additional clues about an individual's T cell function (Roifman et al. 2012). However, such tests remain research-based and not yet widely accessible.

In Canada, there are currently 22 authorized SARS-CoV-2 antibody assays available for clinical use. These measure antibodies (IgM, IgA, IgG) against highly immunogenic, structural components of SARS-CoV-2, such as the surface spike protein and the intracellular nucleocapsid protein (Wang et al. 2020).

The detection of antibodies against the spike protein (elicited by COVID-19 vaccines as well natural exposure to SARS-CoV-2) can indicate an immune response after vaccination, however, can also indicate previous infection with the virus. Conversely, the presence of antibodies against the nucleocapsid protein suggests previous exposure to SARS-CoV-2, but cannot be used as a marker of SARS-CoV-2 post-vaccination responses.

Results of testing must be interpreted with caution, taking into account the patient's baseline immune function and other temporal contexts. For example, test results for either the spike protein or nucleocapsid antibody may be negative in individuals with immune defects, in which the ability to produce antibodies are hampered. Results may also be negative if there has been insufficient time for antibodies to develop (i.e., test is performed too early after vaccination), or if peak antibody levels have already waned (i.e., test is performed beyond 5 mo post-vaccination) (Aziz et al. 2021). While the clinical utility of post-vaccination testing is not yet fully established, for specific cohorts, particularly patients with PID, antibody titer testing can guide discussions on additional measures needed to protect against COVID-19.

SARS-CoV-2 antibody testing for PID patients

Can be used to measure antibody production in response to COVID-19 vaccination, however, should be interpreted with caution as:

- Levels of antibodies required for protection against COVID-19 are not known
- Clinical utility of post-vaccination testing is not yet established
- Variability in detection exists among available assays

Interpretation of SARS-CoV-2 antibody testing results

Spike protein antibody result: positive

Detectable levels of antibodies against SARS-CoV-2 are present (due to vaccine or exposure to the virus)

Additional:

- PID patients should continue to take protective measures against COVID-19 exposure
- A positive result does not equate to complete protection against COVID-19

- Very rarely, a false-positive result may be present if an individual has been previously exposed to other coronaviruses (the nucleocapsid protein in SARS-CoV-2 has 90% homology with another coronavirus, SARS-CoV-1 (Marra 2003)).

Spike protein antibody result: Negative

No detectable levels of antibodies against SARS-CoV-2 are present

Additional:

- PID patients should continue to take protective measures against COVID-19 exposure
- A negative result can occur if testing was done too early (or too late) after vaccination
- The sensitivity of antibody testing in PID patients is not known
- It is not clear how long antibodies remain after vaccination, and can be different in individuals with PID

Nucleocapsid protein antibody result: positive

Detectable levels of antibodies against SARS-CoV-2 are present due to exposure to the virus

Additional:

- Very rarely, a false-positive result may be present if an individual has been previously exposed to other coronaviruses
- A positive nucleocapsid protein antibody titer result, in conjunction with a positive spike protein antibody titer levels, may indicate an immune response due to exposure to the virus (rather than vaccination)

Nucleocapsid protein antibody result: negative

No detectable levels of antibodies against SARS-CoV-2 are present

Additional:

- Individuals with PID may have false negative nucleocapsid antibody titer results
- A negative result in the context of PID should not be used to rule out prior exposure to the virus or infection status

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Children should be offered vaccination against COVID-19

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Since the start of the COVID-19 pandemic, there has been conflicting evidence on SARS-CoV-2 infection and transmission in children (Stawicki et al. 2020; Bialek et al. 2020; Wu and Mcgoogan 2020). Early studies reported only anecdotal outbreaks in school settings and low case numbers in children (Danis et al. 2020), driving speculation that the virus may not be as easily spread in this age group (Cao et al. 2020; Goldstein et al. 2020; Ludvigsson 2020*b*). However, these reports are unlikely to have represented the true frequency of infections, given the widespread school closures implemented to cut transmission opportunities and limitations of swab testing in children (lower uptake, swab volumes) resulting in missed cases (Corman et al. 2021; Han et al. 2021). Indeed, subsequent studies measuring viral load in children reveal similar levels and trajectories as adults, indicating that children can readily transmit the virus (Jones et al. 2021; Jacot et al. 2020, Yonker et al. 2020; Baggio et al. 2021). Moreover, in children under 5 years, significantly higher levels of SARS-CoV-2 viral nucleic acid have been detected (Heald-Sargent et al. 2020), while those under 3 years of age are more likely to transmit the infection compared to older siblings (Paul et al. 2021). Together, current available evidence confirms that children, even if asymptomatic or with mild disease, are an important source of SARS-CoV-2 who can accelerate infections throughout communities.

The highly contagious B.1.617.2 (delta) variant, first identified in December 2020 in India (ECDC 2021), has now been detected in 130 countries worldwide. The delta variant is more transmissible than earlier SARS-CoV-2 strains and confers greater risk of hospitalization, ICU admission and death, particularly in those who are unvaccinated (Sheikh et al. 2021; Fisman and Tuite 2021). In response to a recent outbreak in Massachusetts, U.S., where 74% (346/469) of delta variant-infected cases were found to be fully vaccinated and 79% of those (274/346) were asymptomatic (Brown et al. 2021*a*), the U.S. Centers for Disease Control and Prevention updated their guidance to recommend masking indoors (28th July 2021 (CDC 2021)).

Vaccination against COVID-19, currently authorized for those 12 years and older, remains the most effective way to prevent symptomatic disease and more severe outcomes (Lopez Bernal et al. 2021*b*; Hall et al. 2021; Shrotri et al. 2021; Dagan et al. 2021). In Canada, there are presently 4 COVID-19 vaccines available, two based on mRNA technology (Pfizer-BioNTech (BNT162b2), Moderna (mRNA-1273)), and two utilizing viral-vector platforms (AstraZeneca (ChAdOx1-S), Janssen (Ad26.COV2.S)). A newly developed COVID-19 vaccine by Novovax (NVX-CoV2373), based on the more traditional 'protein-subunit' approach (similar to the pertussis and hepatitis B vaccines), recently reported results of its phase III trials in adults (Heath et al. 2021). Unlike the mRNA or viral-vector COVID-19 vaccines, NVX-CoV2373 contains parts of the recombinant SARS-CoV-2 spike protein and an adjuvant to stimulate host immune responses. The overall efficacy for preventing

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symptomatic disease was shown to be comparable to mRNA vaccines (89%), with the benefit of both fewer and milder adverse effects — an important consideration when deciding among vaccines for children. While not yet authorized for use in Canada, it is expected that NVX-CoV2373 will become available within the coming months.

Since the start of the vaccination program in Canada, SARS-CoV-2 infections have occurred predominately in the unvaccinated population (89.4% versus 0.6% in those who are fully protected). Similarly, of those who were hospitalized or died, unvaccinated individuals accounted for 84.8% and 82.1%, respectively (Goldstein et al. 2020). For the delta variant, studies estimating vaccine efficacy after 2 doses suggest reduced levels of protection against symptomatic disease compared to the original SARS-CoV-2 strain (Pfizer-BioNTech vaccine: 88% vs. 93.7%, respectively; AstraZeneca COVID-19 vaccine: 67% vs. 74.5%, respectively) (Lopez Bernal et al. 2021*a*). Additionally, over a 7-month period in which the predominant SARS-CoV-2 strains changed from the alpha to delta variant, a separate study reported a 2-fold risk reduction against breakthrough infections in those vaccinated with the Moderna compared to the Pfizer-BioNTech vaccines (Puranik et al. 2021). Together with the observed decline of antibody levels over time, a strong argument for a booster shot can be made (Roifman and Vong 2021*a*).

Now, as Canada enters a fourth wave with a strong resurgence of cases, it is children who are most vulnerable to infection and should be fast-tracked for vaccination. Early reports that children don't develop severe symptoms of COVID-19 disease are being surpassed by evidence of long-term effects, some lasting months after the initial infection (Thomson 2021; Buonsenso et al. 2021; Ludvigsson 2020*a*). Serious complications, including multi-system inflammatory syndrome, have also been reported (Riphagen et al. 2020; Waltuch et al. 2020), and there are likely other effects that have yet to be accounted for.

In May 2021, the delta variant accounted for just 8% of positive cases in Ontario, while in July those cases jumped to 78% (Brown et al. 2021*b*). Documentation of 72,000 new pediatric COVID-19 cases in the U.S., the largest weekly increase since the start of the pandemic (American Academy of Pediatrics 2021), prompted the American Academy of Pediatrics and Children's Hospital Association to urge the FDA to fast-track the review of COVID-19 vaccines for pediatric cohorts (<12 years) (Beers 2021). Especially concerning are reports of increased hospitalizations of children infected with the delta variant, with pediatric ICU beds at maximum capacity in numerous hospitals across the U.S. (Conlen et al. 2020). Although the numbers are low compared to adult admissions, to date, they are the highest recorded for children from the start of the pandemic.

Taken together, it is imperative that COVID-19 vaccines are made available for children under 12 years of age as soon as possible. With children returning to school in a matter of weeks, the lack of protection exposes them not only to greater risk of infection and complications, but also of spreading the virus throughout communities and potentially becoming a source for new variants. For those who have compromised immune systems, including children and adults with primary immunodeficiency (Roifman 2020), gatherings among unvaccinated individuals (particularly in the classroom setting) poses a high risk for SARS-CoV-2 transmission (Roifman and Vong 2021*a*, 2021*b*).

Recommendations

1. We urge Health Canada to approve the use of COVID-19 vaccines in school-aged children (5–12 years).
2. We support the recommendations of the American Academy of Pediatrics and Children's Hospital Association in urging the FDA to approve COVID-19 vaccines for school-aged children.
3. Pediatricians should be given the ability to provide off-label COVID-19 vaccine doses for children aged 10–12, if developmentally appropriate.
4. The planned one third dosage of the Pfizer-BioNTech vaccine (10 micrograms compared to 30 micrograms currently administered in adults) for children aged 10-12 may not be sufficient for protection against SARS-CoV-2 and should be investigated further.
5. Given the high reported efficacy and relatively lower side effects identified in clinical studies of the Novavax vaccine, it may, in the future, be considered for use in children. Because it is a more a traditional type of vaccine, it could also be preferred by both parents as well as caregivers.
6. The decision to vaccinate children should be made in consultation with your physician/healthcare

provider. Meanwhile, protective measures including social distancing, hand hygiene, and masking should continue as the mainstay for protection against COVID-19.

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Original Article

A case of common variable immune deficiency with lung disease–not just bronchiectasis

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ABSTRACT

Introduction: Common Variable Immune Deficiency (CVID) is the most prevalent form of severe antibody deficiency in children and adults. Most patients suffer recurrent, mainly sinopulmonary, infections. Despite adequate IVIG replacement therapy, chronic lung disease continues to be a main cause of morbidity and mortality. The term granulomatous-lymphocytic interstitial lung disease (GLILD) is frequently used to describe interstitial lung disease associated with immune dysregulation in primary antibody deficiency, such as CVID.

Aim: To describe the case of a 10-year-old male with CVID who developed GLILD and his response to treatment with Rituximab.

Discussion: Our patient is a young male with CVID and no genetic diagnosis, whose lung functions and general condition continued to deteriorate despite adequate intravenous immunoglobulin replacement therapy and mycophenolate mofetil treatment. After the diagnosis of GLILD, we initiated treatment with a 4-dose weekly course of Rituximab with prompt resolution of his interstitial disease. Although GLILD is a well described condition that accompanies CVID as a manifestation of immune dysregulation, it is still under recognized, especially in the pediatric population. Among experts, there is little uniformity when it comes to diagnostic and treatment approaches. Recent studies showed improved outcomes when using combination therapy with Rituximab, such as in our patient.

Statement of Novelty: We shed light on GLILD, an important condition that accompanies CVID, and demonstrate an excellent response to the steroid sparing agent Rituximab. This is a crucial aspect when considering therapeutic choices for the pediatric population.

Introduction

Common Variable Immune Deficiency (CVID) is the most prevalent form of severe antibody deficiency in children and adults. It is not a single disease but rather a collection of hypogammaglobinemia syndromes resulting from many genetic defects. Despite great advances in genetic sequencing and diagnosis in the last decades, in most cases, the cause is still unknown. CVID is defined by the following laboratory criteria: markedly reduced serum concentrations of immunoglobulin (Ig) G, in combination with low levels of IgA and/or immunoglobulin IgM, poor or absent response to immunizations, and an absence of any other defined

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immunodeficiency state. When these laboratory findings are accompanied by a relevant clinical phenotype, the diagnosis of CVID is confirmed.

Clinical phenotypes of CVID vary. Most patients have recurrent infections, mainly sinopulmonary infections. Other clinical features include lymphoproliferation, autoimmune cytopenia, and granulomatous disease (Bousfiha et al. 2020). The mainstay of treatment consists of antibiotic prophylaxis and IVIG replacement therapy (IGRT). In selected cases HSCT is considered. Despite adequate IGRT, chronic lung disease continues to cause morbidity and mortality in CVID patients.

Respiratory infections can cause structural lung damage that may promote chronic pulmonary disease. Delay in diagnosis is associated with fixed pulmonary obstruction, chronic atelectasis, pulmonary fibrosis, and bronchiectasis. Chronic lung disease may also develop because of immune dysregulation independent of infection or deficiencies in host defense that are not alleviated by IVIG or antibiotic prophylaxis (Maglione 2020). Interstitial lung disease develops in 10-20% of patients with CVID. The term granulomatous-lymphocytic interstitial lung disease (GLILD) is frequently used to describe the interstitial lung disease associated with primary antibody deficiency (Maglione 2020).

We hereby present a patient whose case highlights the importance of recognizing this condition and offer appropriate treatment.

Case Report

A 10-year-old male patient was referred to our immunology clinic in 2018. His prior medical history included atopic dermatitis, recurrent otitis media, and recurrent pneumonia. He presented with recurrent fever and a productive cough. He had no relevant family history and was of Russian decent. His physical exam revealed lymphadenopathy and splenomegaly. Auscultation of the lungs revealed distinct rales.

His laboratory workup showed leukopenia, lymphopenia, and microcytic normochromic anemia. Shortly after initial presentation he also developed thrombocytopenia. His direct and indirect Coombs test was positive. He underwent a basic evaluation for ALPS (autoimmune lymphoproliferative syndrome) which demonstrated a slightly elevated population of DNT cells of 2.7% in addition to elevation of B12, IL-10, SOL–IL2, and FAS-L.

When examining his immunoglobulin levels, we could see that he had a profound antibody deficiency. His IgG levels were 185 (mg/dL), IGA–<6, IgM–21.1, IgE <4.54. He also had a poor immunological response to the vaccinations he received — tetanus, diphtheria, strep. Pneumonia (Table 1). His lymphocyte subpopulations phenotype was normal.

Imaging included a chest X-ray which was prominent for interstitial infiltrates, left lower lobe infiltrate and hilar enlargement. CT scan demonstrated the typical radiological pattern of bronchiectasis in addition to bilateral enlarged hilar lymph nodes, enlarged mediastinal & axillar lymph nodes and a subcarinal mass in the posterior mediastinum, enveloping the main bronchi. There was splenomegaly (16 cm) on abdominal ultrasound. PET CT localized a "hot" axillar gland. A lymph node biopsy ruled out malignancy and demonstrated follicular hyperplasia. Spirometry showed a restrictive pattern with no reversibility, and normal lung volume. Diffusing capacity for carbon monoxide (DLCO) was normal. Bronchoscopy was consistent with chronic airway inflammation. A working diagnosis of CVID was established and he was started on IVIG replacement therapy. He also received azithromycin prophylaxis. Whole exome sequencing did not reveal a recognized genetic disorder.

A year after first presenting to our clinic he developed worsening pancytopenia. Due to his worsening condition, we added mycophenolate mofetil (MMF) to his treatment regimen. He remained cytopenic but with stable counts and his lymphadenopathy lessened.

Unfortunately, due to poor compliance the family stopped treatment with MMF. He presented in 2020 with weight loss and worsening respiratory symptoms. CT demonstrated worsening of all previous findings, further enlargement of the spleen, and diffuse lymphadenopathy (Figure 1). There was a decline in total lung capacity on spirometry and also his DLCO was diminished. His immunoglobulin levels were stable. He had pancytopenia (leukopenia 1000–2000, lymphopenia 500–900, neutropenia 400–600, Hb 10.5–12, PLT 75K). Flow cytometry showed low B cells (CD19–2.5%, CD20–2.5%), and absence of memory B cells (CD27

Table 1: **Immune evaluation of our patient's lymphocyte subpopulations prior to the beginning of his rituximab treatment course and also his immunoglobulin basic levels and response to vaccinations prior to the start of his immunoglobulin replacement therapy.**

Parameter	Result	Normal Range
WBC ($\times 10^3$ cells/μL)	1.85	4.5–13.5
LYM (%)	49	28–45
LYM ($\times 10^3$ cells/μL)	0.9	1.3–6
B LYM CD19 (%)	2.5	5–20
B LYM CD19 (cells/μL)	22.5	
B LYM CD20 (%)	2.5	5–20
B LYM CD20 (cells/μL)	22.5	
HLA DR (%)	6.1	10–28
ACTIVATED LYM HLA DR (cells/μL)	54.9	
T LYM CD2 (%)	92.4	73–95
CD2 (cells/μL)	831.6	
T LYM CD3 (%)	84.2	58–85
T LYM CD3 (cells/μL)	757.8	
T HELPER LYM CD4 (%)	51	32–57
T LYM CD4 (cells/μL)	459	
T SUPPRESSOR CD8 (%)	27.5	15–40
T SUPPRESSOR CD8 (cells/μL)	247.5	
HELPER/SUPPRESSOR	1.85	0.8–2.95
IL-2 RECEPTOR CD25 (%)	1.8	0–5
IL-2 RECEPTOR CD25 (cells/μL)	16.2	
CD3-CD16+CD56+ (%)	12.2	—
IgG (mg/dL)	185	400–1850
IgA (mg/dL)	<6	38–420
IgM (mg/dL)	21.1	28–140
IgE (IU/mL)	<4.54	0–100
Tetanus Antibody (IU/mL)	0.02 mL	—
Diphtheria Antibody (IU/mL)	<0.01 mL	—
Pneumococcal Antibody (mg%)	0.1	2–9999

+CD19−0%) (Table 1). Due to his dramatic clinical decline, we proceeded with a lung biopsy. Pathology showed diffuse lymphoid hyperplasia (Figure 2) with positive immunostains for CD3 and CD20. This is consistent with a diagnosis of granulomatous lymphocytic interstitial lung disease (GLILD).

Due to the presence of both B and T cells in the lung infiltrate of GLILD patients, we added a 4-dose course of Rituximab (dose 1 week apart) to his treatment and also optimized his IGRT to achieve trough levels of at least a 1000 mg/dL. CT scan 2 months after completion of Rituximab therapy showed nearly complete remission of lung disease with residual findings of his bronchiectatic lesions (Figure 1). His lung functions significantly improved.

Discussion

Although GLILD, a chronic lung disease which is a manifestation of the immune dysregulation that accompanies CVID is well described, it is still under recognized especially in the pediatric population.

Alterations in T cell function, generalized immune dysregulation, greater infection susceptibility, and/or the presence of pathogenic B cells may be fundamental to the development of interstitial lung disease in primary antibody deficiency. The presenting symptoms and physical examination are non-specific and high-resolution computed tomography is vital for the evaluation. A lung biopsy confirms the diagnosis while ruling out malignancy. Pathology is typically consistent with one or more forms of benign pulmonary lymphoproliferation. Granulomatous inflammation and organizing pneumonia may also be found in lungs of patients with primary antibody deficiency together with one of the lymphoproliferative pathologies (Maglione 2020).

Predominant cells in the infiltrate are CD4+ T cells, but nodules of CD20+ B cells surrounded by CD4+ T cells are also found, mainly localized to the interstitium. Regulatory T cells are absent in the lungs in GLILD (Baumann et al. 2018).

In patients with CVID, interstitial lung disease frequently occurs in conjunction with lymphoid hyperplasia in other tissues, such as in our patient. The pulmonary lymphoid hyperplasia that characterizes the interstitial lung disease seen in primary antibody deficiency may reflect systemic immune dysregulation inherent to the patient (Maglione 2020).

The European GLILD network (e-GLILDnet) aims to describe how GLILD is currently managed in clinical practice. They developed and conducted an online survey facilitated by the European Society for Immunodeficiencies (ESID) and the European Respiratory Society (ERS) between February and April 2020. There was little uniformity in diagnostic or therapeutic interventions.

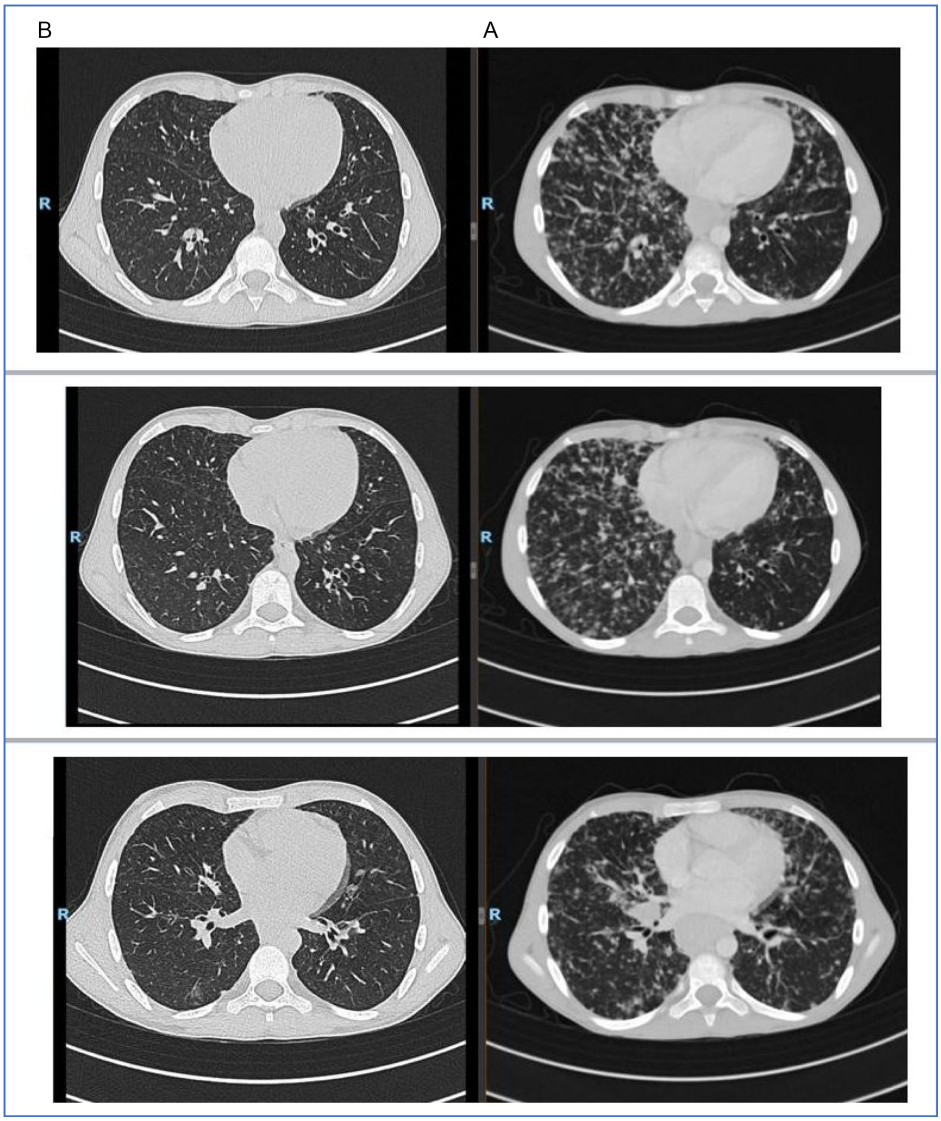

Figure 1: On the right column (A) are the radiological features consistent with his GLILD diagnosis, including prominent lymphadenopathy. On the left (B) are the same CT sections after a treatment course with Rituximab, demonstrating prompt resolution of pulmonary findings.

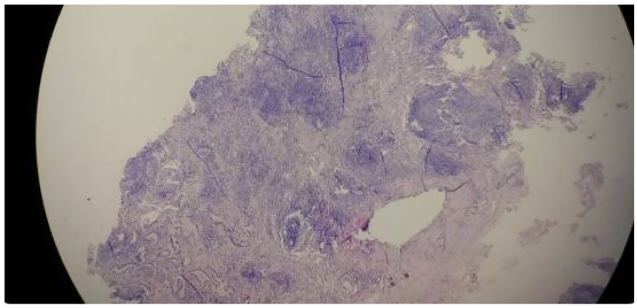

Figure 2: Lung biopsy of our patient. Lung pathology showing diffuse lymphoid hyperplasia /LIP, consistent with a diagnosis of GLILD.

Sixty-six percent used steroids for remission-induction and 47% for maintenance therapy. Azathioprine, Rituximab, and MMF were the most frequently prescribed steroid-sparing agents. Pulmonary function tests were the preferred modality for monitoring patients during follow-up (van de Ven et al. 2020). It should be noted that in a consensus statement from the British Lung Foundation, it is stated that expectant management is a viable option, and not to treat a patient who is asymptomatic with normal and stable lung function (Hurst et al. 2017).

In our case, the patient was symptomatic and had a decline in lung function as his status deteriorated. As such, we decided to add another immunosuppressive agent and he received a course of Rituximab. He continued his other treatments as well. Verbsky et al. (2021) demonstrated in a group of 39 patients who had CVID and GLILD that combination therapy with Rituximab improved lung function and CT scores. Especially in young children, it is important to have a reliable steroid sparing agent and this protocol shows very promising results.

Our patient showed prompt resolution of pulmonary findings after Rituximab therapy and we will continue to monitor long term treatment effects.

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Original Article

Elevated serum gamma globulins in apparently healthy Nigerians living in Ogbomoso: a possible manifestation of phagocytic dysfunction

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ABSTRACT

Background: Serum protein abnormalities, particularly elevated gamma globulins (hypergammaglobulinemia, HGG), have been reported in apparently healthy Nigerians living in Ogbomoso and elsewhere. Since the mechanisms for this phenomenon have not been fully substantiated, we hypothesized that impaired neutrophil phagocytosis could contribute to this condition.

Methods: Healthy humans exhibiting HGG were identified using serum protein electrophoresis performed on cellulose acetate gel in barbital buffer (pH 8.6). GelQuant image analysis and quantitation software were further employed to quantify the gamma globulin fraction. Neutrophils were isolated from K3EDTA anticoagulated peripheral blood using Histopaque neutrophil isolation reagent. Neutrophil phagocytic activity was analyzed using a non-subjective commercial colorimetric phagocytosis assay kit.

Results: The purity and viability of isolated neutrophils were approximately 94% and 92%, respectively. Ex-vivo phagocytic activity of neutrophils isolated from apparently healthy subjects exhibiting HGG, expressed as a percentage of the average absorbance of the control group, was $48.1 \pm 8.6\%$ which was significantly lower ($p < 0.05$) compared to the controls ($98.9 \pm 14.3\%$).

Conclusion: Since neutrophils play crucial roles in innate immune responses, impairment of neutrophil phagocytic activity may lead to persistent antigenic stimulations of the adaptive immune system. This could in turn orchestrate gamma globulins expression leading to HGG.

Statement of novelty: We demonstrated reduced neutrophil phagocytic activity as a possible basis for hypergammaglobulinemia in healthy Nigerians, perhaps for the first time.

Introduction

Phagocytosis is defined as a receptor-mediated process in which targeted particles are engulfed and degraded. Neutrophils are the predominant leukocytes in peripheral blood and from there, they are mobilized to the sites of infection (Teng et al. 2017; Leach et al. 2019). Although neutrophils do not possess the properties for adaptive recognition of antigens, nevertheless, they mediate early innate immune responses to infection and display the capacity to modulate the adaptive immune response (Ley et al. 2018; Papayannopoulos 2018; Silvestre-Roig et al. 2019; Rosales 2020). Neutrophils are also important sources of pro- and anti-inflammatory cytokines, thus participating in host defenses through a variety of phenotypically

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simple but mechanistically complex processes (Tamassia et al. 2018; Gideon et al. 2019; Kumar 2020). Other neutrophil functions include chemotaxis, respiratory burst activity, direct bacterial killing, and antibody-dependent cell-mediated cytotoxicity, and most of these functions can be demonstrated in vitro (Lehman and Segal 2020).

Immunoglobulins are glycoproteins produced by plasma cells and are key effector factors of the adaptive immune system. The Fc regions of IgG molecules are involved in complement and antibody-dependent phagocytosis (Quast et al. 2017). IgG is the most abundant immunoglobulin and therefore constitutes most of the gamma region in serum protein electrophoresis (Adedeji et al. 2014). Hypergammaglobulinemia (HGG) is found in many situations. In the setting of primary immunodeficiency, HGG could, for instance, be related to a defect in T cell function, liver diseases, malignancies, autoimmune diseases, and infections (Upton 2014). Although immunodeficiency may be associated with a significant decrease in plasma IgG, IgM, or IgA isotypes, some immunodeficiency conditions with normal or elevated levels of immunoglobulins have been documented (Conley et al. 1999; Adedeji et al. 2014; Pimenta et al. 2019). Furthermore, neutrophil functions are reduced in the immunodeficiency state, such as in HIV-infection (Dantas et al. 2015). Some immunodeficiency diseases are very well described as having high levels of a particular immunoglobulin. For instance, hyper IgM usually results from the impaired ability of B cells to undergo immunoglobulin class-switching, while IgE usually predominates in hyper IgE syndrome as well as other conditions (Lo et al. 2013).

Previous studies have shown that some healthy humans have exhibited HGG (Buadi et al. 2011; Adedeji et al. 2015). This phenomenon was first recognized in Nigerians living in Britain as far back as the 1950s (Schofield 1957), but the precise immunological basis for this condition is still emerging. Since neutrophils are crucial in the first line of defense and immunoglobulins are expressed following antigenic stimulation, we hypothesized that impaired neutrophil phagocytic function would contribute to this phenomenon. Although phagocyte dysfunction has been reported in pathologic conditions (Carneiro et al. 2012; Teng et al. 2017), data on phagocytic activity of neutrophils isolated from peripheral blood of apparently healthy individuals exhibiting HGG are scarce. Thus, we investigated whether neutrophil dysfunction is associated with HGG in apparently healthy individuals, as this could provide a possible basis for this phenomenon.

Methods

Subject selection

This is a cross-sectional study where 100 healthy undergraduates in the Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso (Supplementary Material[1]), Nigeria, who met the selection criteria were recruited. Volunteers were required to complete a structured questionnaire. Those with a medical history that could influence the results at the time of enrolment were excluded from the study. Blood samples were collected from the participants after overnight fasting. The serum was separated by centrifugation and stored at −20 °C. The study was approved by the Ethical Committee of the Faculty of Basic Medical Sciences and informed consent was obtained from all volunteers before the study was initiated.

Serum protein electrophoresis (SPE)

SPE was performed on cellulose acetate gel in barbital buffer (pH 8.6) at 20 V/cm for 25 minutes. The Helena electrophoresis system (Helena Laboratories, Beaumont, TX) was used to identify participants exhibiting HGG. SPE was performed according to the recommendations provided by the manufacturer. The separated fractions were fixed in 5% acetic acid and visualized with Ponceau S stain. The electropherogram was independently examined by 2 of the authors (ALA and IES) to identify participants exhibiting HGG and any discrepancies were resolved by consensus. To quantitatively define HGG, serum protein fractions were measured both in HGG participants and the controls using GelQuant image analysis and quantitation software, as described by Khakabimamaghani et al. (2013). Absolute protein fractions were calculated from total protein concentration, determined using the Biuret method of Weichselbaum (1946). Serum albumin concentration was determined by the method of Doumas et al. (1971).

[1]Supplementary data are available with the article through the journal Web site at http://lymphosign.com/doi/pdf/10.14785/lpsn-2021-0024.

Serum immunoglobulin estimation

Serum immunoglobulin (total IgG, IgM, and IgA) concentrations were determined by Mancini single radial immunodiffusion (Mancini et al. 1965), modified by Liofilchem S.R.L., Italy.

Liver and renal function assessment

Renal and liver function of the HGG subjects were assessed to rule out liver diseases. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined by the colorimetric method as described by Reitman and Frankel (1957). Serum alkaline phosphatase (ALP) activities were measured by the colorimetric method as described by Plummer (1978) with phenolphthalein monophosphate used as substrate. Serum urea and creatinine concentrations were determined by modified Berthelot reaction (Burtis and Ashwood 1999) and modified Jaffe reaction (Mazzachi et al. 2000) methods, respectively. The reagents used for the enzyme activity, urea, and creatinine assays were obtained from assay kits of Randox Laboratories Ltd., UK.

Isolation of neutrophils

Neutrophils were isolated from whole blood using neutrophil isolation reagent, obtained from Cayman Chemical, USA. The reagent was stored and used according to modified manufacturer instructions. To prevent neutrophil activation during the separation procedure, EDTA anticoagulated blood was used and the washing PBS did not contain Ca^{2+}/Mg^{2+}, since these ions have been shown to prime cells (Oh et al. 2008; Freitas et al. 2008). Briefly, 5 ml of K3EDTA anticoagulated blood diluted 1:2 in cell-based assay buffer (Item No 10009322) was layered on 3.33 mL of cell-based assay neutrophil isolation Histopaque reagent (Item No 600612), in a 15 mL conical tube. It was centrifuged at 500 × g for 25 minutes at 26 °C. The top layers were carefully removed and 10 ml of Red Blood Cell Lysis Buffer (Item No 601077) was added for 10 minutes to lyse the red blood cells. The neutrophil fraction was pelleted at 1200 rpm for 10 minutes. The reddish supernatant was carefully aspirated and the remaining pellet was washed twice in 1.66 ml of RPMI containing 1% BSA. Resuspension of pellets was performed slowly and rested as recommended by Kutscher et al. (2013). The isolated neutrophils were suspended in 5 mL RPMI containing 1% BSA and rested for 10 minutes at 26 °C.

Assessment of viability and purity of isolated neutrophils and phagocytic activity assay

The purity and viability of the isolated neutrophils were evaluated by Leishman's staining and trypan blue dye exclusion method, respectively, as described by Joshi et al. (2020). The neutrophil phagocytic capacity was performed using zymosan (Saccharomyces cerevisiae) commercially prepared from yeast cell wall, which consists of protein-carbohydrate complexes (purchased from Cell Biolabs, USA). The CytoSelectTM 96-well phagocytosis assay that uses prelabelled zymosan particles as a phagocytosis pathogen was employed in the study. The phagocytosed zymosan particles were determined by measuring the absorbance of each well at 405 nm. The results were expressed as a percentage of the average absorbance of the control group.

Statistical analysis

Descriptive analysis and student t-test were used for the comparison of data. Spearman correlation was used to test the association between variables, using Graphpad® 5 software (San Diego, CA). *P*-values <0.05 were considered significant.

Results

Study participants

Seven (7%) of a total of 100 healthy participants initially recruited were identified to exhibit HGG. Seven participants exhibiting normal gamma globulin bands were randomly selected as controls. The renal and liver indices and other details are presented in Table 1.

Serum protein pattern and neutrophil phagocytic activity

Serum proteins and albumin concentrations in HGG were not significantly ($p < 0.05$) different from the controls whereas the concentrations of IgG and gamma globulin were significantly ($p < 0.05$) higher in HGG compared with the control. The changes in other immunoglobulin concentrations were not significant ($p > 0.05$). The purity and viability (94% and 92%, respectively) of isolated neutrophils were adequate for the in vitro phagocytosis assay. Figure 1 shows that the phagocytic activity of neutrophils isolated from apparently healthy participants exhibiting HGG is significantly lower than the controls ($p < 0.05$).

Table 1: **Characteristics of study participants exhibiting HGG.**

Parameter	*Control	*HGG	*p*-value
N	7	7	—
Female to male ratio	3/4	4/3	0.185
Age (Years)	24 ± 2.5	25 ± 1.8	0.452
Body mass index (Kg/m^2)	21.1 ± 1.1	21.7 ± 2.0	0.873
Serum aspartate aminotransferase (U/L)	6.6 ± 2.9	6.5 ± 2.5	0.979
Serum alanine aminotransferase U/L)	15.0 ± 3.7	13.1 ± 5.2	0.771
Serum alkaline phosphatase (U/L)	139 ± 12.5	153.3+14.2	0.487
Serum rreatinine (mg/dL)	0.88 ± 0.10	1.02 ± 0.04	0.261
Serum urea (mg/dL)	26.1 ± 1.2	24.3 ± 0.8	0.233
HIV status	Negative	Negative	—

Note: *Data are mean ± SD. HGG = hypergammaglobulinemia.

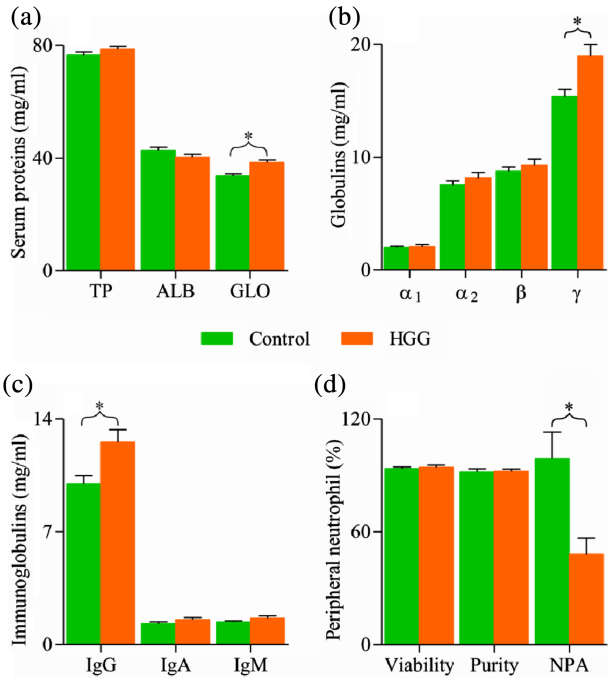

Figure 1: Neutrophil phagocytic activity and immunoglobulins in participants exhibiting hypergammaglobulinemia. Bars with error bars are mean±SEM. HGG= hypergammaglobulinemia; TP = total proteins; ALB = albumin; GLO: globulins; α_1 = alpha-1 globulin; α_2 = alpha-2 globulin; β = beta globulin; γ = gamma globulins; IgG = immunoglobulin G, IgM = immunoglobulin M, IgA = immunoglobulin A; NPA = neutrophil phagocytic activity. Where indicated, * denotes $p < 0.05$ significance level of HGG compared to control.

Association between neutrophil phagocytic activity and serum immunoglobulins

Figure 2 shows the correlation of neutrophil phagocytic activity (NPA) with immunoglobulins in HGG and the controls. IgG positively correlated with gamma globulin ($R = 0.920$; $p = 0.003$) and ($R = 0.867$; $p = 0.014$), respectively, in HGG and controls. No significant correlation was observed between neutrophil phagocytic activity and gamma globulin, IgG, in both the HGG and control group ($p > 0.05$).

Discussion

In this report, we demonstrated reduced phagocytotic activity in neutrophils isolated from apparently healthy participants exhibiting HGG compared with the controls. We first identified healthy participants showing HGG by SPE, and subsequently confirmed HGG by quantifying the gamma globulin band using GelQuant image analysis and quantitation software (Khakabimamaghani et al. 2013).

Since HGG has been reported in patients with chronic liver disease (Fallatah and Akbar 2010), we ruled out this condition by studying the liver and renal indices such as plasma AST, ALT, ALP, urea and creatinine. The liver and renal indices and HIV status reports confirmed that HGG is not due to underlying liver disease in the study participants. Other demographic parameters in both HGG and control participants were essentially the same ($p > 0.05$) (Table 1).

SPE is invaluable in health and diseases. It has 3 traditional applications; the first application is in the identification and characterization of serum protein abnormalities, the second application is in identifying the transition from monoclonal gammopathy of undetermined significance to multiple myeloma, and the third application is in disease activity or treatment in multiple myeloma (Katzmann et al. 1997). The procedure is now very useful in identifying healthy humans exhibiting HGG. Different varieties of SPE have been

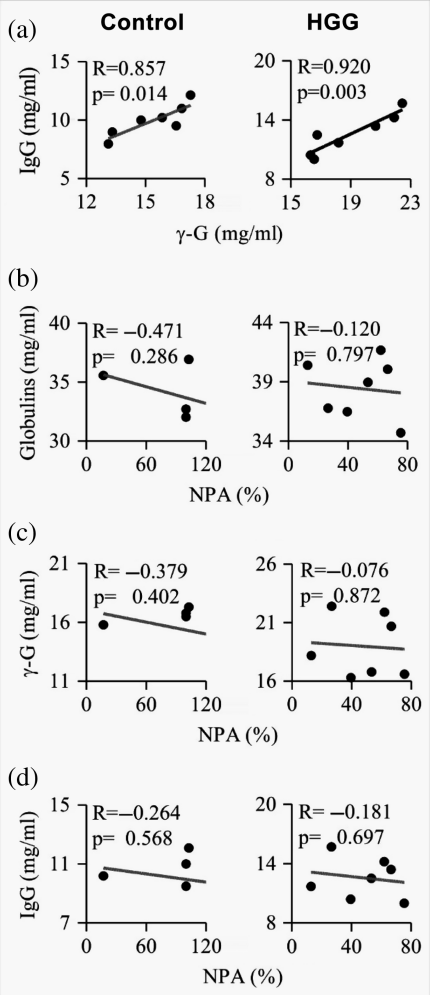

Figure 2: Comparison of serum globulins and neutrophil phagocytic activity in hypergammaglobulinemia. *a*: Association between gamma globulin (γ-G) and immunoglobulin G (IgG) in control (left panel) and HGG (right panel). *b*: Association between NPA and total globulins in control (left panel) and HGG (right panel). *c*: Association between NPA and γ-G in control (left panel) and HGG (right panel). *d*: Association between NPA and IgG in control (left panel) and HGG (right panel). Regression lines and Pearson *R*-values are shown for correlations. $p < 0.05$ were considered significant.

reported depending on the nature of the support medium employed. The 3 commonly used support media, cellulose acetate, agarose, and capillary, each have different specificity and sensitivity in determining serum protein abnormalities (Katzmann et al. 1997). It is noteworthy that the cellulose acetate support medium used in this study has been reported to have excellent specificity.

The concentration of serum gamma globulin and IgG were significantly ($p < 0.05$) higher in the HGG group compared to the control. Immunofixation electrophoresis (IFE) is a research tool for identifying the electrophoretic mobility of serum protein fractions in blood and other biological fluids. With the use of IFE, several proteins have been identified to migrate in the gamma band of SPE. Notably amongst them is IgG. Others are IgA, IgM, and complement C3 (Yousif et al. 2018). In this study, gamma globulin and total IgG were positively correlated and it is evident from previous reports that the major component of the gamma band is IgG. Phagocytic activity of neutrophils was demonstrated to be significantly reduced in participants exhibiting HGG. Anomalies in neutrophil phagocytic function have been observed in several common medical and surgical conditions (Engelich et al. 2001). Its occurrence among participants exhibiting HGG is still emerging.

As is generally known, phagocytosis is initiated by the interaction of phagocytic receptors with ligands on the surface of target microbes. Then, receptors aggregate to initiate signaling pathways that regulate the actin cytoskeleton, so that the phagocyte can produce membrane protrusions to engulf microbes. Lastly, the microbes are encircled in a new vesicle that protrudes out from the plasma membrane. The impairment in NPA may result from 1 or more of the many well-defined and complex processes that have been systematically divided into 4 principal steps: recognition of pathogens, activation of the internalization process, formation of the phagosome, and phagolysosome maturation. These processes have been recently reviewed (Nordenfelt and Tapper 2011; Rosales and Uribe-Querol 2017; Liew and Kubes 2019). Further studies will be needed to demonstrate precisely the molecules that are defective in this condition. However, phagocytic defects are generally a consequence of impaired actin polymerization around phagosomes (May and Machesky 2001; Baranov et al. 2016).

The correlation studies show that IgG is implicated in HGG and significant correlation was observed between total IgG and gamma globulins (Figure 1), and these may be expressed against microbes that breached the first line of defense. Determination of specific

antibodies would add an interesting dimension to this study. Furthermore, evaluation of the IgG subtype may reveal a predominant subclass in this condition. For instance, IgG1 and IgG3 subclass predominate in HIV infection and liver cirrhosis, respectively (Riggione et al. 1983; Kekow et al. 1988).

Since neutrophils play crucial roles as effector molecules in the first line of defense in humans, impairment of neutrophil phagocytic activity may favour persistent antigenic stimulation of the adaptive immune system. This in turn could orchestrate gamma globulin expression leading to HGG. Consequently, there might be a reduced ability of neutrophils, in this subset of young individuals, to combat microbial infections. However, since no peculiar symptom is associated with reduced neutrophil phagocytosis in apparently healthy individuals considered in this study, this phenomenon could be termed reduced neutrophil phagocytic activity of undetermined significance.

Conflict of interest

The authors declare no conflict of interest. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author's contribution

ALA conceived and designed the study, and collected data. JAB contributed to laboratory reagents and analysis tools. JD and IOB collected data and performed data analysis. IES drafted and revised the manuscript. OGA supervised laboratory analysis and revised the manuscript.

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Wiskott-Aldrich syndrome caused by a novel mutation in the *WAS* gene and presenting with a mild phenotype

Jenny Garkaby* and Julia Upton

ABSTRACT

Background: Wiskott-Aldrich syndrome (WAS) is an X-linked recessive disorder associated with combined immunodeficiency, microthrombocytopenia, eczema, and an increased risk of autoimmunity and cancer.

Aim: To report the clinical presentation, immune features, and genetic mutation in a patient with a novel mutation in the Wiskott-Aldrich syndrome (*WAS*) gene, causing a mild phenotype of WAS.

Methods: The patient's chart was reviewed. We report the phenotypical and laboratory characteristics of a patient with a mild phenotype of WAS identified by *WAS* gene sequence analysis.

Results: Our patient presented with thrombocytopenia and 3 episodes of otitis media at 24 months of age, with no other significant manifestations suggestive of immunodeficiency or immune dysregulation. A missense mutation was found in exon 12 of the *WAS* gene, C1498>T, leading to a Trp500Arg amino acid change. Currently, he is 15 years old and remains in good health, free of infections or other complications to date.

Conclusion: Genetic analysis is helpful for the diagnosis of WAS; our patient's mutation was found to cause a mild phenotype.

Statement of novelty: We describe a patient with a mild phenotype of WAS with a novel mutation in the *WAS* gene, thus, expanding the spectrum of *WAS* gene mutations.

Introduction

Wiskott-Aldrich syndrome (WAS) is a rare X-linked primary immunodeficiency disorder characterized by recurrent infection, eczema, microthrombocytopenia and an increased risk of autoimmune disorders and malignancies, mostly lymphoma Buchbinder et al. 2014. The Wiskott-Aldrich syndrome gene (*WAS*) is located at Xp11.22–p11.23 and contains 12 exons, encoding the Wiskott-Aldrich syndrome protein (WASp) — a 502-amino acid intracellular multi-domain protein, which has a crucial role in cytoskeleton organization and cell signaling (Massaad et al. 2013; Kirchhausen and Rosen 1996). WASp contains 5 distinct structural domains: the N-terminal WASP-homology domain 1 (WH1), the GTPase-binding domain (GBD), the proline-rich region (PRR), the ver-prolin homology domain (V), and the cofilin-homology sequence (C), and a C-terminal acidic (A) domain (Kim et al. 2000; Massaad et al. 2013) (Figure 1).

The N-terminal domain of WASp is important for IL-2 signaling while the WH1 domain is the binding site of WASp-interacting protein (WIP). WIP is crucial for the stability of WASp. The inactive state of WASp is maintained by autoinhibition with binding of the VCA

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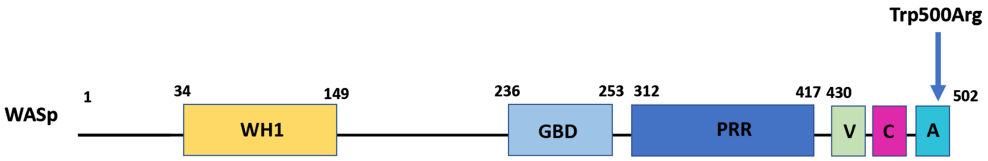

Figure 1: Schematic representation of WASp, encoded by *WAS*, with our patient's mutation. The N-terminal WASP-homology domain 1 (WH1), GTPase-binding domain (GBD), proline-rich region (PRR), verprolin homology domain (V), cofilin-homology sequence (C), and C-terminal acidic (A) domain are shown.

domain to the GBD domain. The PRR domain serves as a docking site for other signaling and adaptor proteins. The C-terminal VCA domain is the functional unit that has a critical role in the process of actin polymerization (Kim et al. 2000; Chou et al. 2006). There are multiple immunological functions which are dependent on the normal actin cytoskeleton, including lymphoid cell proliferation, immune synapse assembly and signaling, lymphoid and myeloid cell migration, as well as natural killer cell cytolytic activity and phagocytosis (Rivers et al. 2017).

The clinical manifestations of WAS include microthrombocytopenia associated with increased risk of bleeding, eczema in different degrees of severity, and recurrent infections. Infections may include severe life-threatening viral infections, bacterial, and opportunistic infections (Imai et al. 2004). Furthermore, patients with WAS are at high risk of developing autoimmune complications such as autoimmune hemolytic anemia, arthritis, inflammatory bowel disease, and vasculitis (Dupuis-Girod et al. 2003).

Different mutations in the *WAS* gene have been identified, resulting in various phenotypes and a broad range of disease severity (Imai et al. 2003). Most of the missense mutations identified in WAS patients are located in exons one to four, affecting the WH1 domain, leading to poor binding of WIP to the WH1 domain and WASp instability (Luthi et al. 2003). Missense mutations usually result in normal-sized WASp, often with reduced protein expression and were considered to cause a clinical phenotype less severe than patients with no WASp expression, with some exceptions. In contrast, patients with nonsense mutations either lack WASp or express a truncated protein, and were considered to present with the classical phenotype of WAS. However, data provided in published reports have so far failed to demonstrate a clear correlation of genotype/protein expression and phenotype (Imai et al. 2003, 2004; Albert et al. 2010).

In some cases, WAS can be fatal without hematopoietic stem cell transplantation early in life, whereas milder cases can be managed symptomatically with clinical follow up and/or immunoglobulin replacement therapy (Imai et al. 2004). In this case, we report on a young male with WAS caused by a novel mutation. He presented with a mild phenotype and has no WAS-related complications.

Methods

Patient chart review and targeted sequencing of the *WAS* gene were performed following informed consent, in accordance with a research ethics approved protocols. Following our patient's diagnosis, further genetic testing was performed on the patient's mother, maternal grandfather, and sibling for the known familial mutation. Targeted sequencing was the preferred method at the time of diagnosis as commercially available genetic panels were not available.

Results

Case presentation

Our patient, currently a 15-year-old male, presented at the age of 2 years with thrombocytopenia and mild bruising. He was initially diagnosed with immune thrombocytopenic purpura but further findings of persistent low platelet count, microthrombocytopenia on blood smear, and 3 episodes of otitis media prompted immunological evaluation. He did not experience any severe or deep-seated infections, nor dermatitis, or diarrhea. He was born to non-consanguineous healthy parents. His family history is significant for a maternal grandfather with Crohn's disease and frequent epistaxis. His physical exam was within normal limits

Table 1: **Immune evaluation.**

	Age 5 years	Reference range	Age 10 years	Reference range	Age 13 years	Reference range
WBC (cells/L)	4.7	5.14–13.38	6.1	4–10 × 10^9/L	4.5	3.84–9.84 × 10^9/L
Hemoglobin (g/L)	125	102–127	134	120–160	130	110–145
Platelets (cells/L)	78	202–403	93	150–400 × 10^9/L	74	175–332 × 10^9/L
Neutrophils (cells/mL)	2.46	1.54–7.92	3.14	2–7.5 × 10^9/L	2.5	1.54–7.04 × 10^9/L
Lymphocytes (cells/mL)	1.61	1.13–1.52	1.88	1.50–7 × 10^9/L	1.35	0.97–3.26 × 10^9/L
Monocytes (cells/mL)	0.54	0.19–0.94	0.64	0.05–0.8 × 10^9/L	0.48	0.18–0.78 × 10^9/L
Eosinophils (cells/mL)	0.05	0.03–0.53	0.34	0.02–0.5 × 10^9/L	0.11	0.04–0.38 × 10^9/L
CD3+ (cells/mL)	903	1578–3707	937	800–3500	762	954–2332
CD3+ /CD4+ (cells/mL)	582	870–2144	637	400–2100	517	610–1446
CD3+ /CD8+ (cells/mL)	192	472–1107	203	200–1200	172	282–749
CD19+ (cells/mL)	452	434–1274	503	200–600	350	173–685
NK (cells/mL)	244	155–565	312	70–1200	214	87–504
PHA stimulation index	1046	>50% of control or >200	1337	>50% of control or >200	788	>50% of control or >200
IgG (g/L)	4.1	5.4–13.6	5.9	6.6–15.3	6	6.6–15.3
IgM (g/L)	0.5	0.4–1.5	0.3	0.4–1.5	0.3	0.4–1.5
IgA (g/L)	0.2	0.3–1.5	0.8	0.5–2.2	0.9	0.5–2.2
IgE (IU/mL)	4	<450	<25	<450	<25	<450
Isohemagglutinins (anti B)	1:32	—	1:8	—	1:64	—
Anti-tetanus Ab (IU/mL)	0.49	—	1.87	—	1.25	—
Measles, Mumps, Rubella Ab	Positive	—	Positive	—	Positive	—

except for mild bruising. He had normal growth and development.

Investigations

Immune evaluation revealed normal white blood count (WBC), no anemia, and thrombocytopenia of 50×10^9/L. Total lymphocyte and T cell counts were normal (Table 1), but over time, a gradual reduction in both CD4+ and CD8+ cells was observed. PHA stimulation index was slightly low, however, repeated testing was within the normal range. Serum concentrations of immunoglobulins (IgG, IgA, and IgM) were normal for age and vaccine responses to Measles, Mumps, Rubella and Tetanus toxoid were protective. *WAS* gene sequencing revealed a missense mutation of C1498>T, leading to a Trp500Arg amino acid change. This mutation has not been described in the literature previously. Flow cytometry showed WASp was present, but reduced expression of WASp in leucocytes was noted.

Further genetic testing via sequencing the region covering the known familial mutation was performed on the patient's maternal grandfather, healthy mother, and healthy sibling. Both the grandfather and sibling were found to be negative, and both had normal platelet counts. The patient's mother was found to be a carrier of the same mutation. She is otherwise well and healthy.

Outcome

Further evaluation revealed that the patient's brother was not a match for hematopoietic stem cell transplantation. The challenge of future management for this patient stemmed from the fact that, at the time, his prognosis was uncertain given that his mutation had not been previously described in the literature. Since he did not have a matched-sibling donor, nor a matched-unrelated donor, hematopoietic stem cell transplantation did not become part of his treatment. Over the following 13 years, he continued to be well, with no recurrent infections. He did not develop clinical evidence suggestive of immune dysregulation, i.e., inflammatory bowel disease, arthritis, vasculitis, autoimmune hemolytic anemia etc. His immunological evaluation is significant for stable thrombocytopenia and T cell lymphopenia, borderline immunoglobulins levels and normal vaccines response.

Discussion

WAS is a rare immunodeficiency with estimated prevalence of ~1:250,000, male births in Canada and the U.S. (Perry et al. 1980). It is characterized by recurrent infections, eczema and microthrombocytopenia, as well as an increased risk for cancer and autoimmunity

(Massaad et al. 2013; Rivers et al. 2017). The causative gene, *WAS*, encodes WASp, which is a hematopoietic cell specific protein and is involved in actin polymerization, cytoskeletal rearrangement, and signaling events (Massaad et al. 2013).

Our patient's mutation is at the C-terminal acidic A domain which is required for the initiation of actin polymerization and thus normal cell motility. Deletion of the VCA domain results in complete loss of WASp mediated actin polymerization (Massaad et al. 2013). Another important role of the VCA domain is in WASp autoinhibition, which occurs upon GBD site binding to the C-terminal VCA region, resulting in autoinhibition of the stimulatory activity of the protein (Scherl et al. 2002).

The mild phenotype observed in our patient is most probably associated with intact WASp functions, such as the WIP/WH1 domain interaction and protein stabilization, signaling through the GBD domain, and normal docking at the PRR domain, as those are not affected by the far C-terminal acidic mutation. Phosphorylation of WASp on tyrosine 291, a location that is most probably not affected by our patient's mutation, was shown to enhance the actin polymerization activity of WASp via the Actin Related Protein (Arp) 2/3 complex. Moreover, immune functions such as T cell differentiation, memory B cell activation, and transcription of inflammatory cytokines are independent of the normal actin polymerization by WASp (Rivers et al. 2017).

According to the scoring system suggested by Zhu et al. (1997), patients considered to have an X-linked thrombocytopenia (XLT) phenotype are assigned a score of one to two, whereas patients considered to have WAS are assigned a score of three to four. XLT and WAS patients who develop autoimmunity and/or malignancies at a later stage in life progress to a score of five (Imai et al. 2004).

As our patient has only thrombocytopenia, mild eczema with no infections or evidence of immune dysregulation he was assigned severity score of 2 according to the scoring system suggested by Zhu et al. (1997) Gene therapy has focused on patients with severity score from 3–5 who display a WAS phenotype characterized by bleeding, severe eczema, and severe infections (Abina et al. 2015).

Mutations in the *WAS* gene result in a broad range of disease severity and can be divided in different groups according to their effect on WASp expression. Mutations include null mutations (nonsense mutations, deletions, insertions with frameshift) - 46%, missense mutations - 42%, and splice anomalies - 12%. As previously published, most missense mutations are located in exons 1 through 4, in the WH1 domain resulting in an XLT phenotype or low severity WAS score, while nonsense mutations were observed as causing a severe phenotype (Imai et al. 2003, 2004). The genotype-phenotype correlation in WAS is not absolute. Other published data failed to demonstrate a clear correlation of missense mutations genotype/protein expression and phenotype, with only half of the patients carrying missense mutations exhibiting the XLT phenotype and detectable WASp (Liu et al. 2015). Clinicians often rely on published case report series to determine prognosis, given the wide clinical spectrum of WAS. Thus, it is important to broaden the genotypic and phenotypic spectrum of *WAS* mutations. We have shown a novel mutation causing a mild phenotype of WAS/XLT, our findings also support the notion of genetic testing in patients with thrombocytopenia and mild symptoms to ensure an early diagnosis of inborn errors of immunity.

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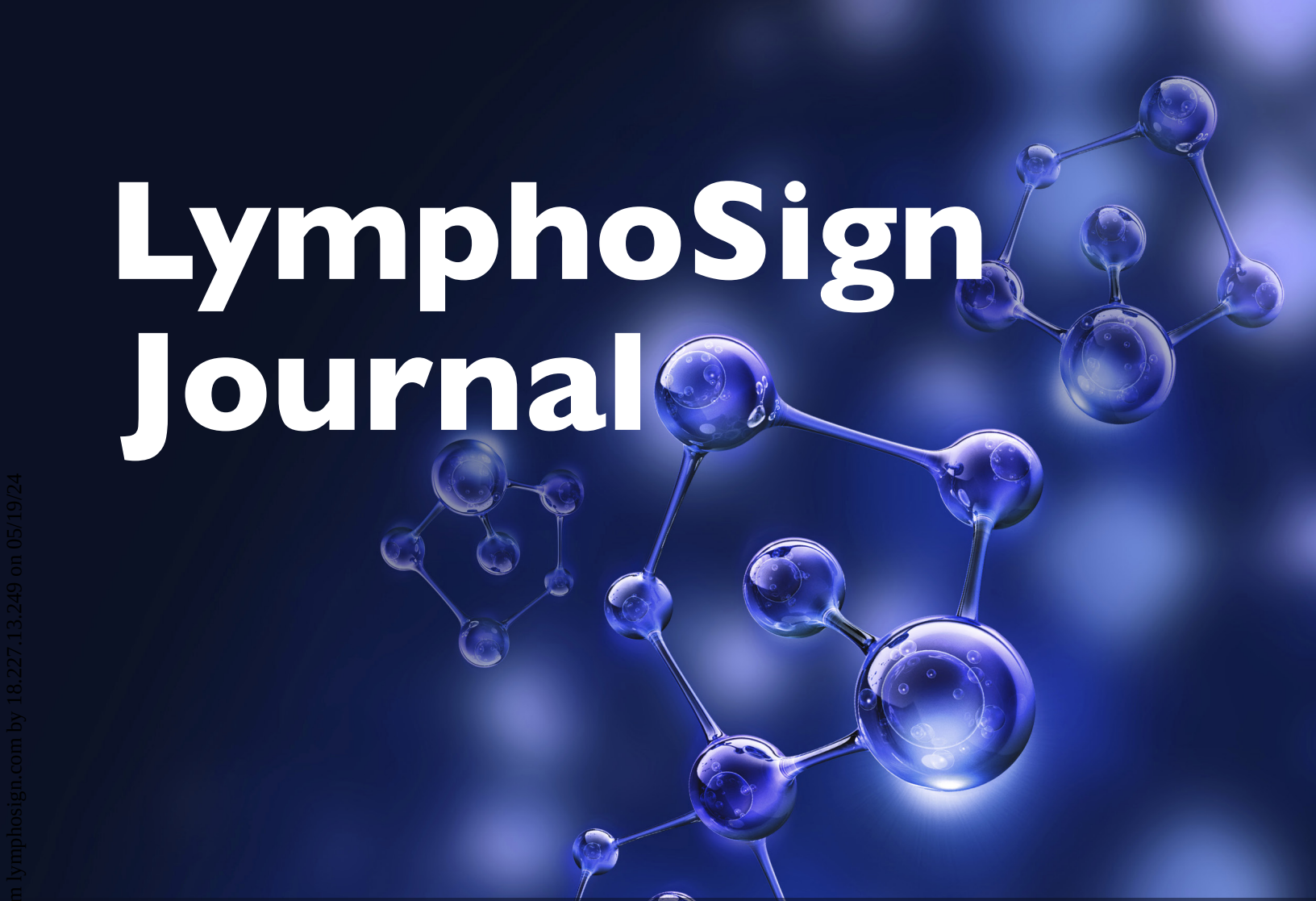

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