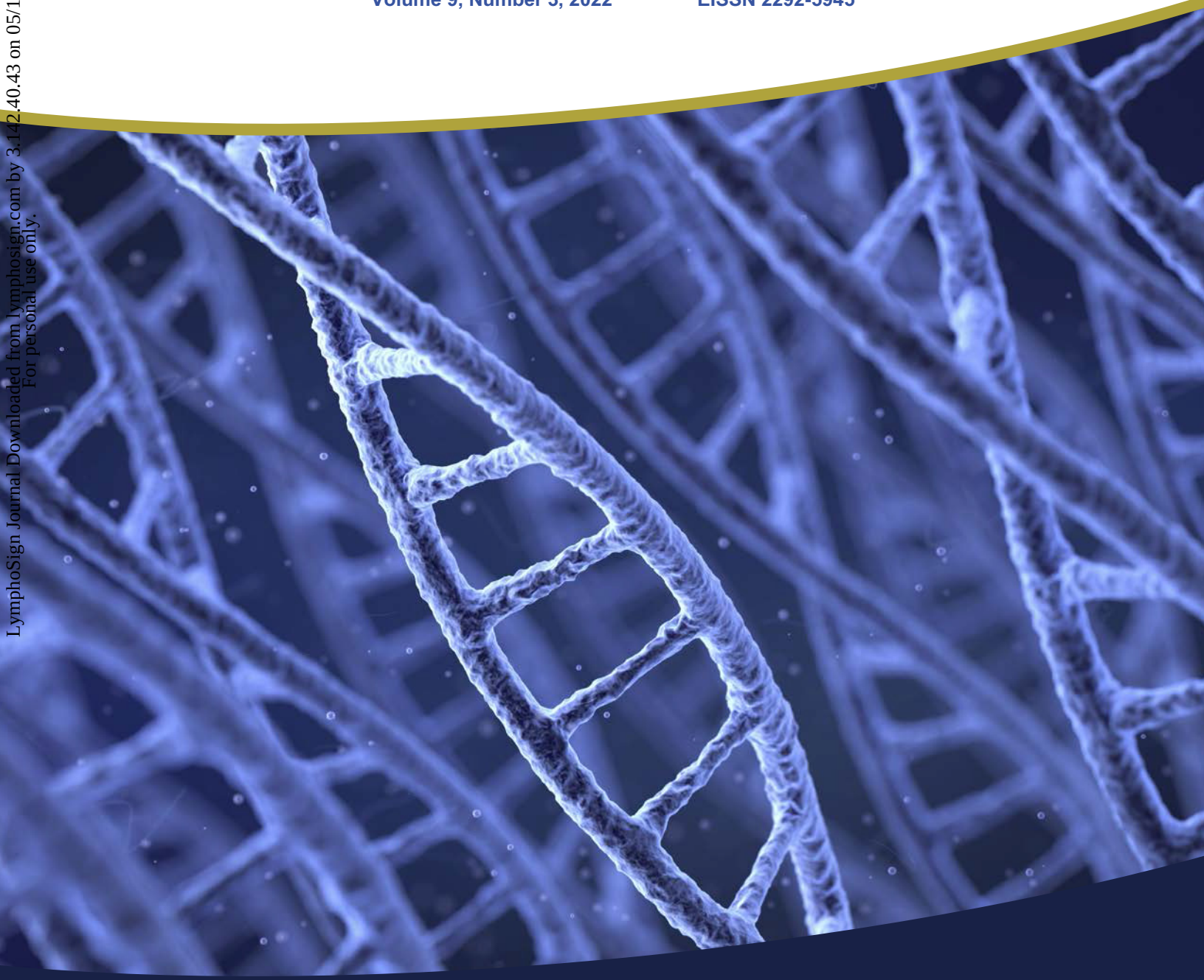
LymphoSign Journal

The journal of inherited immune disorders

Volume 9, Number 3, 2022 EISSN 2292-5945



Immunodeficiency Canada

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.

Vision

To cure Primary Immunodeficiency (PI)

Mission

To improve the lives of people with Primary Immunodeficiency (PI) by promoting early diagnosis and effective treatments through leadership in research, education and advocacy in partnership with health-care professionals, volunteers, industry and government

The Immunodeficiency Canada addresses its mission through four core programs:

- Network
- Education
- Research
- Patient Support

Programs and Services

- Emergency Financial Assistance
- Patient Support
- Primary Immunodeficiency Information
- Publications
- Resources
- Social Support

For Healthcare Professionals

Chaim Roifman Scholar Award for research

Supported by Baxter, CSL Behring, Grifols, Octapharma and Immunodeficiency Canada

- for Senior Investigators
- for Young Investigators

Conferences, seminars and symposiums
Immunodeficiency Canada-Grifols Fellow Training Program
Immunodeficiency Canada Network

- Consultations
- Education
- Immunologists network grants
- PI video conferences
- Healthcare facility sites and affiliates

Medical Advisory Board
Physicians Login (for diagnostic tools, case studies and resources)
PI Nurses Network
PI Social Workers Network

Additional Resources

Canadian, International resources and links to condition-specific information can be found at **www.immunodeficiency.ca**

Immunodeficiency Canada
Immunodéficience Canada

Providing patient support, education and research to cure Primary Immunodeficiency

Suite 848, 439 University Ave, Toronto, Ontario M5G1Y8
Tel 416-964-3434 **Fax** 416-964-6594
contactus@immunodeficiency.ca
charitable # 87276 0897 RR0001

www.immunodeficiency.ca

LymphoSign Journal

Volume 9, Number 3, 2022 EISSN 2292-5945

TABLE OF CONTENTS

LymphoSign Journal (ISSN electronic 2292-5945) is published quarterly in March, June, September, and December by LymphoSign Journal Limited Partnership, Toronto, Ontario, Canada; Tel.: 416-986-3508; Fax: 416-964-6594.

Service information

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.

Subscriptions

Requests for subscriptions, renewals, and single or back issues, and all remittances should be addressed to: Subscription Office, Canadian Science Publishing, PO Box 361, Birmingham, AL 35283-0399, USA; Toll-free: 1-800-852-7404; Outside North America: 205-995-1567; Fax: 205-995-1588; Email: nrcresearchpress-csp@subscriptionoffice.com. 2020 subscriptions rates are as follows: Institution: electronic $525.00, Personal: electronic $150.00.

Submissions

To submit, use the journal's online submission system at http://mc06.manuscriptcentral.com/lpsn. The Instructions to Authors are available at http://lymphosign.com/page/lpsn-authors. All correspondence concerning submissions should be addressed to the Editorial Office at editorialoffice@lymphosign.com.

Offprints and reprints

Offprint and reprint requests should be directed to the Editorial Office at editorialoffice@lymphosign.com.

Permissions

For permission to reprint materials, contact the Editorial Office at editorialoffice@lymphosign.com.

Advertising sales

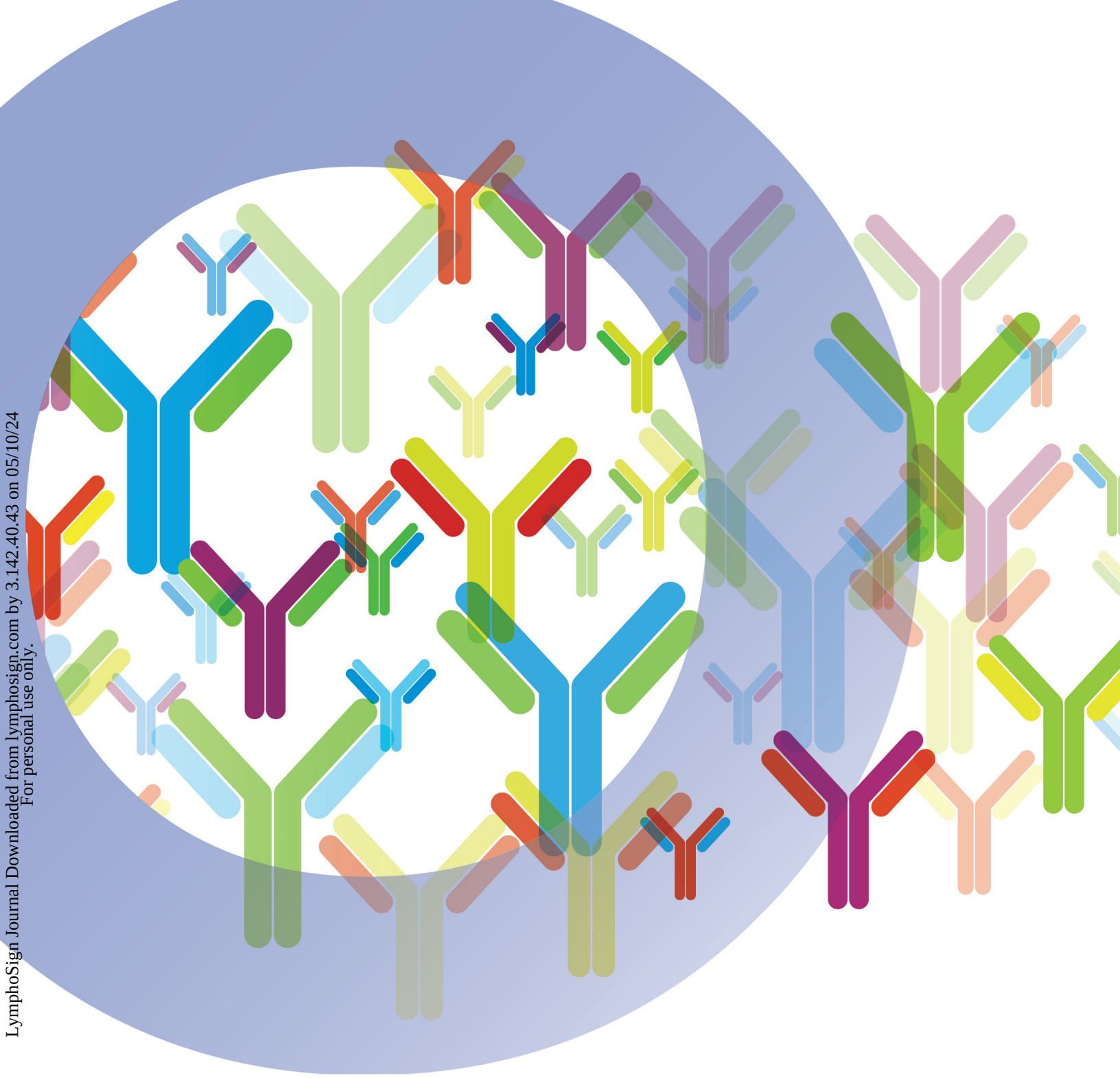
Advertising inquiries should be directed to the Editorial Office at editorialoffice@lymphosign.com. Acceptance of or the publishing of advertisements does not imply endorsement by either the Journal's Editors or the Publisher.

Publishing services

Publishing services are provided by Canadian Science Publishing, 1840 Woodward Drive, Suite 1, Ottawa, ON K2C 0P7, Tel: 613-656-9846; www.cdnsciencepub.com. Scientific Publishing Editor: Lianne Johnsen, lianne.johnsen@cdnsciencepub.com; Manager, Production: Judy Hum-Delaney, judy.hum-delaney@cdnsciencepub.com; Director, Publishing Operations: Judy Busnarda, judy.busnarda@cdnsciencepub.com.

Indexing

CrossRef, Google Scholar, HINARI, J-Gate, OCLC, ReadCube, Ulrich's, and Emerging Sources Citation Index.



LymphoSign Journal

Volume 9, Number 3, 2022 EISSN 2292-5945

COVID-19 treatments

Linda Vong[a*] and Chaim M. Roifman[a,b]

The evolving race to protect against severe outcomes of coronavirus disease 2019 (COVID-19), caused by SARS-CoV-2, has spurred the rapid development and authorization of novel vaccines and treatments worldwide (Roifman and Vong 2021*b*). Individuals infected with SARS-CoV-2 may experience a wide spectrum of symptoms, from nil (asymptomatic), mild (fever, cough, and dyspnea), to more severe clinical course (including acute respiratory distress, pneumonia, renal failure, and death). Comorbidities which predispose to complications and severe illness include older age (>65 y), males, obesity, cancer, serious cardiovascular disease, chronic obstructive pulmonary disease, type II diabetes mellitus, and immunodeficiency (including primary immunodeficiency; PID) (Chen et al. 2020, Huang et al. 2020, Li et al. 2020).

While uptake of the recommended vaccines exceeds 83% (fully vaccinated) within the Canadian population, levels of protection vary, especially in patients with PID who have abnormal humoral and cellular immune responses (Vong and Roifman 2022; Roifman and Vong 2021*a*). Moreover, attempts to use existing treatments to combat COVID-19, including metformin, ivermectin, and fluvoxamine, have shown little to no effectiveness against infection with SARS-CoV-2 (Abdool Karim and Devnarain 2022). At present, there are 6 drug modalities for the prevention and treatment of COVID-19 authorized for use in Canada (with variable guidance across provinces and territories). These include (*i*) neutralizing antibodies that target the spike protein of SARS-CoV-2 (Cilgavimab/Tixagevimab; Sotrovimab; Casirivimab/Imdevimab; Bamlanivimab), and (*ii*) antivirals which inhibit the ability of the SARS-CoV-2 virus to replicate (Remdesivir; Nirmatrelvir/Ritonavir). The indications for treatment and modes of administration have similarly evolved to enable easier access and targets populations that are most at-risk, including the availability of at-home versus hospital treatment as well as pre-exposure prophylaxis for those who are immunocompromised or unable to be vaccinated against SARS-CoV-2. However, in practice there remain barriers to getting hold of these treatments. The prevalence of SARS-CoV-2 variants must also be taken into account, particularly given that some treatments exhibit waning activity against recent circulating variants and subvariants (Takashita et al. 2022). To date, there are no real-world comparative studies for the use of these medications.

We provide here a brief overview of the indications and dose of the current available treatments. It is important to note that assessment for suitability of use, particularly for those with immunodeficiency, should be performed on an individual basis by a healthcare provider.

Neutralizing monoclonal antibodies against SARS-CoV-2

Cilgavimab and Tixagevimab (Evusheld; AstraZeneca Canada Inc.)

Cilgavimab/tixagevimab are recombinant human IgG1 monoclonal antibodies that bind to the spike protein receptor binding domain (RBD) of SARS-CoV-2,

[a]Canadian Centre for Primary Immunodeficiency and the Division of Immunology & Allergy, Department of Paediatrics, The Hospital for Sick Children, Toronto, ON; [b]University of Toronto, Toronto, ON

*Corresponding author: Linda Vong/linda.vong@sickkids.ca.

Submitted 29 August 2022
Accepted 31 August 2022
Available online 31 August 2022

LymphoSign Journal 9:57–61 (2022)
dx.doi.org/10.14785/lymphosign-2022-0012

preventing interaction with the ACE2 receptor and thus virus attachment and entry into healthy cells. The long-acting combination of cilgavimab/tixagevimab provides passive humoral immunity, and was authorized for use by Health Canada on 14 April 2022, representing the first pre-exposure treatment for immunocompromised individuals and those unable to be vaccinated. Clinical trial results for the combination of cilgavimab/tixagevimab support a reduction in the proportion of individuals who developed symptomatic laboratory-confirmed COVID-19, with a relative risk reduction of 82.8% (Levin et al. 2022).

Indication: Adults and adolescents (12 y and older, ≥40 kg) who have not had a known recent exposure to an individual infected with SARS-CoV-2 and (*i*) who are immune compromised and unlikely to mount an adequate immune response to COVID-19 vaccination, or (*ii*) for whom COVID-19 vaccination is not recommended.

Dose: 300 mg administered intramuscularly as 2 separate, 1.5 mL, sequential injections of 150 mg of cilgavimab (100mg/mL) and 150 mg of tixagevimab (100 mg/mL). Repeat dosing every 6 mo.

Considerations: Due to decreased in vitro neutralization activity of cilgavimab/tixagevimab against Omicron subvariants an increase in dose to 600 mg may be considered. Guidance for use was revised in British Columbia in August 2022, in light of the predominance of the BA.4/BA.5 variants to which cilgavimab/tixagevimab have reduced neutralizing activity.

Sotrovimab (GlaxoSmithKline Inc.)

Sotrovimab is a recombinant human IgG1 monoclonal antibody that binds to the spike protein RBD of SARS-CoV-2, but does not compete with ACE2 receptor binding. It was authorized for the treatment of mild to moderate COVID-19 by Health Canada on 30 July 2021. Clinical trials on the efficacy of sotrovimab demonstrated that in a cohort of participants with at least 1 risk factor for severe COVID-19 (diabetes, obesity (BMI>30), chronic kidney disease, congestive heart failure, chronic obstructive pulmonary disease, moderate to severe asthma; or subjects aged 55 y and older regardless of other comorbidities), a significantly greater percentage of participants receiving the placebo were hospitalized and (or) developed severe and (or) critical respiratory COVID-19 (Gupta et al. 2022, 2021).

Indication: Treatment of adults and adolescents (12 y and older, ≥40 kg) with mild to moderate COVID-19, confirmed by SARS-CoV-2 viral testing, who are at high risk for progressing to hospitalization and/or death.

Dose: 500 mg single intravenous infusion to be administered within 7 d after the onset of symptoms.

Considerations: Reduced neutralization of Omicron subvariants (BA.2) has been reported.

Casirivimab and Imdevimab (Hoffman-La Roche Limited)

Casirivimab/imdevimab are 2 non-competing recombinant human IgG1 monoclonal antibodies that bind to regions of the spike protein RBD, preventing interaction with the ACE2 receptor and infection of host cells. The combination was authorized for use by Heath Canada on 9 June 2021. Clinical studies on the efficacy of casirivimab/imdevimab demonstrated significantly reduced numbers of hospitalizations and deaths among those who received the treatment versus placebo control (risk reduction of 71.3%) (Weinreich et al. 2021*a*, Weinreich et al. 2021*b*).

Indication: Treatment of adults and adolescents (12 y and older, ≥40 kg) with mild to moderate COVID-19, confirmed by SARS-CoV-2 viral testing, who are at high risk for progressing to hospitalization and/or death.

Dose: Single intravenous infusion of 1200 mg of casirivimab (10 mL; 120 mg/mL) and 1200 mg of imdevimab (10 mL; 120 mg/mL) over a minimum infusion time of 60 min and maximum infusion rate of 270 mL/hr. To be administered as soon as possible after the onset of symptoms.

Considerations: Potential failure of treatment reported against Omicron variants.

Bamlanivimab (Ely Lilly Canada Inc.)

Bamlanivimab is a recombinant human IgG1 monoclonal antibody directed against the spike protein of SARS-CoV-2, preventing virus attachment and entry into host cells. It was authorized for use by Health

Canada on 20 November 2020. Phase 2 clinical studies demonstrated that a lower proportion of participants who received bamlanivimab progressed to hospitalization compared to placebo control (Chen et al. 2021). While a subsequent combination of bamlanivimab plus estevimab was shown to reduce both COVID-19-related hospitalization and death as well as SARS-CoV-2 viral load (Dougan et al. 2021), estevimab has not yet been authorized for use in Canada.

Indication: Treatment of adults and adolescents (12 y and older, ≥40 kg) with mild to moderate COVID-19, who are at high risk of progressing to severe COVID-19 illness and/or hospitalization.

Dose: Single intravenous infusion of 700 mg bamlanivimab (35 mg/mL), administered withing 10 d of clinical signs and symptoms onset.

Considerations: Loss of activity observed in some SARS-CoV-2 lineages.

Antiviral medications against SARS-CoV-2

Nirmatrelvir and Ritonavir (Paxlovid; Pfizer Canada ULC)

Nirmatrelvir prevents viral replication by inhibiting the SARS-CoV-2 3C-like protease, required for processing of polyprotein precursors. Concurrently, ritonavir inhibits the cytochrome P450 3A-mediated breakdown of nirmatrelvir, leading to increased plasma concentrations. Authorized for use by Health Canada on 17 January 2022, this is the first treatment that can be taken orally at home. Clinical studies reported a lower incidence of hospitalization as well as low viral load in participants administered nirmatrelvir and ritonavir compared to placebo control (89% lower risk) (Hammond et al. 2022).

Indication: Treatment of mild to moderate COVID-19 in adults with positive results of direct SARS-CoV-2 viral testing, and who are at high risk for progression to severe COVID-19, including hospitalization or death.

Dose: Oral administration of 300 mg nirmatrelvir (2 × 150 mg tablet) and 100 mg ritonavir (1 × 100 mg tablet), twice daily for 5 d. To be administered within 5 d of symptom onset.

Considerations: Lower dosing is available for those with renal impairment. There is also potential for drug interactions given the effect on cytochrome P450 3A. The impact on rates of hospitalization and death appears greater in the aging (>65 year) at-risk population (Arbel et al. 2022).

Remdesivir (Veklury; Gilead Sciences Canada Inc.)

Remdesivir inhibits SARS-CoV-2 viral replication and RNA synthesis. It is metabolized within host cells to form remdesivir triphosphate, an analog of adenosine triphosphate (ATP), which competes with ATP incorporated into RNA chains by the SARS-CoV-2 RNA-dependent RNA polymerase. Remdesivir was first authorized for use by Health Canada on 27 July 2020, approximately 4 mo after COVID-19 was declared a pandemic. Clinical trial results reported reduced time to recovery and higher odds of improvement in participants administered remdesivir versus placebo (Beigel et al. 2020).

Indication: Treatment of hospitalized adults and adolescents (12 y and older, ≥40 kg) with pneumonia requiring oxygen, or non-hospitalized adults with positive results of COVID testing, at high risk of progression to severe COVID, including hospitalization and death.

Dose: Single intravenous infusion of a loading dose of remdesivir (200 mg) on day 1, and thereafter a once daily infusion of 100 mg, for a maximum duration of 10 d (i.e., initial 200 mg and 9 × 100 mg). Hospitalized adults/adolescents with pneumonia requiring supplemental oxygens should receive remdesivir for at least 5 d up to a maximum of 10 d. The treatment duration for non-hospitalized adults is 3 d, to be administered within 7 d of symptom onset.

Considerations: Anti-viral activity maintained against Omicron variants.

REFERENCES

Abdool Karim, S.S., and Devnarain, N. 2022. Time to Stop Using Ineffective Covid-19 Drugs. N. Engl. J. Med. **387**: 654–655. PMID: 36070715. doi: 10.1056/NEJMe2209017.

Arbel, R., WolffSagy, Y., Hoshen, M., Battat, E., Lavie, G., Sergienko, R., Friger, M., Waxman, J.G., Dagan, N.,

Balicer, R., Ben-Shlomo, Y., Peretz, A., Yaron, S., Serby, D., Hammerman, A., and Netzer, D. 2022. Nirmatrelvir Use and Severe Covid-19 Outcomes during the Omicron Surge. N. Engl. J. Med. **387**: 790–798. PMID: 36001529. doi: 10.1056/NEJMoa2204919.

Beigel, J.H., Tomashek, K.M., Dodd, L.E., Mehta, A.K., Zingman, B.S., Kalil, A.C., Hohmann, E., Chu, H.Y., Luetkemeyer, A., Kline, S., , Lopez de castilla, D., Finberg, R.W., Dierberg, K., Tapson, V., Hsieh, L., Patterson, T.F., Paredes, R., Sweeney, D.A., Short, W.R., Touloumi, G., Lye, D.C., Ohmagari, N., Oh, M.-D., Ruiz-Palacios, G.M., Benfield, T., Fätkenheuer, G., Kortepeter, M.G., Atmar, R.L., Creech, C.B., Lundgren, J., Babiker, A.G., Pett, S., Neaton, J.D., Burgess, T.H., Bonnett, T., Green, M., Makowski, M., Osinusi, A., Nayak, S., and Lane, H.C. 2020. Remdesivir for the Treatment of Covid-19 — Final Report. N. Engl. J. Med. **383**: 1813–1826. PMID: 32445440. doi: 10.1056/NEJMoa2007764.

Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., Qiu, Y., Wang, J., Liu, Y., Wei, Y., Xia, J., Yu, T., Zhang, X., and Zhang, L. 2020. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet, **395**: 507–513. PMID: 32007143. doi: 10.1016/S0140-6736(20)30211-7.

Chen, P., Nirula, A., Heller, B., Gottlieb, R.L., Boscia, J., Morris, J., Huhn, G., Cardona, J., Mocherla, B., Stosor, V., Shawa, I., Adams, A.C., Van Naarden, J., Custer, K.L., Shen, L., Durante, M., Oakley, G., Schade, A.E., Sabo, J., Patel, D.R., Klekotka, P., and Skovronsky, D.M. 2021. SARS-CoV-2 Neutralizing Antibody LY-CoV555 in Outpatients with Covid-19. N. Engl. J. Med. **384**: 229–237. PMID: 33113295. doi: 10.1056/NEJMoa2029849.

Dougan, M., Nirula, A., Azizad, M., Mocherla, B., Gottlieb, R.L., Chen, P., Hebert, C., Perry, R., Boscia, J., Heller, B., Morris, J., Crystal, C., Igbinadolor, A., Huhn, G., Cardona, J., Shawa, I., Kumar, P., Adams, A.C., Van Naarden, J., Custer, K.L., Durante, M., Oakley, G., Schade, A.E., Holzer, T.R., Ebert, P.J., Higgs, R.E., Kallewaard, N.L., Sabo, J., Patel, D.R., Dabora, M.C., Klekotka, P., Shen, L., and Skovronsky, D.M. 2021. Bamlanivimab plus Etesevimab in Mild or Moderate Covid-19. N. Engl. J. Med. **385**: 1382–1392. PMID: 34260849. doi: 10.1056/NEJMoa2102685.

Gupta, A., Gonzalez-Rojas, Y., Juarez, E., Crespo Casal, M., Moya, J., Falci, D.R., Sarkis, E., Solis, J., Zheng, H., Scott, N., Cathcart, A.L., Hebner, C.M., Sager, J., Mogalian, E., Tipple, C., Peppercorn, A., Alexander, E., Pang, P.S., Free, A., Brinson, C., Aldinger, M., and Shapiro, A.E. 2021. Early Treatment for Covid-19 with SARS-CoV-2 Neutralizing Antibody Sotrovimab. N. Engl. J. Med. **385**: 1941–1950. PMID: 34706189. doi: 10.1056/NEJMoa2107934.

Gupta, A., Gonzalez-Rojas, Y., Juarez, E., Crespo Casal, M., Moya, J., Rodrigues Falci, D., Sarkis, E., Solis, J., Zheng, H., Scott, N., Cathcart, A.L., Parra, S., Sager, J.E., Austin, D., Peppercorn, A., Alexander, E., Yeh, W.W., Brinson, C., Aldinger, M., Shapiro, A.E., Free, A., Johnson, K., Cordasco, E., Little, R., Bajwa, A., Doshi, A., Focil, A., Hussain, R., Bostick, G., Somodevilla, G., Ali, H., Kowalczyk, J., Mittal, S., Caso, J., Goisse, M., Espinal, L., Zepeda, L., Nguyen, T., Martinez, L., Alvarez, G., Pucillo, R., Seep, M., Parikh, N., Escobar, V., Curra, A., Dal Maso, V., O'mahony, J., Ramacciotti, E., Diaz, J., Luz, K., Ruane, P., Mochlera, B., Roldan Sanchez, J., Hernandez, L., Fernandez, A., Leavitt, G., Azizad, M., Afzal, H., Fatakia, A., Narejos Perez, S., Stadnik, C.M., Gorgos, L., Sachdeva, Y., Segura, P., Quandros, C., Perry, R., and Sher, L. 2022. Effect of Sotrovimab on Hospitalization or Death Among High-risk Patients With Mild to Moderate COVID-19. Jama, **327**: 1236.

Hammond, J., Leister-Tebbe, H., Gardner, A., Abreu, P., Bao, W., Wisemandle, W., Baniecki, M., Hendrick, V.M., Damle, B., Simón-Campos, A., Pypstra, R., and Rusnak, J.M. 2022. Oral Nirmatrelvir for High-Risk, Nonhospitalized Adults with Covid-19. N. Engl. J. Med. **386**: 1397–1408. PMID: 35172054. doi: 10.1056/NEJMoa2118542.

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., Cheng, Z., Yu, T., Xia, J., Wei, Y., Wu, W., Xie, X., Yin, W., Li, H., Liu, M., Xiao, Y., Gao, H., Guo, L., Xie, J., Wang, G., Jiang, R., Gao, Z., Jin, Q., Wang, J., and Cao, B. 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet, **395**: 497–506. PMID: 31986264. doi: 10.1016/S0140-6736(20)30183-5.

Levin, M.J., Ustianowski, A., De Wit, S., Launay, O., Avila, M., Templeton, A., Yuan, Y., Seegobin, S., Ellery, A., Levinson, D.J., Ambery, P., Arends, R.H., Beavon, R., Dey, K., Garbes, P., Kelly, E.J., Koh, G.C.K.W., Near, K.A., Padilla, K.W., Psachoulia, K., Sharbaugh, A., Streicher, K., Pangalos, M.N., and Esser, M.T. 2022. Intramuscular AZD7442 (Tixagevimab–Cilgavimab) for Prevention of Covid-19. N. Engl. J. Med. **386**: 2188–2200. PMID: 35443106. doi: 10.1056/NEJMoa2116620.

Li, Q., Guan, X., Wu, P., Wang, X., Zhou, L., Tong, Y., Ren, R., Leung, K.S.M., Lau, E.H.Y., Wong, J.Y., Xing, X., Xiang, N., Wu, Y., Li, C., Chen, Q., Li, D., Liu, T., Zhao, J., Liu, M., Tu, W., Chen, C., Jin, L., Yang, R., Wang, Q., Zhou, S., Wang, R., Liu, H., Luo, Y., Liu, Y., Shao, G., Li, H., Tao, Z., Yang, Y., Deng, Z., Liu, B., Ma, Z., Zhang, Y., Shi, G., Lam, T.T.Y., Wu, J.T., Gao, G.F., Cowling, B.J., Yang, B., Leung, G.M., and Feng, Z. 2020. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia. N. Engl. J. Med. **382**: 1199–1207. PMID: 31995857. doi: 10.1056/NEJMoa2001316.

Roifman, C.M., and Vong, L. 2021*a*. COVID-19 post-vaccination recommendations in primary immunodeficiency. LymphoSign J. 8: 69–74. doi: 10.1056/NEJMc2209952.

Roifman, C.M., and Vong, L. 2021*b*. COVID-19 vaccination for patients with primary immunodeficiency. LymphoSign J. **8**: 37–45. doi: 10.14785/lymphosign-2021-0020.

Takashita, E., Kinoshita, N., Yamayoshi, S., Sakai-Tagawa, Y., Fujisaki, S., Ito, M., Iwatsuki-Horimoto, K., Halfmann, P., Watanabe, S., Maeda, K., Imai, M., Mitsuya, H., Ohmagari, N., Takeda, M., Hasegawa, H., and Kawaoka, Y. 2022. Efficacy of Antiviral Agents against the SARS-CoV-2 Omicron Subvariant BA.2. N. Engl. J. Med. **386**: 1475–1477. PMID: 35263535. doi: 10.1056/NEJMc2201933.

Vong, L., and Roifman, C.M. 2022. COVID-19 outcomes in immunocompromised individuals: seroconversion and vaccine effectiveness. LymphoSign J. **9**: 33–39. doi: 10.14785/lymphosign-2022-0006.

Weinreich, D.M., Sivapalasingam, S., Norton, T., Ali, S., Gao, H., Bhore, R., Musser, B.J., Soo, Y., Rofail, D., Im, J., Perry, C., Pan, C., Hosain, R., Mahmood, A., Davis, J.D., Turner, K.C., Hooper, A.T., Hamilton, J.D., Baum, A., Kyratsous, C.A., Kim, Y., Cook, A., Kampman, W., Kohli, A., Sachdeva, Y., Graber, X., Kowal, B., Dicioccio, T., Stahl, N., Lipsich, L., Braunstein, N., Herman, G., and Yancopoulos, G.D. 2021*a*. REGN-COV2, a Neutralizing Antibody Cocktail, in Outpatients with Covid-19. N. Engl. J. Med. **384**: 238–251. doi: 10.1056/NEJMoa2035002.

Weinreich, D.M., Sivapalasingam, S., Norton, T., Ali, S., Gao, H., Bhore, R., Xiao, J., Hooper, A.T., Hamilton, J.D., Musser, B.J., Rofail, D., Hussein, M., Im, J., Atmodjo, D.Y., Perry, C., Pan, C., Mahmood, A., Hosain, R., Davis, J.D., Turner, K.C., Baum, A., Kyratsous, C.A., Kim, Y., Cook, A., Kampman, W., Roque-Guerrero, L., Acloque, G., Aazami, H., Cannon, K., Simón-Campos, J.A., Bocchini, J.A., Kowal, B., Dicioccio, A.T., Soo, Y., Geba, G.P., Stahl, N., Lipsich, L., Braunstein, N., Herman, G., and Yancopoulos, G.D. 2021*b*. REGEN-COV Antibody Combination and Outcomes in Outpatients with Covid-19. N. Engl. J. Med. **385**: e81. doi: 10.1056/NEJMoa2108163.

Original Article

Case series of COVID-19 outcomes in adult patients with inborn errors of immunity

Jenny Garkaby[a,b]*, Ori Scott[a,b], Laura Abrego Fuentes[a,b], Linda Vong[a,b], Jessica Willett Pachul[b], Myra Pereira[b], Vy Hong-Diep Kim[b], and Chaim M. Roifman[a,b]

ABSTRACT

Background: Since the onset of the COVID-19 pandemic, a main challenge for clinicians and public health decision-makers has revolved around risk stratification in vulnerable populations, in particular individuals with inborn errors of immunity (IEI). However, available reports of the clinical course of COVID-19 in patients with IEI show wide variability, from a complete lack of symptoms to severe and complicated disease.

Objective: To present the clinical features and outcomes of SARS-CoV-2 infection in adult patients with IEI.

Methods: We performed a retrospective chart review documenting patient characteristics and clinical course of SARS-CoV-2 infection between December 2021 and July 2022.

Results: Ten adult patients with IEI followed in our center were diagnosed with COVID-19, as determined by RT-PCR or rapid antigen testing. IEI in this cohort included those with humoral and combined immunodeficiencies, as well as phagocytic defects. An underlying lung comorbidity was identified in 3 patients. Symptoms were mostly mild and self-limiting, and no severe outcomes, complications, or mortality were noted in this study.

Conclusions: We suggest that patients affected by a wide range of both humoral and combined IEI may demonstrate resilience, while highlighting the possible protective effects of vaccination and immunoglobulin replacement in this population.

Statement of Novelty: We report on the mild COVID-19 clinical course of 10 adults with IEI.

Introduction

In March 2020, Coronavirus disease 2019 (COVID-19) caused by the novel coronavirus SARS-CoV-2 was declared a global pandemic (Cucinotta and Vanelli 2020; Kahn and Mcintosh 2005). Among previously healthy individuals, the clinical spectrum of COVID-19 is broad, ranging from asymptomatic infection to severe respiratory complications, myocarditis, and multi-system failure. Given the high burden of disease seen in many immunocompetent hosts, concern has risen in regards to the possible impact of COVID-19 on immunocompromised patients. Indeed, reports published during the early stages of the pandemic showed evidence of hyperinflammation, acute respiratory distress syndrome, cytopenias, and increased mortality among patients with inborn errors of immunity (IEI) (van Damme et al. 2020; Jin et al. 2020; Castano-Jaramillo et al. 2021; Shields et al. 2021; Meyts et al. 2021). Additionally, a prolonged recovery period of up to 60 days was reported in patients with

[a]Canadian Center for Primary Immunodeficiency and the Jeffrey Modell Research Laboratory for the Diagnosis of Primary Immunodeficiency, The Hospital for Sick Children and Research Institute, Toronto, ON; [b]Division of Immunology and Allergy, Department of Paediatrics, The Hospital for Sick Children and the University of Toronto, Toronto, ON

*Corresponding author: Jenny Garkaby/jenn.1184@gmail.com

Submitted 10 August 2022
Accepted 29 August 2022
Available online 31 August 2022

LymphoSign Journal 9:62–66 (2022)
dx.doi.org/10.14785/lymphosign-2022-0009

X-linked agammaglobulinemia (Giardino et al. 2022; Esenboga et al. 2021).

Since the publication of the above reports, additional variables have entered the clinical equation of COVID-19 in IEI patients. These have included the availability of vaccines against SARS-CoV-2, specific therapies such as monoclonal neutralizing antibodies, and the rise of new variants and subvariants. Thus, it is pertinent to continuously re-evaluate the severity of COVID-19 in IEI patients and determine the need for targeted interventions in this population. We hereby review our center's recent experience in following 10 adult patients with a wide variety of IEI affected by COVID-19. All patients had been fully vaccinated against SARS-CoV-2, and most had also been receiving immunoglobulin replacement. Our patients, including those with underlying lung disease, overall showed mild disease symptoms with no complications or need for hospital admission.

Methods

In this retrospective study, we reviewed the charts of patients with IEI followed in our center who were diagnosed with COVID-19 infection between December 2021–July 2022. Patients were included if they consented to participate in the Canadian Centre for Primary Immunodeficiency Registry and Tissue Bank, approved by the Hospital for Sick Children Research Ethics Board (REB protocol # 1000005598). Information regarding patient demographics, underlying immune diagnoses, presence of an underlying lung disease, clinical course of COVID-19 infection, laboratory evaluation and therapeutic measures used, was collected.

Results

Patient characteristics

Between December 2021 and July 2022, 10 adult patients (7 females and 3 males, aged 23–69 years, median age: 38 years) followed by our center for IEI were diagnosed with COVID-19, as determined by RT-PCR or rapid antigen testing (Table 1). With regards to underlying immune diagnosis, 5 patients had a humoral immunodeficiency (CVID, Roifman syndrome), 2 patients had a combined immunodeficiency, 2 patients had immune dysregulation features (currently undergoing genetic work up), and 1 patient had chronic granulomatous disease (CGD). Three patients had an underlying lung condition, including asthma and (or) interstitial lung disease.

Clinical course, immunoglobulin replacement, and vaccination

The most frequent presenting symptoms were upper respiratory tract symptoms such as cough and coryza, while fever was present only in 3 patients. Respiratory complications or asthma exacerbations were not observed in any of the patients. Duration of symptoms ranged from 3 days to 3 weeks (median: 10 days). All patients were treated as outpatients. All patients had been vaccinated with 2–4 doses of specific vaccines against SARS-CoV-2. Eight patients had been receiving regular immunoglobulin replacement therapy at the time of acquiring SARS-CoV-2 infection. P10, a 35-year-old woman with CGD, contracted COVID-19 twice; the first infection occurred 5 months post-vaccination and included 3 days of fever, cough and upper respiratory illness (URI), while the second episode included mild URI symptoms.

COVID-19 treatments

Specific treatments were given to 4 out of 10 patients. P1, a 64 year-old female with recurrent opportunistic lung infections and an unknown genetic diagnosis, was treated with an anti-SARS-CoV-2 monoclonal antibody (sotrovimab) on day 7 of her symptoms and reported improvement 24 hours post-treatment. Sotrovimab was also given to P5, a 39-year-old female with DOCK8 deficiency, and P7, a 41-year-old female with immune dysregulation disorder, autoimmune hemolytic anemia, dysgammaglobulinemia and status post-splenectomy, who both had a mild course. P6, a 23-year-old female with Roifman Syndrome received paxlovid and remained well. Non-specific therapies included empiric antimicrobials and prednisone for P2 due to a prolonged course of disease, prescribed by his family physician for presumed bronchitis.

Follow up

At the time of study conclusion, median follow-up period from the time of infection has been 4 months (range: 0.5–7 months). At present, all patients remain complication-free following infection, with full symptom resolution and no long-term sequelae.

Table 1: **Patients characteristics and clinical features.**

Patient number	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
Gender	F	M	M	F	F	F	F	F	M	F
Age (years)	64	69	45	28	39	23	41	37	27	35
IEI diagnosis	CID	CVID	Roifman syndrome	Humoral immunodeficiency	Hyper Ige syndrome, DOCK8 deficiency	Roifman syndrome	Immune dysregulation, NOD2 variant	Immune dysregulation	CVID	CGD
COVID-19 test method	RT-PCR	Rapid antigen test	Rapid antigen test	Rapid antigen test + PCR	RT-PCR	Rapid antigen test	RT-PCR	Rapid antigen test	Rapid antigen test	Rapid antigen test
Presenting symptoms	Cough, URI fatigue	Cough, headache, loss of smell and taste, fatigue	Cough, URI	Cough, URI, fatigue	Cough, headache	Fever, cough, URI, fatigue	fever, cough, URI, headache	URI	fever, cough, arthralgia	fever, cough, URI (first infection), Two months later brief URI
Duration of symptoms	3 weeks	2 weeks	1 week	5 days	10 days	NA	1 week	2.5 weeks	3-4 days	3 days, 1-2 days
Other health problems	Asthma	OSA, liver cirrhosis, anemia		Hypothyroidism, global developmental delay	Barter syndrome with renal calcifications, liver cirrhosis, hypersplenism, IBD	Mild asthma, CNS vasculitis, seizures	AIHA, liver cirrhosis, s/p splenectomy	Multiple Sclerosis, ITP, AIHA, Myasthenia Gravis		Hypothyroidism
Chronic treatment and prophylaxis	none	IVIG	IVIG	IVIG	IVIG, Filgrastim, Prednisone, MMF	IVIG	IVIG, Prednisone	IVIG	IVIG	none
Vaccination status	3 doses	3 doses	3 doses	2 doses	3 doses	3 doses	4 doses	3 doses	3 doses	3 doses
COVID-19 treatment	anti-COVID19 mAB (sotrovimab)	Empiric antibiotics, Prednisone	none	none	anti-SARS-CoV-2 mAB (sotrovimab)	Paxlovid	anti-SARS-CoV-2 mAB (sotrovimab)	none	none	none
Follow up (months)	4	6	4	7	4	0.5	6	6	1	2

Note: CID, combined immunodeficiency; CVID, common variable immune deficiency; URI, upper respiratory illness; NA, not available; OSA, obstructive sleep apnea; IBD, inflammatory bowel disease; CGD, chronic granulomatous disease; ITP, idiopathic thrombocytopenic purpura; AIHA, autoimmune hemolytic anemia; MMF, Mycophenolate mofetil; mAB, monoclonal antibodies.

Discussion

We report the clinical manifestations and outcomes of 10 adult patients with IEI. This heterogeneous cohort includes patients with humoral immunodeficiency such as hypogammaglobulinemia, combined immunodeficiency such as DOCK8 deficiency, syndromic immunodeficiency (Roifman syndrome), immune dysregulation disorders, and CGD. All patients had an uncomplicated clinical course without the need for hospital admission, even in those with underlying lung disease.

Compared with previous reports of higher disease severity in IEI patients, a number of protective factors may have contributed to milder disease in our cohort. First, all patients in our cohort were diagnosed with COVID-19 between December 2021–July 2022, a period of predominance of the omicron variant, as opposed to the alpha and delta strains likely impacting patients in earlier reports (Araf et al. 2022). Additionally, 7 of 10 patients had been receiving immunoglobulin replacement, likely providing patients with some degree of passive immunity. It is noteworthy that our center has diligently recommended vaccination against SARS-CoV-2 for all patients with IEI, including those with humoral deficiency. Indeed, while specific antibody responses may be variable in such patients, cellular immunity may still be highly beneficial. Finally, specific treatment with sotrovimab or paxlovid were initiated by community practitioners in 50% of our patients, although it remains unclear whether mild symptoms and disease resolution occurred in these patients due to this treatment. It remains unclear under what circumstances and in which IEI patients this treatment should be administered.

We conclude that patients affected by IEI may show clinical resilience in the face of COVID-19 infection, with vaccination against SARS-CoV-2 and immunoglobulin replacement therapy representing possible protective factors in this patient population. Diligent long-term follow-up studies are warranted and are ongoing to ensure no severe or unique complications develop in this patient population.

REFERENCES

Araf, Y., Akter, F., Tang, Y.-D., Fatemi, R., Parvez, M.S.A., Zheng, C., and Hossain, M.G. 2022. Omicron variant of SARS-CoV-2: genomics, transmissibility, and responses to current COVID-19 vaccines. J. Medical Virol. doi: 10.1002/jmv.27588.

Castano-Jaramillo, L.M., Yamazaki-Nakashimada, M.A., O'Farrill-Romanillos, P.M., Muzquiz Zermeño, D., Mendoza, S.C.S., Montoya, E.V., Campos, J.A.G., Sánchez-Sánchez, L.M., González, L.B.G., López, J.M.R., Ogando, JCB, Vásquez-Echeverri, E., Torres, E.A.M., Lopez-Herrera, G., Galicia, L.B., Ruiz, L.B., Staines-Boone, A.T., Espinosa-Padilla, S.E., Mendez, N.H.S., and Reyes, S.O.L. 2021. COVID-19 in the context of inborn errors of immunity: a case series of 31 patients from Mexico. J. Clin. Immunol. **41**: 1463–1478. PMID: 34114122. doi: 10.1007/s10875-021-01077-5

Cucinotta, D., and Vanelli, M. 2020. WHO declares COVID-19 a pandemic. Acta. Biomedica. **91**: 157–160. doi: 10.23750/abm.v91i1.9397.

Esenboga, S., Ocak, M., Akarsu, A., Bildik, H.N., Cagdas, D., Iskit, A.T., and Tezcan, I. 2021. COVID-19 in patients with primary immunodeficiency. J. Clin. Immunol. **41**, 1515–1522. doi: 10.1007/s10875-021-01065-9.

Giardino, G., Milito, C., Lougaris, V., Punziano, A., Carrabba, M., Cinetto, F., Scarpa, R., Dellepiane, R.M., Ricci, S., Rivalta, B., Conti, F., Marzollo, A., Firinu, D., Cirillo, E., Lagnese, G., Cancrini, C., Martire, B., Danieli, M.G., Pession, A., Vacca, A., Azzari, C., Fabio, G., Soresina, A., Agostini, C., Spadaro, G., Badolato, R., Cicalese, M.P., Aiuti, A., Plebani, A., Quinti, I., and Pignata, C. The impact of SARS-CoV-2 infection in patients with inborn errors of immunity: the experience of the italian primary immunodeficiencies network (IPINet). J. Clin. Immunol. **42**(5): 935–946. PMID: 35445287. doi: 10.1007/s10875-022-01264-y.

Kahn, J.S., and Mcintosh, K. 2005. History and Recent Advances in Coronavirus Discovery. J. Pediatr. Infect. Dis. **24**: S223–S227. PMID: 16378050. doi: 10.1097/01.inf.0000188166.17324.60.

Jin, H., Reed, J.C., Liu, S.T.H., Ho, H-E., Lopes, J.P., Ramsey, N.B., Waqar, O., Rahman, F., Aberg, J.A., Bouvier, N.M., and Cunningham-Rundles, C., and Mount Sinai Health System Convalescent Plasma Team. 2020. Three patients with X-linked agammaglobulinemia hospitalized for COVID-19 improved with convalescent plasma. J. Allergy Clin. Immunol. Pract. **8**: 3594–3596.e3. PMID: 32947026. doi: 10.1016/j.jaip.2020.08.059.

Meyts, I., Bucciol, G., Quinti, I., Neven, B., Fischer, A., Seoane, E., Lopez-Granados, E., Gianelli, C., Robles-

Marhuenda, A., Jeandel, P-Y., Paillard, C., Sankaran, V.G., Demirdag, Y.Y., Lougaris, V., Aiuti, A., Plebani, A., Milito, C., Dalm, V., Guevara-Hoyer, K., Sánchez-Ramón, S., Bezrodnik, L., Barzaghi, F., Gonzalez-Granado, L.I., Hayman, G.R., Uzel, G., Mendonça, L.O., Agostini, C., Spadaro, G., Badolato, R., Soresina, A, Vermeulen, F., Bosteels, C., Lambrecht, B.N., Keller, M., Mustillo, P.J., Abraham, R.S., Gupta, S., Ozen, A., Karakoc-Aydiner, E., Baris, S., Freeman, A.F., Yamazaki-Nakashimada, M., Scheffler-Mendoza, S., Espinosa-Padilla, S., Gennery, A.R., Jolles, S., Espinosa, Y., Poli, M.C., Fieschi, C., Hauck, F., Cunningham-Rundles, C., Mahlaoui, N., IUIS Committee of Inborn Errors of Immunity, Warnatz, K., Sullivan, K.E., and Tangye, S.G. 2021. Coronavirus disease 2019 in patients with inborn errors of immunity: an international study. J. Allergy. Clin. Immunol. **147**: 520–531. PMID: 32980424. doi: 10.1016/j.jaci.2020.09.010.

Shields, A.M., Burns, S.O., Savic, S., and Richter, UK PIN COVID-19 Consortium. 2021. COVID-19 in patients with primary and secondary immunodeficiency: The United Kingdom experience. J. Allergy Clin. Immunol. **147**: 870–875.e1. PMID: 33338534. doi: 10.1016/j.jaci.2020.12.620.

van Damme, K.F.A., Tavernier, S., Roy, N.V., Leeuw, E.D., Declercq, J., Bosteels, C., Maes, B., Bruyne, M.D., Bogaert, D, Bosteels, V., Hoste, L., Naesens, L., Maes, P, Grifoni, A., Weiskopf, D., Sette, A, Depuydt, P., Braeckel, E.V., Haerynck, F, and Lambrecht, B.N. 2020. Case report: convalescent plasma, a targeted therapy for patients with cvid and severe COVID-19. Front. Immunol. **11**: 596761. PMID: 33329586. doi: 10.3389/fimmu.2020.596761.

Original Article

Association between *NOD2* and autoinflammation presenting as Yellow Nail Syndrome

Laura Edith Abrego Fuentes[a] and Mohammad Alsalamah[b,c,d*]

ABSTRACT

Background: Yellow Nail Syndrome is defined as a triad of lymphedema, respiratory symptoms, and nail discolouration. The precise etiology remains unknown, however it has been reported alongside a broad spectrum of conditions including malignancies, autoinflammatory diseases, and immunodeficiencies.

Aim: To highlight the association between defects in the intracellular bacterial sensor gene *NOD2* and Yellow Nail Syndrome.

Methods: A retrospective review of the patient's chart was performed, including family history, characteristics, immune laboratory evaluation, and genetics.

Results: A 65-year-old female was referred to our centre for lymphedema and bronchiectasis. She had recurrent episodes of pneumonia, cellulitis, and oral ulcers. Bilateral lymphedema on her lower limbs up to the hip and discoloured yellow nails were reported. Given her clinical picture, she was diagnosed with Yellow Nail Syndrome. The immunological evaluation was unremarkable overall, with normal T cell subsets and function and adequate antibody titers. Genetic testing identified a heterozygous mutation in the *NOD2* gene, c.2107C>T (p.Arg703Cys), considered a variant of uncertain significance.

Conclusion: Heterozygous variants in *NOD2* can result in a spectrum of autoimmune and autoinflammatory disorders, including Yellow Nail Syndrome.

Statement of novelty: We describe a patient with Yellow Nail Syndrome, presenting with the classic triad of clinical features. Genetic evaluation identified a heterozygous variant in *NOD2*, which has been extensively associated with several autoinflammatory diseases, but not Yellow Nail Syndrome.

Introduction

Yellow Nail Syndrome (YNS) was initially described in 1964, consisting of discoloured nails and lymphedema in a group of 13 patients (Samman and White 1964). The clinical features have since evolved to include the triad of discoloured nails, lymphedema, and respiratory symptoms (including pleural effusion, bronchiectasis, sinusitis, and chronic cough) (Hiller et al. 1972; Emerson 1966). At least two of these three manifestations must be present, with nail abnormalities being a major criterion, as the manifestations may not always be present simultaneously (Vignes and Baran 2017). It is reported almost exclusively in adults over 50 years of age, without sex predilection (Hoque et al. 2007; Maldonado et al. 2008; Piraccini et al. 2014). While extremely rare, YNS has also been documented in pediatric populations (Vignes and Baran 2017).

[a]Division of Clinical Immunology and Allergy, Department of Paediatrics, Hospital for Sick Children and University of Toronto, Toronto, ON; [b]College of Medicine, King Saud Bin Abdulaziz University for Health Sciences, Riyadh; [c]King Abdullah International Medical Research Center, Riyadh; [d]Clinical Immunology and Allergy Division. Pediatrics Department, King Abdullah Specialist Children's Hospital, Ministry of National Guard - Health Affairs, Riyadh

*Corresponding author: Mohammad Alsalamah/alsalamahmo@ngha.med.sa

Submitted 21 August 2022
Accepted 28 August 2022
Available online 30 August 2022

LymphoSign Journal 9:67–71 (2022)
dx.doi.org/10.14785/lymphosign-2022-0011

To date, the precise etiology of YNS remains unclear. It has been associated with malignancy (including bronchial carcinoma, breast, non-Hodgkin's lymphoma, gallbladder, larynx, renal cell carcinoma, endometrium, melanoma, multiple myeloma after hematopoietic stem cell transplantation or precancerous mycosis fungoides), leading to suggestions that YNS is a paraneoplastic syndrome (Hoque et al. 2007; Maldonado et al. 2008; Piraccini et al. 2014; Vignes and Baran 2017). The symptoms of lymphedema, nail discolouration, and pleural effusions (specifically chylothorax) have also been ascribed to lymphatic impairment, yet this does not explain the chronic cough and sinus infections. Microvasculopathy with protein leakage rather than functional lymphatic impairment has also been considered (Maldonado et al. 2008).

An underlying immunological association has previously been proposed (Siegelman et al. 1969). Reports of YNS in conjunction with immunodeficiencies such as common variable immunodeficiency and combined T and B cell deficiency may explain the sinus and pulmonary infections (Vignes and Baran 2017). Yet, immune investigations among these patients are scarce, and the few reported cases are remarkable for severe lymphopenia, deficiency of naive CD4+ and CD8+ T cells and total B cells. Increased transitional B cells were observed, as well as abnormal T cell response to mitogens and antibody deficiency (Gupta et al. 2012).

Some studies have highlighted FOXC2, a member of the forkhead domain family of transcription factors, as a possible genetic cause. Variable phenotypical presentations were described, including lymphedema-distichiasis syndrome (OMIM 153400), Meige lymphedema (OMIM 153200), and lymphedema ptosis (OMIM 15300) (Finegold et al. 2001; Brice 2002).

Here, we describe a patient with YNS who presented with the classic triad of clinical features. Genetic investigations identified a heterozygous mutation in the *NOD2* gene, which has been extensively associated with autoinflammatory disease and immunodeficiency.

Case presentation

Patient

Our patient is a 65-year-old female of Italian descent who first presented at the age of 59 years with recurrent X-ray-confirmed pneumonias, occurring 2–3 times per year, which required management with oral antibiotics. She has mild bronchiectasis affecting the right upper and lower lobe, as well as the left lower lobe. Chronic sinusitis was noted, with sinus CT showing mild polypoid hypertrophy of the right inferior nasal turbinate and significant mucosal thickening affecting most of the sinuses. She experiences postnasal drip and chronic cough managed by a corticosteroid inhaler.

Over the past 7 years, she has had 3 separate episodes of cellulitis affecting her left leg, right leg, and once involving her right shin (each treated with IV or oral antibiotics). From an autoimmune standpoint, she suffers recurrent oral ulcers and has osteoarthritis of the hips, knees, and proximal interphalangeal and distal interphalangeal joints. She also has Raynaud's phenomenon in both hands, triggered by cold weather. An abdominal CT scan demonstrated extensive colon diverticulosis.

On physical examination, the patient was found to have thick yellow nails, inflammatory hyperpigmentation and lichenification, severe lymphedema and lipedema bilaterally on both her lower limbs up to her hips. Given the characteristic symptoms, she was subsequently diagnosed with YNS.

The patient's family history is remarkable for consanguinity, autoimmunity, and malignancy. Her parents are third cousins. Father suffers from congestive heart disease and hypertension, while the mother has hypothyroidism, hypertension, and arthritis. The patient's sister passed away from pancreatic cancer around age 60. She has another half-sister and a brother who has hypertension and a history of bladder cancer. The patient has two sons, one had thyroid cancer, and the other suffered from chronic urticaria. Extensive oncology workup of our patient, including whole body CT and mammography, did not show any evidence of malignancy.

Immune evaluation

Immune investigations showed normal immunoglobulins (IgG: 13.6 g/L, IgA 1.9 g/L, IgM: 0.5 g/L) while specific antibody titers against mumps, measles, rubella, tetanus, and varicella were protective (Table 1). Lymphocyte immunophenotyping was unremarkable overall, although slightly low B cell numbers of 83 cells/μL (normal: 91–610 cells/μL) were detected,

Table 1: **Immune evaluation of our patient with Yellow Nail Syndrome.**

Parameter	Reference Range	Patient (age 62 y)
CD3+ (%)	62–87	85.8
CD3+ Absolute (cells/μL)	570–2400	1620
CD3+CD8+ (%)	15–46	15.0
CD3+CD8+ Absolute (cells/μL)	210–1200	283
CD3+CD4+ (%)	32–64	67.8 (H)
CD3+CD4+ Absolute (cells/μL)	430–1800	1,280
CD19+ (%)	6–23	4.4 (L)
CD19+ Absolute (cells/μL)	91–610	83 (L)
CD16+56+ (%)	4–26	8.3
CD16+56+ Absolute (cells/μL)	78–470	157
CD4/CD8 Ratio	0.80–3.90	4.5 (H)
Total Protein (g/L)		72
Albumin (g/L)		44
IgA (g/L)	0.8–4	1.9
IgG (g/L)	6.0–16.0	13.6
IgM (g/L)	0.5–2.0	0.5

which is not expected to be significant given normal humoral investigations. T cell subsets were within normal range (CD3+CD8+: 283 cells/μL, normal: 200–900 cells/μL; CD3+CD4+ 1280 cells/μL, normal: 300–1400 cells/μL) with a slight predominance of CD4+ over CD8+ T cells, at a ratio of 4.5 (normal: 0.8–3.9). NK cell numbers were unremarkable. Lymphocyte proliferation to the mitogen phytohemagglutinin (PHA) was normal, with a stimulation index of 1039.

Genetic investigations

A 17-gene Autoinflammatory Disease panel detected a heterozygous variant of uncertain significance in the *NOD2* gene (NM_022162.1), c.2107C>T (p.Arg703Cys), resulting in the substitution of an arginine residue with cystine at position 703. In silico programs (Sift, Polyphen, MutationTaster) found no consensus regarding the pathogenicity of this variant.

Discussion

We report a novel association between YNS and the *NOD2* gene, encoding NOD2 (nucleotide-binding oligomerization domain-containing protein 2). Previously referred to as CARD15 (caspase recruitment domain-containing protein 15), NOD2 belongs to the intracellular NOD-like receptor family of pattern recognition receptors. These serve as intracellular sensors that detect patterns in bacterial peptidoglycan (i.e., muramyl dipeptide) and promote bacterial clearance through activation of the NF-kappa-B pathway and autophagy. Mutations in NOD2 are linked with several chronic inflammatory diseases, suggesting that balanced signalling is critical for maintaining immune homeostasis (Negroni et al. 2018). NOD2 is expressed primarily in monocytes but also in macrophages, dendritic cells, lymphocytes, epithelial and endothelial cells, and intestinal paneth cells.

The *NOD2* gene maps to chromosome 16q12 and comprises 12 coding exons. Structurally, NOD2 consists of 2 N-terminal Caspase Recruitment Domains (CARD), followed by a Nucleotide Binding Domain (NBD, residues 195–425), Helicoidal Domain 1 (HD1, residues 426–485), Winged-helix Domain (WHD, residues 486–602), Helicoidal Domain 2 (HD2, residues 2603–743) and a C-terminal leucine-rich repeat domain (LRR, residues 744–1,020) (Maekawa et al. 2016) (Figure 1). The CARD domains are responsible for signal transduction, the NBD through HD2 domains together form the NOD domain with ATPase activity, while the LRR is essential for recognizing ligands.

To date, pathogenic mutations in NOD2 have been associated with Blau Syndrome (OMIM 186580) (Miceli-Richard et al. 2001) and Early Onset Sarcoidosis, Crohn's Disease (OMIM 266600) (Ogura et al. 2001; Hugot et al. 2001; King et al. 2006), and NOD Associated Autoinflammatory Disease (NAID)/Yao

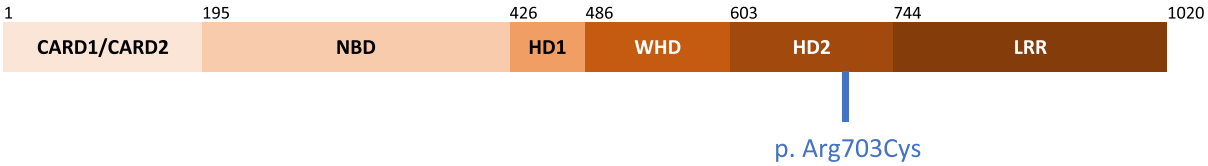


Figure 1: Schematic diagram of NOD2. The NOD2 receptor is comprised of 2 N-terminal CARD domains, a NOD domain encompassing the Nucleotide Binding Domain (NBD), Helicoidal Domain 1 (HD1), Winged-Helix Domain (WHD), and Helicoidal Domain (2), and a C-terminal leucine-rich repeat (LRR) domain. The mutation identified in our patient c.2107C>T leading to p.Arg703Cys is shown in blue.

Syndrome (OMIM 617321) (Yao et al. 2011). In our patient, the heterozygous NOD2 variant, c.2107C>T (p.Arg703Cys), affects the HD2 domain. Mutations targeting this domain have been reported in patients with the above conditions, resulting in either gain- or loss-of-function (Tanabe et al. 2004).

Blau Syndrome and Early Onset Sarcoidosis are the heritable and sporadic forms, respectively, of an autoinflammatory disease caused by NOD2 gain-of-function mutations. These are characterized by non-caseating granulomatous arthritis, uveitis, and dermatitis. Cranial neuropathies, sarcoidosis, and a high incidence of Crohn's colitis are reported (Tangye et al. 2022). The majority of NOD2 mutations that lead to these conditions are concentrated on or near the domain interfaces of NBD, HD1, WHD and HD2: 3 mutations have been reported in the NBD, 7 in HD1, 3 in the WHD, and 1 in HD2. While arthritis involving wrists, ankles, knees, and interphalangeal joints in Blau syndrome/Early Onset is reminiscent of the arthritic features in our patient, the other more common features were absent.

The NOD2 variants associated with Crohn's disease, of which the majority are loss-of-function, can be found scattered throughout the NOD2 protein in all domains except HD1; 13 mutations mapped to the NBD, 1 to the WHD, 7 to HD2 and 10 to the LRR domain (Maekawa et al. 2016). Of note, a polymorphism associated with Crohn's disease, p.Arg702Trp, lies within just one residue of the mutation identified in our patient. Yet, the only gastrointestinal finding in our patient was diverticulosis.

The p.Arg702Trp is also present in patients described with NAID/Yao Syndrome, an autoinflammatory disease characterized by periodic fever, dermatitis, arthritis, and swelling of the distal extremities, as well as gastrointestinal and sicca-like symptoms (Yao et al. 2013, 2019). The clinical picture of our patient does not fit the NAID/Yao Syndrome criteria.

The features of YNS in our patient may be an atypical or mild presentation of one or more of the diverse conditions attributed to NOD2. Paradoxically, her immune evaluation was not consistent with the previously described immune defects associated with YNS. It is not uncommon for genes located on the same chromosome to share overlapping phenotypes. For example, inflammatory disorders categorized under the umbrella of Systemic Autoinflammatory Diseases (SAIDs), which encompasses a group of genetically diverse and heterogeneous inflammatory disorders resulting from abnormal innate immunity, have been attributed to both *NOD2* and *MEFV* genes (Aksentijevich 2015; Yao et al. 2019). Both are present in chromosome 16. Interestingly, *FOXC2*, linked to YNS (OMIM 602402), is also located on chromosome 16.

In sum, we describe a patient with YNS whom, upon genetic evaluation, we identified a heterozygous mutation in NOD2. To our knowledge, this is the first report of such an association. Further investigations are required to delineate the role of NOD2 in YNS. With the widespread availability of genetic tools, including next-generation sequencing, attaining a genetic diagnosis should be prioritized to provide tailored and effective treatment options.

REFERENCES

Aksentijevich, I. 2015. Update on genetics and pathogenesis of autoinflammatory diseases: the last 2 years. Semin. Immunopathol. **37**: 395–401. PMID: 25860799. doi: 10.1007/s00281-015-0478-4.

Brice, G. 2002. Analysis of the phenotypic abnormalities in lymphoedema-distichiasis syndrome in 74 patients with FOXC2 mutations or linkage to 16q24. J. Med. Genet. **39**: 478–483. PMID: 12114478. doi: 10.1136/jmg.39.7.478.

Emerson, P.A. 1966. Yellow nails, lymphoedema, and pleural effusions. Thorax, **21**: 247–253. PMID: 5914998. doi: 10.1136/thx.21.3.247.

Finegold, D.N., Kimak, M.A., Lawrence, E.C., Levinson, K.L., Cherniske, E.M., Pober, B.R., Dunlap, J.W., and Ferrell, R.E. 2001. Truncating mutations in FOXC2 cause multiple lymphedema syndromes. Hum. Mol. Genet. **10**: 1185–1189. PMID: 11371511. doi: 10.1093/hmg/10.11.1185.

Gupta, S., Samra, D., Yel, L., and Agrawal, S. 2012. T and B Cell Deficiency Associated with Yellow Nail Syndrome. Scand. J. Immunol. **75**: 329–335. PMID: 21995335. doi: 10.1111/j.1365-3083.2011.02653.x.

Hiller, E., Rosenow, E.C., and Olsen, A.M. 1972. Pulmonary Manifestations of the Yellow Nail Syndrome. Chest, **61**: 452–458. PMID: 5046843. doi: 10.1378/chest.61.5.452.

Hoque, S.R., Mansour, S., and Mortimer, P.S. 2007. Yellow nail syndrome: not a genetic disorder? Eleven new cases and a review of the literature.

Br. J. Dermatol. **156**: 1230–1234. PMID: 17459037. doi: 10.1111/j.1365-2133.2007.07894.x.

Hugot, J.P., Chamaillard, M., Zouali, H., Lesage, S., Cezard, J.P., Belaiche, J., Almer, S., Tysk, C., O'morain, C.A., Gassull, M., Binder, V., Finkel, Y., Cortot, A., Modigliani, R., Laurent-Puig, P., Gower-Rousseau, C., Macry, J., Colombel, J.F., Sahbatou, M., and Thomas, G. 2001. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature, **411**: 599–603. PMID: 11385576. doi: 10.1038/35079107.

King, K., Sheikh, M.F., Cuthbert, A.P., Fisher, S.A., Onnie, C.M., Mirza, M.M., Pattni, R.C., Sanderson, J., Forbes, A., Mansfield, J., Lewis, C.M., Roberts, R.G., and Mathew, C.G. 2006. Mutation, selection, and evolution of the Crohn disease susceptibility gene CARD15. Hum. Mutat. **27**: 44–54. PMID: 16278823. doi: 10.1002/humu.20264.

Maekawa, S., Ohto, U., Shibata, T., Miyake, K., and Shimizu, T. 2016. Crystal structure of NOD2 and its implications in human disease. Nat. Commun. **7**: 11813. PMID: 27283905. doi: 10.1038/ncomms11813.

Maldonado, F., Tazelaar, H.D., Wang, C.-W., and Ryu, J.H. 2008. Yellow Nail Syndrome. Chest, **134**: 375–381. PMID: 18403655. doi: 10.1378/chest.08-0137.

Miceli-Richard, C., Lesage, S., Rybojad, M., Prieur, A.M., Manouvrier-Hanu, S., Hafner, R., Chamaillard, M., Zouali, H., Thomas, G., and Hugot, J.P. 2001. CARD15 mutations in Blau syndrome. Nat. Genet. **29**: 19–20. PMID: 11528384. doi: 10.1038/ng720.

Negroni, A., Pierdomenico, M., Cucchiara, S., and Stronati, L. 2018. NOD2 and inflammation: current insights. J. Inflamm. Res., **11**: 49–60. PMID: 29483781. doi: 10.2147/JIR.S137606.

Ogura, Y., Bonen, D.K., Inohara, N., Nicolae, D.L., Chen, F.F., Ramos, R., Britton, H., Moran, T., Karaliuskas, R., Duerr, R.H., Achkar, J.P., Brant, S.R., Bayless, T.M., Kirschner, B.S., Hanauer, S.B., Nunez, G., and Cho, J.H. 2001. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature, **411**: 603–606. PMID: 11385577. doi: 10.1038/35079114

Piraccini, B.M., Urciuoli, B., Starace, M., Tosti, A., and Balestri, R. 2014. Yellow nail syndrome: Clinical experience in a series of 21 patients. JDDG: J. Dtsch. Dermatol. Ges. **12**: 131–137. PMID: 24134631. doi: 10.1111/ddg.12216.

Samman, P.D., and White, W.F. 1964. The "Yellow Nail" Syndrome. Br. J. Dermatol. **76**: 153–157. PMID: 14140738. doi: 10.1111/j.1365-2133.1964.tb14499.x.

Siegelman, S.S., Heckman, B.H., and Hasson, J. 1969. Lymphedema, Pleural Effusions and Yellow Nails. Dis. Chest. **56**: 114–117. PMID: 5822545. doi: 10.1378/chest.56.2.114.

Tanabe, T., Chamaillard, M., Ogura, Y., ZHU, L., Qiu, S., Masumoto, J., Ghosh, P., Moran, A., Predergast, M.M., Tromp, G., Williams, C.J., Inohara, N., and Núñez, G. 2004. Regulatory regions and critical residues of NOD2 involved in muramyl dipeptide recognition. EMBO J. **23**: 1587–1597. PMID: 15044951. doi: 10.1038/sj.emboj.7600175.

Tangye, S.G., Al-Herz, W., Bousfiha, A., Cunningham-Rundles, C., Franco, J.L., Holland, S.M., Klein, C., Morio, T., Oksenhendler, E., Picard, C., Puel, A., Puck, J., Seppänen, M.R.J., Somech, R., Su, H.C., Sullivan, K.E., Torgerson, T.R., and Meyts, I. 2022. Human Inborn Errors of Immunity: 2022 Update on the Classification from the International Union of Immunological Societies Expert Committee. J. Clin. Immunol. **24**: 1–35. doi: 10.1007/s10875-022-01289-3

Vignes, S., and Baran, R. 2017. Yellow nail syndrome: a review. Orphanet. J. Rare. Dis. **12**(1): 42. PMID: 28241848. doi: 10.1186/s13023-017-0594-4.

Yao, Q., Li, E., and Shen, B. 2019. Autoinflammatory disease with focus on NOD2-associated disease in the era of genomic medicine. Autoimmunity, **52**: 48–56. PMID: 31084224. doi: 10.1080/08916934.2019.1613382.

Yao, Q., Su, L.-C., Tomecki, K.J., Zhou, L., Jayakar, B., and Shen, B. 2013. Dermatitis as a characteristic phenotype of a new autoinflammatory disease associated with NOD2 mutations. J. Am. Acad. Dermatol. **68**: 624–631. PMID: 23102769. doi: 10.1016/j.jaad.2012.09.025.

Yao, Q., Zhou, L., Cusumano, P., Bose, N., Piliang, M., Jayakar, B., Su, L.C., and Shen, B. 2011. A new category of autoinflammatory disease associated with NOD2 gene mutations. Arthritis. Res. Ther. **13**: R148. PMID: 21914217. doi: 10.1186/ar3462.

A novel variant in *RUNX1* in a patient with refractory eosinophilic gastrointestinal disease and long-term clinical response to ketotifen

Ori Scott[a], Jenny Garkaby[a], Laura Abrego Fuentes[a], Chaim M. Roifman[a,b]*, and Jessica Willett Pachul[a]

ABSTRACT

Background: Eosinophilic gastrointestinal disease (EGID) is an umbrella term for a heterogeneous group of disorders affecting the GI tract. In contrast to the relatively common eosinophilic esophagitis (EoE), eosinophilic gastroenteritis (EGE) remains poorly understood in terms of both its pathophysiology and genetic etiology, while treatment options remain limited.

Aim: To expand the genotypic spectrum of EGE and describe our long-term experience of treatment with ketotifen.

Methods: Case report of a patient with EGE followed by our team for over 27 years.

Results: Our patient was diagnosed with EGE at the age of 4 years, accompanied by multiple other atopic manifestations and serum eosinophilia. He was later diagnosed with a heterozygous variant in *RUNX1*, a gene implicated in multi-lineage hematopoiesis, inhibition of Th2 polarization and T regulatory cell function. The patient has experienced long-term symptom improvement while treated with the mast cell stabilizing H1 antihistamine, ketotifen, with substantial symptomatic worsening after this agent was briefly stopped.

Conclusion: We expand the genotypic spectrum of EGID etiology to include mutations in *RUNX1*, and suggest ketotifen as a viable option for patients with treatment-refractory EGE.

Statement of novelty: This case reports on a possible novel genetic cause of EGID and describes long-term successful clinical management with ketotifen.

Introduction

Eosinophilic gastrointestinal disease (EGID) represents a wide spectrum of conditions marked by inflammatory eosinophilic infiltration of the GI tract, including eosinophilic esophagitis (EoE), gastroenteritis (EGE), and colitis (EoC). EoE is the most common form of EGID, with an estimated prevalence of 1:2000, while EGE remains rarer (estimated prevalence of 2–5:100 000), although this latter figure likely represents a substantial underestimation due to heterogeneity of symptoms and a lack of clear, standardized diagnostic criteria (Gonsalves 2019; Rossi et al. 2022; Dellon and Spergel 2022). In line with our incomplete clinical understanding of EGID, its genetic etiology has not been fully elucidated. Single nucleotide polymorphisms (SNPs) in genetic loci associated with barrier response to tissue damage and Th2 immunity have been implicated in

[a]Division of Immunology and Allergy, Department of Paediatrics, Hospital for Sick Children and University of Toronto, Toronto, ON; [b]The Canadian Centre for Primary Immunodeficiency and The Jeffrey Modell Research Laboratory for the Diagnosis of Primary Immunodeficiency, The Hospital for Sick Children, Toronto, ON

*Corresponding author: Chaim M. Roifman/chaim.roifman@sickkids.ca

Submitted 15 August 2022
Accepted 28 August 2022
Available online 30 August 2022

LymphoSign Journal 9:72–76 (2022)
dx.doi.org/10.14785/lymphosign-2022-0010

EoE, with notable examples including *CAPN14, STAT6*, and *TSLP* (Ryu et al. 2020). However, much less is known about the genetics of other forms of EGID.

Further to the above challenges in diagnosing EGID and establishing its etiology, therapeutic options for patients with EGID remain limited. In EoE patients, current approaches may involve both dietary and pharmaceutical interventions, including various degrees of elimination diets, proton pump inhibitors, steroids (topical and/or systemic), leukotriene receptor antagonists, mast cell stabilizers, and more recently biologic agents (Gonsalves 2019; Dellon and Spergel 2022). However, well-established and effective treatment protocols are still lacking for EGE.

The current report described our experience of caring for a patient with EGE over a period of 27 years. The patient was diagnosed with a variant in the *RUNX1* gene, potentially accounting for his presentation. He had substantial clinical and endoscopic improvement on ketotifen, with rapid symptom recurrence after treatment was briefly stopped.

Methods

Patient and blood samples

Data compiled prospectively and retrospectively from patient medical records were entered into the Canadian Centre for Primary Immunodeficiency Registry and Tissue Bank, which has been approved by the SickKids Research Ethics Board (protocol No. 1000005598). Patient and family members provided written informed consent.

Genetic diagnosis and sequencing confirmation

Genomic DNA was isolated from patient peripheral blood leukocytes using the Geneaid genomic DNA extraction kit (Geneaid Mini Kit; Sensi Capital Corp, Toronto, Ontario, Canada). The patient's mutation was identified via whole exome sequencing using Illumina HiSeq2500 GATK 1.1.28 sequencing platform, and confirmed by Sanger sequencing using DTCS Quick Kit on an automated sequencer (Beckman-Coulter CEQ 8000).

Clinical case

A male patient was first referred to our practice at the age of 4 years, and is still currently followed at the age of 31 years. He was first referred with a history of difficult-to-control EGID, other atopic features, and peripheral hypereosinophilia. His EGID presenting complaints included poor growth, abdominal pain, severe reflux, dysphagia, and vomiting, and he was identified on an upper endoscopy to have marked distal esophagitis with deep linear ulcers of the antrum extending into the duodenum. On biopsy, he had clusters and aggregates of eosinophils in the distal esophagus, stomach, and duodenum, with foci of blunted villi in the duodenum. Review of past medical history revealed that the patient had other atopic features including asthma and mild eczema, and he later developed allergic rhinitis. He had no history of recurrent or unusual infections, and no autoimmune or inflammatory features. He had received all childhood vaccines uneventfully. On review of family history, his parents were noted to be non-consanguineous of European descent. His father had a similar history of eosinophilic esophagitis/EGID leading to extensive scarring and fibrosis, as well as peripheral hypereosinophilia, anaphylaxis to insect venom, multiple food allergies, and Addison's disease. His mother was healthy. The patient's brother had a diagnosis of psoriasis. There was no family history of malignancy. No abnormalities were noted on physical exam with the exception of mild eczema. Initial laboratory evaluation revealed peripheral hypereosinophilia of 1.9×10^9 cells/L but otherwise normal complete blood count and differential, and, unexpectedly, hypogammaglobulinemia, with IgG of 5.1 g/L and normal IgA (0.8 g/L) and IgM (0.8 g/L). His specific vaccine responses at the time to polio, measles, mumps, rubella, and tetanus were all reactive.

In regard to managing the patient's EGID, initial treatments included a strict six-food elimination diet, multiple courses of systemic steroids, proton pump inhibitors, swallowed steroids, and a trial of montelukast, with minimal symptomatic improvement. Ultimately, substantial clinical relief was noted when he was treated with a combination of ketotifen and an extensive elimination diet. A repeat endoscopy and biopsy at the age of 12 years while on ketotifen showed some improvement in duodenal architecture and a reduction in eosinophilic infiltration of his esophagus, although gastric eosinophilia had not improved. At the age of 27, the patient discontinued his treatment of ketotifen due to daytime somnolence, headaches, and flushing. Shortly thereafter he began experiencing fatigue, shortness of breath and developed peripheral edema. Work-up identified profound anemia with a hemoglobin of 50 g/L, as well as hypoalbuminemia.

Repeat endoscopy showed extensive inflammation of his mid esophagus, gastric antrum, gastric body, and duodenum, and >15 eos/hpf in all the above regions. He was suspected to have protein-losing enteropathy precipitated by uncontrolled EGID. He was re-started on ketotifen and experienced a rapid improvement in his symptoms, with normalization of his hemoglobin and serum albumin.

At the age of 29, given persistence of ketotifen side-effects, the patient was trialed on Dupilumab, of which he received 2 infusions. However, following his second infusion he experienced a rise in his serum eosinophils from the usual range of $2-3 \times 10^9$ cells/L up to 6×10^9 cells/L, without any evidence of end-organ damage related to hypereosinophilia. He therefore did not receive any further Dupilumab infusions. He has resumed treatment with ketotifen and remains on an extensive elimination diet. On his most recent laboratory assessment, he was noted to have hypereosinophilia of 2.6×10^9 cells/L (back to his baseline) but otherwise normal complete blood counts and differential, with normal lymphocyte subsets. His IgG was again low at 2.76 g/L with normal IgA and IgM. He had protective specific vaccine titres to measles, mumps, and varicella, while rubella titres were equivocal. His anti-A isohemagglutinin titre was normal at 1:128.

Genetic evaluation

The patient was first assessed via an inborn errors of immunity panel, which did not reveal any variants that could explain his phenotype. He was subsequently evaluated using research whole exome sequencing and was found to have a novel heterozygous variant in the *RUNX1* gene, NM_001754: exon 9: c.T1270C (p.S424P). This variant was not identified in large population databases, and was predicted *in-silico* to be borderline/deleterious. It is expected to alter the RUNX1-Foxp3 interaction domain, therefore preventing inhibition by T regulatory cells. The variant has previously been reported and classified as a variant of unknown significance in ClinVar, with no patient phenotype reported (ClinVar allele ID 958947).

Discussion

We report on a patient with EGE who was found to harbour a variant in the *RUNX1* gene, coding for Runt-related transcription factor 1 (RUNX1), also known as acute myeloid leukemia 1 (AML1). RUNX1 plays a variety of roles in multi-lineage hematopoietic development, differentiation of myeloid and lymphoid cells, and its interaction with Foxp3 is essential for carrying out the suppressive role of T regulatory cells (Tregs) (Ono et al.2007; Cohen 2009). Additionally, RUNX1 inhibits differentiation of naïve CD4+ T cells into Th2 via repression of GATA3 (Komine et al. 2003). Somatic mutations in *RUNX1* have been implicated in the development of various hematopoietic malignancies, and are also commonly seen in cases of systemic mastocytosis (Holmes et al. 2014; Schwaab et al. 2020; Di Giacomo et al.2022). Heterozygous germline mutations in *RUNX1* have been associated with familial platelet disorder with associated myeloid malignancy (OMIM: 601399), a disorder marked by incomplete penetrance and broad phenotypic spectrum (Schmit et al. 2015; Kanagal-Shamanna et al. 2017; Simon et al. 2020; Li et al. 2021; Tang et al. 2022). Our patient has never displayed any abnormalities in platelet number or size; rather, his presentation is of systemic and gastrointestinal hypereosinophilia. Notably, bone marrow eosinophilia has been identified in 6 of 11 patients with germline *RUNX1* mutations in one case series (Kanagal-Shamanna et al. 2017), while eosinophilic leukemia was reported in another study in a patient with a germline *RUNX1* mutation (Tang et al. 2022). The potential mechanism of eosinophilia may relate to loss-of-function of RUNX1, leading to reduced inhibition of GATA3 and therefore excessive Th2 polarization.

Our patient's mutation has not been reported as causing familial platelet disorder with associated myeloid malignancy; however, it is possible that mutations affecting different amino acid residues of *RUNX1* may lead to a variable phenotype. In this regard, a recent study identified that while some *RUNX1* mutations caused haploinsufficiency others resulted in a dominant negative effect with differential transcriptional outputs (Li et al. 2021). The current mutation, Ser424Pro, affects the transcriptional activation domain at the site of Foxp3 interaction and it is possible therefore that it results in decreased Treg inhibitory capacity, resulting in a "Tregopathy", although this will require molecular functional validation. The patient presented with unexpected hypogammaglobulinemia, though his specific vaccine responses have been intact. Work in an experimental model of *runx1* loss-of-function in zebrafish revealed a humoral immunodeficiency, with failure of V(D)J recombination in B cells but not in T cells.

However, no human evidence is available to date to suggest an equivalent phenotype.

Our patient showed substantial clinical improvement while on treatment with ketotifen, and minimal response to all other treatments. To date, there are no standardized treatment protocols for patients with EGE. A number of ongoing clinical trials have investigated the potential use of biologic agents in EGID, with one of the most promising agents being the IL-4 receptor alpha chain antagonist, Dupilumab, currently approved in the United States for treatment of EoE (Dellon and Spergel 2022). However, evidence for use of biologics in EGE specifically is still lacking. Our patient showed a dramatic rise in serum eosinophils when treated with Dupilumab leading to treatment discontinuation, suggesting that we still do not fully understand the pathophysiology of EGE or how it differs from that of EoE. Other trials in EGE are ongoing for Lirentelmab (Siglec-8 antagonist) and Benralizumab (IL-5 receptor antagonist) (Dellon and Spergel 2022). In regard to evidence for ketotifen use in EGE, and in particular the long-term use of this agent, evidence is still scarce beyond our report of using this agent in 6 patients with EGE in 1991 (Melamed and Roifman 1991). Short-term ketotifen use for a period of 30 days has been reported to result in resolution of acute EGE symptoms, with a patient in clinical remission up to 10 months later (Bolukbas et al. 2004). Another report described a 44-year-old patient with difficult to control EGE who had been treated with ketotifen for over 20 years with excellent symptomatic relief, although histologic findings were suggestive of continued inflammation of his upper GI tract despite the patient being asymptomatic (Freeman 2019). This raises the importance of a combination of periodic clinical and endoscopic surveillance of EGE patients, regardless of treatment modality. While treatment with ketotifen may be a compelling option given the experience of our center and others, a systematic trial assessing the effectiveness and long-term impact of this agent in patients with EGID is warranted. Further points which would require more systematic large-scale evaluation include optimization of treatment dose and standardization of treatment response assessment.

We conclude that EGID is a broad-spectrum group of disorders, with EGE possibly having distinct genetic etiology and pathophysiology as well as differential treatment response compared with the more common EoE. While several genome-wide association studies have identified various risk loci associated with EoE (Kottyan et al. 2021; Chang et al.2022), enabling identification genes and risk variants (Ryu et al. 2020), there is a substantial lag in our knowledge of genetic predisposition to other EGID such as EGE. To date, no clear risk loci have been identified in association with forms of EGID other than EoE. We suggest that mutations in *RUNX1,* previously associated with other hematologic abnormalities such as eosinophilia and malignancy, may also underlie a presentation of tissue eosinophilia such as EGID. We further suggest that in refractory EGE cases, the mast cell stabilizer H1 antihistamine ketotifen may be considered as a therapeutic option.

Ongoing surveillance of patients is warranted to assess for clinical symptoms and histologic evidence of disease.

REFERENCES

Bolukbas, F.F., Bolukbas, C., Uzunkoy, A., Baba, F., Horoz, M., and Ozturk, E. 2004. Case report: a dramatic response to ketotifen in a case of eosinophilic gastroenteritis mimicking abdominal emergency. Dig. Dis. Sci. **49**(11–12): 1782–1785. PMID: 15628703. doi: 10.1007/s10620-004-9570-7.

Chang, X., March, M., Mentch, F., Nguyen, K., Glessner, J., Qu, H., Liu, Y., Furuta, G., Aceves, S., Gonsalves, N., Nadeau, K., Cianferoni, A., Spergel, J., Sleiman, P., and Hakonarson, H. 2022. A genome-wide association meta-analysis identifies new eosinophilic esophagitis loci. J. Allergy Clin. Immunol. **149**(3): 988–998. PMID: 34506852. doi: 10.1016/j.jaci.2021.08.018.

Cohen, M.M. Jr. 2009. Perspectives on RUNX genes: an update. Am. J. Med. Genet. A. **149A**(12): 2629–2646. PMID: 19830829. doi: 10.1002/ajmg.a.33021.

Dellon, E.S., and Spergel, J.M. 2022. Biologics in eosinophilic gastrointestinal diseases. Ann. Allergy Asthma Immunol. **20**: S1081-1206(22)00536-1. PMID: 35738437. doi: 10.1016/j.anai.2022.06.015.

Di Giacomo, D., Quintini, M., Pierini, V., Pellanera, F., La Starza, R., Gorello, P., Matteucci, C., Crescenzi, B., Fiumara, P.F., Veltroni, M., Borlenghi, E., Albano, F., Forghieri, F., Maccaferri, M., Bettelli, F., Luppi, M., Cuneo, A., Rossi, G., and Mecucci, C. 2022. Genomic and clinical findings in myeloid neoplasms with PDGFRB rearrangement. Ann. Hematol. **101**(2): 297–307. PMID: 34859285. doi: 10.1007/s00277-021-04712-8.

Freeman, H.J. 2009. Longstanding eosinophilic gastroenteritis of more than 20 years. Can. J. Gastroenterol. **23**(9): 632–634. PMID: 19816628. doi: 10.1155/2009/565293.

Gonsalves, N. 2019. Eosinophilic gastrointestinal disorders. Clin. Rev. Allergy Immunol. **57**(2): 272–885. PMID: 30903439. doi: 10.1007/s12016-019-08732-1.

Holmes, A., Coviello, J., and Velagaleti, G. 2014. Acute multilineage (B/myeloid) leukemia with RUNX1 duplication/amplification and hypereosinophilia. Eur. J. Haematol. **93**(5): 449–452. PMID: 24912843.

Kanagal-Shamanna, R., Loghavi, S., DiNardo, C.D., Medeiros, L.J., Garcia-Manero, G., Jabbour, E., Routbort, M.J., Luthra, R., Bueso-Ramos, C.E., and Khoury, J.D. 2017. Bone marrow pathologic abnormalities in familial platelet disorder with propensity for myeloid malignancy and germline RUNX1 mutation. Haematologica. **102**(10): 1661–1670. PMID: 28659335. doi: 10.3324/haematol.2017.167726.

Komine, O., Hayashi, K., Natsume, W., Watanabe, T., Seki, Y., Seki, N., Yagi, R., Sukzuki, W., Tamauchi, H., Hozumi, K., Habu, S., Kubo, M., and Satake, M. 2003. The Runx1 transcription factor inhibits the differentiation of naive CD4+ T Cells into the Th2 Lineage by repressing GATA3 expression. J Exp Med. **198**(1): 51–61. PMID: 12835475. doi: 10.1084/jem.20021200.

Kottyan, L.C., Trimarchi, M.P., Lu, X., Caldwell, J.M., Maddox, A., Parameswaran, S., Lape, M., D'Mello, R.J., Bonfield, M., Ballaban, A., Mukkada, V., Putnam, P.E., Abonia, P., Morgenstern, N.B-B, Eapen, A.A., Wen, T., Weirauch, M.T., and Rothenberg, M.E. 2021. Replication and meta-analyses nominate numerous eosinophilic esophagitis risk genes. J. Allergy Clin. Immunol. **147**(1):255–266. PMID: 33446330. doi: 10.1016/j.jaci.2020.10.018.

Li, Y., Yang, W., Devidas, M., Winter, S.S., Kesserwan, C., Yang, W., Dunsmore, K.P., Smith, C., Qian, M., Zhao, X., Zhang, R., Gastier-Foster, J.M., Raetz, E.A., Carroll, W.L., Li, C., Liu, P.P., Rabin, K.R., Sanda, T., Mullighan, C.G., Nichols, K.E., Evans, W.E., Pui, C.H., Hunger, S.P., Teachey, D.T., Relling, M.V., Loh, M.L., and Yang, J.J. 2021. Germline RUNX1 variation and predisposition to childhood acute lymphoblastic leukemia. J. Clin. Invest. **131**(17): e147898. PMID: 34166225. doi: 10.1172/JCI147898.

Melamed, I., and Roifman, C.M. 1991. Benefit of ketotifen in patients with eosinophilic gastroenteritis. Am. J. Med. **90**(3): 310–314. PMID: 2003512.

Ono, M., Yaguchi, H., Ohkura, N., Kitabayashi, I., Nagamura, Y., Nomura, T., Miyachi, Y., Tsukada, T., and Sakaguchi, S. 2007. Foxp3 controls regulatory T-cell function by interacting with AML1/Runx1. Nature. **446**(7136): 685–689. PMID: 17377532. doi: 10.1038/nature05673.

Rossi, C.M., Lenti, M.V., Merli, S., Licari, A., Votto, M., Marseglia, G.L., and Sabatino, A.D. 2022. Primary eosinophilic gastrointestinal disorders and allergy: clinical and therapeutic implications. Clin Transl Allergy [Internet]. [cited 2022 Jul 7]; **12**(5). doi: 10.1002/clt2.12146.

Ryu, S., Lee, K.H., Tizaoui, K., Terrazzino, S., Cargnin, S., Effenberger, M., Shin, J.I., and Kronbichler, A. 2020. Pathogenesis of eosinophilic esophagitis: a comprehensive review of the genetic and molecular aspects. Int J Mol Sci. **21**(19):7253. PMID: 33008138. doi: 10.3390/ijms21197253.

Schmit, J.M., Turner, D.J., Hromas, R.A., Wingard, J.R., Brown, R.A., Li, Y., Li, M.M., Slayton, W.B., and Cogle, C.R. 2015. Two novel RUNX1 mutations in a patient with congenital thrombocytopenia that evolved into a high grade myelodysplastic syndrome. Leuk Res Rep. **4**(1): 24–27. PMID: 25893166. doi: 10.1016/j.lrr.2015.03.002.

Schwaab, J., Cabral do O Hartmann, N., , Naumann, N., Jawhar, M., Weiß, C., Metzgeroth, G., Schmid, A., Lübke, J., Reiter, L., Fabarius, A., Cross, N.C.P., Sotlar, K., Valent, P., Kluin-Nelemans, H.C., Hofmann, W-K., Horny, H-P., Panse, J., and Reiter, A. 2020. Importance of adequate diagnostic workup for correct diagnosis of advanced systemic mastocytosis. J. Allergy Clin. Immunol. Pract. **8**(9): 3121–3127.e1. PMID: 32422371. doi: 10.1016/j.jaip.2020.05.005.

Simon, L., Spinella, J.F., Yao, C.Y., Lavallée, V.P., Boivin, I., Boucher, G., Audemard, E., Bordeleau, M-E., Lemieux, S., Hébert, J., and Sauvageau, G. 2020. High frequency of germline RUNX1 mutations in patients with RUNX1-mutated AML. Blood. **135**(21): 1882–1886. PMID: 32315381. doi: 10.1182/blood.2019003357.

Tang, K.H., Li, S., Khodadadi-Jamayran, A., Jen, J., Han, H., Guidry, K., Chen, T., Hao, Y., Fedele, C., Zebala, J.A., Maeda, D.Y., Christensen, J.G., Olson, P., Athanas, A., Loomis, C.A., Tsirigos, A., Wong, K-K., and Neel, B.G. 2022. Combined inhibition of SHP2 and CXCR1/2 promotes antitumor T-cell response in NSCLC. Cancer Discov. **12**(1): 47–61. PMID: 34353854. doi: 10.1158/2159-8290.CD-21-0369.

LymphoSign Journal

Working together for patients with PI

Immunodeficiency Canada
Immunodéficience Canada

jmcn Jeffrey Modell Centers Network

Canadian Science Publishing