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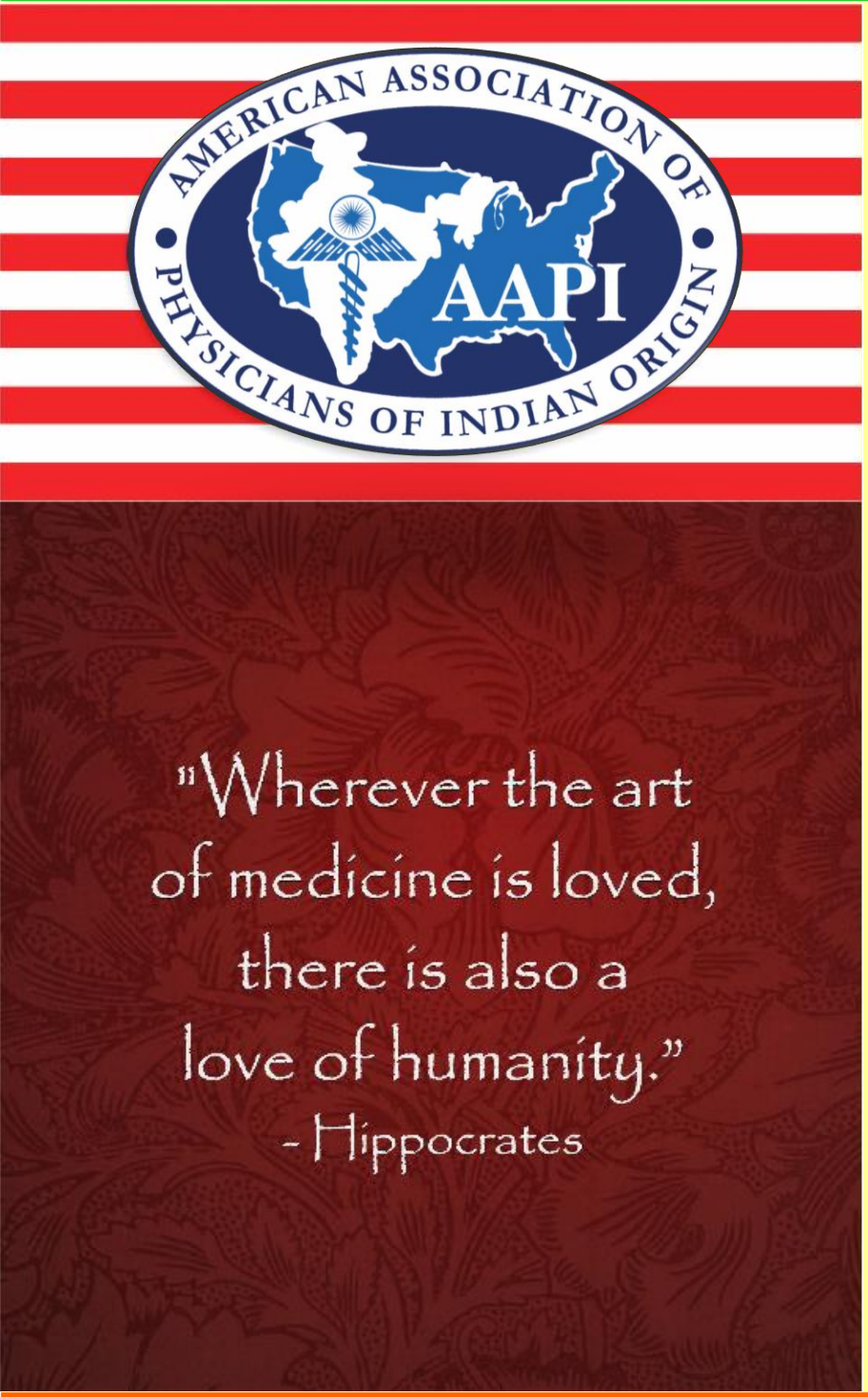

In this Issue...

This Winter Issue of JAAPI is Dedicated to

Sir Ronald Ross, FRS, FRCS

Nobel Laureate 1902 (Medicine)

(1857 -1932)

Image: NIH - in Public Domain

Physician, Surgeon, Mathematician, Epidemiologist, Sanitarian, Editor, Novelist, Dramatist, Poet, Amateur Musician, Composer, and Artist.

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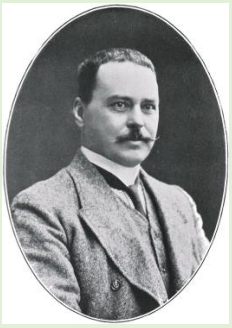

Pioneers in Medicine and Healthcare

Sir Ronald Ross, FRS, FRCS

Nobel Laureate 1902 (Medicine or Physiology)

Physician, Surgeon, Mathematician, Epidemiologist, Sanitarian, Editor, Novelist, Dramatist, Poet, Amateur Musician, Composer and Artist.

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About 10 million children under age 5 die each year. About 10% of them die due to malaria

Peter Agre, M.D., Nobel Laureate & Director of Johns Hopkins Malaria Research Institute

As the world is battling against COVID-19 pandemic, with renewed concerns about the new Omicron mutant of SARS-CoV-2 virus, unnoticed by the rest of the world and the mainstream media, in sub-Saharan Africa, more people are dying due to malaria driven by shortfalls in prevention and treatment efforts. The World Health Organization (WHO) has warned these "additional" deaths due to malaria will probably dwarf direct deaths from COVID-19. According to the WHO, in 2020 there were about 214 million malaria cases resulting in 627,000 deaths worldwide. African countries carry a disproportionately high share (95%) of global malaria burden. Malaria is the fourth leading cause of death in children under 5 years worldwide.

In 19th century, Sir Ronald Ross, a British Physician working in India, discovered the life cycle of malarial parasite in mosquitoes, which opened avenues to prevent malaria. Sir Ronald Ross was born in Almora, India in 1857. At the age or eight he went to England for studying. As a child, Ronald developed interest in poetry, literature, music, and mathematics, which he passionately pursued throughout his life. Ronald did not plan to study medicine, but at 17 as per the wish of his father, he pursed medical education. In 1881, at the age of 24, he entered the Indian Medical Service. Later he obtained a Diploma in Public Health and studied bacteriology. Ronald Ross became interested in malaria in 1892 and worked to prove the hypothesis that mosquitoes are related to the propagation of malaria. While dissecting and examining the stomach contents of an *Anopheles* mosquito on August 20, 1897, in Secunderabad, India, Ross made his landmark discovery. Having found the malarial parasite in the mosquitoes, Ross proved the role of *Anopheles* mosquito in the transmission of malaria in humans. Experimenting on birds as model hosts for malaria, by July 1898 Ross could prove that malarial parasites develop in the mosquitoes and migrate to the insect's salivary grands, from where they are injected into the hosts during bites.

In 1899 Ross moved to England, where he established Liverpool School of Tropical Medicine, and taught there as a Professor of Tropical Medicine. Under his leadership, the school investigated and devised anti-malaria schemes and projects in West Africa. That was the first major international effort to address the problem of malaria in Africa. In 1901 Ross was elected as a Fellow of the Royal Society and Royal College of Surgeons of England. In 1902 he received Nobel Prize in Medicine or Physiology for his work on malaria. In the same year Ross was appointed a Companion of the Most Honourable Order of Bath by His Majesty the King of Great Britain. In 1911, Ross was elevated to the rank of Knight Commander. During the World War I (1914-1918) Ross was appointed as a Consultant Physician of Tropical Diseases to Indian troops. In 1926 the Ross Institute and Hospital for Tropical Diseases was opened on Putney Heath, London with Ross as the Director-in-Chief. He held that position until his death in 1932. In 1934 this Institute was formally incorporated into the London School of Hygiene & Tropical Medicine.

Ross wrote extensively on malaria and other subjects. He also wrote several novels – to name a few *The Child of the Ocean, Spirit of the Storm, The Revels of Orsera.* Despite his illustrious life with rich contributions, unfortunately in 1928 Ross had to sell his papers for £2,000 to provide for his wife and family. These papers now in the possession of the London School of Hygiene & Tropical Medicine, may sell for millions of dollars today if auctioned. Sir Ronald Ross was a versatile genius and a humanitarian. In August 1897, following his discovery of malarial parasite in Anopheles mosquito, he wrote a poem. A fragment of it, cited widely, reads as follows. Incidentally, these words apply today to SARS-CoV-2 virus as well.

With tears and toiling breath
I find thy cunning seeds
O million-murdering Death…

Article Contributed by: Bellamkonda K. Kishore, M.D.

Editorial Perspective

Best Practices in Healthcare

International Standards, Guidelines, and Recommendations
Implementation Challenges and Critical Role of International Collaboration

Vemuri S. Murthy, M.D., M.S.
Advisor, Editorial Board, JAAPI

The Need for a Tailored Approach: "Think Global, Act Local"

"Best Practices in Healthcare" enhance global health. The core components of "Best Practices" include "Health Technology Assessment," "Evidence-Based Medicine," and "Clinical Practice Guidelines." The efforts of International Health Organizations to improve patient outcomes through evidence-based recommendations for best practices are relentless. This editorial column takes an objective look at the realities (challenges) in implementing these standards in resource-limited countries and underserved communities to yield sustainable results.

Human ailments of different etiologies have been wreaking havoc for centuries, impacting longevity and the quality of human lives. The innovative human mind has been striving to reduce the impact of diseases through the medium of organized knowledge, which we call "Science." The continuing human survival depends on the fruits of scientific research such as vaccines, antibiotics, chemotherapeutic agents, and other therapeutic measures.

As inventions emerged in every branch of science, the medical community focused more and more on basic and applied research with experimental trials. Of course, knowledge has always been there. However, the recommendations by the scientific community are solely driven by "evidence-based" knowledge that helps predict outcomes more accurately than ever before. As a result, they became benchmarks for quality healthcare and patient outcomes. "Translational Medicine," the interdisciplinary branch of biomedicine with three pillars of "bench," "bedside," and "community," has become the new buzzword in the scientific community promoting the application of laboratory research and preclinical studies to develop human trials.

The globalization of medicine is a boon to humanity. Sharing evidence-based scientific information among international peers with open communication channels leads to faster management of diseases with better patient outcomes. As a result, international affiliations and partnerships emanate rapidly during this century, unifying "silos of knowledge" from various parts of the world. The recent fast-track development of the COVID-19 vaccine exemplifies such global efforts.

Strategic planning of any successful global health project based on international guidelines needs "fine-tuning" in the real world as these recommendations face challenges for successful implementation in resource-limited countries and even in developed countries within their underserved communities. This is obvious when the same healthcare project design yields better patient outcomes in a well-served area compared to an underserved area. Even though basic healthcare access is considered a "human right" by global consensus, healthcare rationing is well-known in economically backward areas.

Preparing the ground is crucial before implementing any new undertaking. Healthcare projects are no exceptions. A comprehensive feasibility study is a prerequisite for initiating any project. In addition, local living standards, ease of access, and acceptance decide the survival of the projects in any community. Therefore, timely community feedback-based modifications must be made at every implementation phase. The following factors need to be considered in designing and initiating a practical and viable project.

• Available resources, especially funding • Governmental support and local legal requirements • Critical assessment of existing infrastructure and fundamental changes needed to jumpstart the project • Ease of implementation and available help from all stakeholders involved • Financial burden on the uninsured and under-insured community members • Literacy, cultural and religious beliefs • Community awareness

In conclusion, the healthcare models that can be successfully implemented in resource-rich countries and affluent communities may not be sustainable in financially strapped territories and underserved communities. The healthcare initiatives need to be "tailored" to the local needs and resources. Collaborations with global peers and organizations with a performance history will enhance patient outcomes through shared knowledge and combined efforts, and they have a track record in doing just that!

"Science knows no country because knowledge belongs to humanity and is the torch which illuminates the world." - Louis Pasteur

From the Editorial Desk

First Volume of JAAPI Completed

Bellamkonda K. Kishore, M.D., Ph.D., MBA
Editor-in-Chief of JAAPI

As the world has completed two years of COVID-19 pandemic, JAAPI, launched during the pandemic has successfully completed its first volume comprising three issues – *the Inaugural, Summer, and Winter*. The first volume has 41 articles, of which 12 are reviews, 2 case reports, 1 clinical study, 2 commentaries, 3 updates/guidelines, 2 brief report/analysis, 1 regulatory compliance, 5 pioneer profiles, 5 editorials/invited editorials, 7 symposia synopses, and 1 set of abstracts of Winter Medical Research conference. That is a very impressive repertoire within a year for a new peer-reviewed scientific journal. The credit for this achievement goes to the entire Editorial Board, authors who contributed, reviewers who reviewed the articles, the AAPI Leadership and administrative office for their unconditional support to JAAPI. We will work with renewed enthusiasm and rigor to elevate JAAPI as a premier academic journal of AAPI.

As we are entering the second year of publication, there are a few important developments. First, JAAPI has a Manuscript Management Platform, leased from a company in Denmark. This platform will be linked to the JAAPI page in AAPI website, which is https://www.aapiusa.org/jaapi/ This platform will function as the virtual office of JAAPI, where all peer-review work will take place and the documents stored securely. Prospective authors should submit their manuscripts through this management platform after creating an account. One can continue to use the email ID jaapi@aapiusa.org to contact the Editor-in-Chief directly for any questions, but not for submission of manuscripts. Second, to organize and streamline editorial work, JAAPI has recruited an Editorial Executive, Ms. Constance Kimball, a very efficient and experienced administrator. Third, with the success of the first two issues of JAAPI, we are able to attract outstanding academicians as editorial board members in the United States and India. Some of them commit time as Deputy Editors, while others would like to work as Guest Editors whose expertise and services will be utilized when there is a need. Thus, the editorial board of JAAPI is expanding to cover different specialties of clinical medicine. We thank the new board members for their support. They are listed in the Editorial Board section of JAAPI.

Another important change is presentation of credentials and affiliations of board members. In line with the practice in the publication world, starting with this Winter issue, JAAPI is using ORCID (**O**pen **R**esearcher and **C**ontributor **Id**entifier) numbers of the Editorial Board members. ORCID system is for identifying and accessing the profiles, such as qualifications, positions, affiliations, publications of editors, reviewers, and authors in the publication world.

Last but not the least, this Winter Edition is dedicated to Sir Ronald Ross, Nobel Laureate in Medicine or Physiology. About 125 years ago, working in India, Dr. Ross discovered how malarial parasite is transmitted through mosquitoes. Since then, despite all our efforts, many countries in Asia and Africa are still plagued by malaria with significant mortality. We knew for long time that sub-Saharan Africa bears the brunt of malaria. But the COVID-19 pandemic precipitated another crisis in Africa. Due to disruption of healthcare delivery systems and supply chains, the number of deaths due to malaria have increased substantially, and most of the dead are children. What is alarming is in sub-Saharan Africa, number of deaths due to malaria are dwarfing the deaths due to COVID-19. This is not acceptable. As exemplified in the blog of Richard Smith, M.D., former editor of the British Medical Journal reproduced in this issue, and the Editorial Perspective by Vemuri S. Murthy, M.D., Editorial Advisor of JAAPI, globalization of healthcare has both pros and cons, depending on the nature of the disease and whether it is endemic, epidemic, or pandemic as well available resources. COVID-19 pandemic has exposed these fault lines regarding both management of COVID-19 disease and prevention of SARS-CoV-2 virus infection by vaccines. Even frontline health workers in many countries suffered considerably due to short supply of PPE and other essentials. It is a wake-up call for all developing nations as well as emerging economies to proactively address their healthcare problems and be prepared by developing their own strategies and backup systems, as globalized healthcare delivery systems may not work as expected, especially during pandemics, when the demand overwhelms the supply chain, and the whole world faces the same problem.

https://blogs.bmj.com/bmj/2021/10/21/academic-medicine-and-publishing-from-developing-countries/

Academic Medicine and Publishing from Developing Countries

Richard Smith, M.D.
Editor of the British Medical Journal (until 2004)

October 21, 2021

Samiran Nundy, Atul Kakar, and Zulfi Bhutta have published a book titled *How to Practice Academic Medicine and Publish from Developing Countries? A Practical Guide*. It's a book that will be extremely useful to the growing number of academics working in low- and middle-income countries. The book is published by Springer and will from 30 October be available open access, meaning you can access it for free as often as you want. Hard copies will also be available for a fee. I felt privileged to be asked to write the foreword, and what follows is an edited version of my foreword.

When I became an assistant editor at *The BMJ* in 1979 and began to process scientific papers submitted to the journal it was extremely unusual to see, let alone publish, a paper from a low-income country. I remember being surprised by high quality papers coming from Bangladesh, a country that Henry Kissinger called a "basket case." Those papers came from the International Centre for Diarrhoeal Disease Research, Bangladesh, which I learnt later was a creature of the Cold War and might cruelly be called a "branch office of Johns Hopkins."

Years later, in 2013, I became the chair of the board of what was by then called icddr,b. The centre has now published major vaccine trials led by Bangladeshi scientists in the *New England Journal of Medicine*,[1] and I was privileged to be on the steering committee of a major trial, also published in the *New England Journal of Medicine*, of a system for managing hypertension in rural Pakistan, Bangladesh, and Sri Lanka led by scientists from those countries.[2] Last year I was delighted to see a major trial of the polypill for the prevention of cardiovascular disease led from India and also published in the *New England Journal of Medicine*.[3]

High quality research relevant to the needs of low- and middle-income countries is much commoner now than it was 40 years ago, and China has become a scientific leader. But, as the editors of this book describe in the introduction, there is still not nearly as much good research as there should be from the part of the world that carries most of the disease burden. Even worse, the medicine practised in some of these countries is disconnected from research and teaching, and driven more by profit than what is best for patients and the population. I have Indian friends who are terrified of seeing a cardiologist for fear that they will be given treatments they don't need.

I agree with the editors' diagnosis that "the main reasons for our having sunk into this deep morass is not because we are poor but because we have not intelligently examined, evaluated and investigated how we could use our own resources more effectively. We have tended to blindly follow what is being done in richer countries instead of trying to provide healthcare to our population which is accessible, affordable and, most importantly, appropriate even if this means deploying and working with informal healthcare providers."

Other people's research can be valuable, but it can never be as valuable as your own addressing the problems that matter to your people with relevant methods and the tools you have. And we know that the very act of researching brings improvement, and (as I know to my cost) you can never learn about research from reading about it: you need to do it.

I've never quite understood why people in low- and middle-income countries would want to replicate the health systems of high-income countries. Not only are those systems not relevant to the needs and circumstances of the low- and

middle-income countries, but the systems in high income countries are increasingly unaffordable and unsustainable and not meeting the needs of their own populations.

Health systems in high income countries were developed decades ago and were designed to respond to the infectious disease and trauma that were then the main causes of suffering and death. Those problems could be cured, but now non-communicable disease is the main cause of suffering and death. Such disease cannot be cured and needs a different approach.

Non-communicable disease is now also the main cause of suffering and death in low- and middle-income countries (apart from some sub-Saharan countries, but even there it will soon be the main cause). The epidemiological transition happened very fast in low- and middle-income countries: in Bangladesh non-communicable disease caused about 10% of deaths in 1986 but nearer 80% by 2006.[4] I spent years working with 11 centres in low- and middle-income countries that were doing research, building capacity, and advising on policy in relation to non-communicable disease. We envisioned what a better system in low- and middle-income countries might look like—with an emphasis on public health, the social determinants of health, prevention, primary care, patient empowerment, and widespread use of evidence-based guidelines.[5] (Such guidelines were developed by academics in South Africa as part of a package that allows good primary care where doctors are few or unavailable.[6])

We should have said more about the use of technology. Most people in low- and middle-income countries, even some of the poorest, now have mobile phones, which has meant that people can communicate without having to connect every house by wires, as happened with terrestrial phone systems in high income countries. Low- and middle-income countries can in this way "leapfrog" over a stage that was needed in high income countries, and the same can be done for health—not least by using mobile phones to provide access to care. Similarly, health systems in low- and middle-income countries might create health record systems where patients, not healthcare providers, own and control the records. Health systems in high income countries are just beginning to recognise the importance and inevitability of giving patients ownership and control of their records. (I have a conflict of interest here as I'm the chair of Patients Know Best, a company that gives patients in Britain and some other countries control of their records and data.)

Health systems in high income countries are actually sickness systems, and low- and middle-income countries would be wise to concentrate more on health. Only a small part of health comes from the health system, but politicians, citizens, and even many health professionals seem unaware of the fact. Consequently, health and healthcare are treated as if they are synonymous. Those countries that currently have poorly developed health systems have the opportunity to build systems that pay more attention to health than healthcare, as indeed was the case in many traditional and ancient health systems. Physicians to Chinese emperors were paid only if the emperor was well.

Such developments in health and health systems can be achieved only through research conducted in low- and middle-income countries by researchers from those countries. And, I suggest, we need a new way of doing science, and researchers in low- and middle-income countries should take the lead. I have recently been part of a discussion on the future of the UK Academy of Medical Sciences, and people are advocating a new way of doing science that will be much more transdisciplinary and global with more involvement of citizens. A broader range of methods will be needed, together with a greater willingness to bring together different kinds of studies and data to reach conclusions. Without curiosity-driven research being neglected, there might be more emphasis on research that brings social benefit. Implementation of research findings will become as important as discovery, and the hierarchy of science that ranks genetics above social science will disappear.

Secondary aspects of the new science might be universal data sharing, greater transparency throughout the research process, immediate open access to all research, and the final abandoning of publications and the place of publication as the main way to measure academic success. In addition, scientific integrity (and its dark twin, misconduct) will be taken much more seriously, as will the commitment to explaining science and how it works to the public.

As part of the debate over the future of the academy there has been discussion on priorities, and two of the priorities that are widely advocated—climate change and inequalities—are even more relevant to low- and middle-income countries than to high income ones. It's a huge global injustice that most of the greenhouse gases that are causing climate change have been produced in high income countries, but the resulting harm will be experienced mostly in low and middle income countries. A third of Bangladesh, already a densely crowded country, is set to disappear under water, and temperature increase and drought will reduce crop yields in many low- and middle-income countries, forcing people to migrate. Health academics must pay attention to climate change, which will mean forming new, unfamiliar research partnerships with climate, agricultural, social, and political scientists.

Academics must also recognise the huge role that inequalities in wealth, income, education, and opportunity play in health. The covid-19 pandemic has brutally illustrated the importance of inequality, in both high and low- and middle-income countries. Most low- and middle-income countries have even greater inequality within the countries than do high income countries. Health researchers in some high-income countries, including Britain, have done a good job of measuring and describing the harm to health from inequalities but have done less well in reducing the harm. Researchers in low- and middle-income countries have an opportunity to do better.

The world faces considerable problems, and what is clear is that research and teaching will be essential in tackling those problems. It's also clear that the research and teaching must be undertaken by researchers and teachers within countries, producing responses and using methods that are right for their countries. This book will be a great aid to researchers and teachers. The result should be better health and sustainable health systems. The opportunities are greater than the problems.

Richard Smith *was the editor of The BMJ until 2004.*

Conflict of Interest: RS is the unpaid chair of Patients Know Best, but he has equity in the company. He is the unpaid chair of the UK Health Alliance on Climate Change, but he has shares in the UnitedHealth Group. He was not paid for writing the foreword to the book and will not benefit from whatever sales there might be.

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Editorial Board Members

Editorial Interns

This is for Medical Students and Residents.
Recruitment Criteria is in Preparation.

Journal of the American Association of Physicians of Indian Origin (AAPI)

Vision and Mission: JAAPI is a peer-reviewed medical and healthcare journal published by the AAPI. In line with the vision and mission of AAPI, JAAPI is dedicated to facilitating physicians to excel in patient care, teaching and research, and thus pursue their aspirations in professional and community affairs. JAAPI is open to contributions from physicians and scientists of all backgrounds and from all over the world.

Scope of JAAPI: JAAPI publishes a variety of articles, such as original research articles, clinical studies, reviews, perspectives, commentaries, case studies etc., covering all aspects of medical sciences, clinical specialties, and healthcare, including epidemiology, and policy, regulatory and legislative issues. Articles submitted to the JAAPI must be original and should not have been published or under consideration for publication elsewhere, except in abstract form in proceedings of conferences or meetings. Based on type of the article, the length and specifications vary. Only manuscripts that meet professional and scientific standards will be accepted for publication. Review process is single fold blinded on the authors side. But after acceptance of papers, the names of the handling Editors and Reviewers will be published on the front page of the article. This new trend started by some European journals is gaining momentum as it gives due credit to the Editors and Reviewers and ensures fair review process.

Publication Model: JAAPI is published as completely Open Access in electronic form (PDF). These will be archived in AAPI website (https://www.aapiusa.org/jaapi/), and the link to URL for each issue will be emailed to AAPI Members. A few hard copies will be printed for promotional purposes and for displaying at AAPI Conventions and other professional meetings or for distributing to libraries or dignitaries. There will be no submission fee or publication charges to the authors. Although materials published are copyrighted by the AAPI, others can cite or reproduce figures, schemes and pictures published in JAAPI without paying fee, but by giving due credit to JAAPI. This does not apply for materials reproduced in JAAPI from other journals, which are copyrighted by the original publisher.

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Editorial Board: The Editorial Board of JAAPI consists of one Editor-in-Chief, several Deputy Editors and Guest Editors covering different areas of medicine and health care, Editorial Board Members and Editorial Interns. In addition, there are Editorial Advisors to oversee performance and stability of JAAPI and to help the Editorial Board Members in logistics, administrative and fiscal issues. The Deputy Editors and Guest Editors will handle review process of submitted papers helped by internal (Editorial Board Members) and external reviewers. Editorial Interns are medical students or residents who would like to obtain training in editing for journals. They will work with the Deputy Editors. AAPI membership is required for all Editorial Board Members, except Guest Editors, who are expected to promote the vision and mission of AAPI through JAAPI.

CME Credits for Peer-Review Process: After indexing by PubMed, working through AAPI, JAAPI will obtain CME Credit eligibility for its reviewers by the Accreditation Council for Continuing Medical Education of the American Medical Association.

Journal Periodicity: Initially, JAAPI will have three issues per year (Spring, Summer, and Winter). As the journal picks up momentum and article submissions increase, the periodicity will be quarterly.

Types of Articles JAAPI Accepts:

- **Original Research Articles:** These describe original scientific or clinical research conducted on in vitro or animal models or human subjects after obtaining approval by the concerned institutional animal care and use committees or human subjects research review boards. The research should comply with the guidelines and regulations of US Public Health Service. The original research articles can be up to 3,000 to 4,000 words in length, excluding title page, abstract, legends and references. Maximum 7 figures or tables are allowed. Additional figures or tables need to be justifiable for the article. Supplemental Information (SI) containing data and text, such as methods, are allowed for deposition.
- **Review Articles:** The review articles can address any contemporary issue in medical or clinical sciences, or healthcare, including epidemiology, and policy, regulatory and legislative issues. The reviews should provide in depth analysis of the topics but should not be just presenting catalog of information. The review articles should be balanced and should cite literature without bias. The review articles can be 3,000 to 5,000 words, excluding title page, abstract, references, and legends. Not over 5 figures and tables combined. There is no limit on the number of references, but they should be recent and relevant ones.
- **Clinical Studies:** Clinical studies can be observational or retrospective analysis of data or prospective randomized studies. All clinical studies should be conducted under the regulations and guidelines, documenting informed consent, protection of research subjects, inclusion of minorities etc., as per the guidelines of the US Public Health Service. Rigorous statistical analysis should be followed. Raw data should be provided for analysis if required. These articles can be up to 5,000 words, excluding title page, abstract, tables, legends, and references. Maximum number of figures or tables are 7 combined. Additional figures or tables should be justifiable for the study. Supplemental Information (SI) is allowed for deposition.
- **Brief Reports:** Brief reports of contemporary issues of high significance are accepted to disseminate information. These reports are up to 1,500 words in length, excluding title page, abstract, legends and references. About 4 tables or figures combined are permitted. Maximum 15 references are allowed.
- **Letters to the Editor:** Letters to the editors on topics of high importance or on the articles published in JAAPI are welcome. These should be focused and carry significant take home message, rather than a simple presentation of one's own perspective on the topic. These can be up to 600 words in length with 6 references, 2 small tables or figures maximum. The authorship should be limited to 2 or 3. No abstracts are allowed.
- **Articles on Diagnosis and Treatment Review:** Article describing latest methods, approaches and technologies in diagnosis and treatment can be up to 2,000 words, excluding title page, abstract, references, and legends. Figures and tables should be limited to five combined.
- **Case Studies or Clinical Challenges:** Case presentation with about 300 to 400 words, followed by discussion of 500-600 words, 1-2 small figures, and less than 10 references, are welcome. The authorship should be limited to 3 unless it involves trainees. Proof of patient consent should be provided.
- **Perspectives on Contemporary or Controversial Topics:** These should be thought-provoking with intuitive analysis rather than presentation of facts. Some speculation and hypothesis are permitted provided they are supported by rational analytical base. These articles can be up to 1,200 words, excluding title page, abstract, legends and references. Less than 3 tables or figures combined are allowed. References should be limited to the required ones.
- **Commentaries on Published Papers:** Commentaries on published papers are accepted if they provide a significant perspective or missed findings in the original publications. These can either positively or negatively

affect the original publication. But the emphasis is how the original publication can affect clinical practice or evidence-based medicine. These can be up to 1,200 words in length with one or two figures or tables, and limited references. No abstract is allowed. Authorship should be limited to one or two.

- **Bench-to-Bedside or Bedside-to-Bench:** Authors can take laboratory findings to clinical settings or bring clinical dilemmas to laboratory research. Special emphasis should be made on moving the subject from bench to bedside or vice versa. This type of articles can be up to 1,200 words in length, excluding title page, abstract, legends and references. Not over 3 tables or figures combined are allowed. References should be limited to the required ones.

References Style: JAAPI follows the same style as JAMA for presentation of references, which can be found in the following URL. https://www.bibguru.com/c/jama-citation-generator/

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Review Article

Clinical Usefulness of Left Atrial Strain Imaging by Echocardiography

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Abstract: Left atrial strain (LAS) is a measure of left atrial (LA) muscle deformation which is a functional component of the LA. LA has a unique phasic function and the phasic LAS is represented as LA reservoir strain, LA conduit strain and LA contractile (booster) strain measured during left ventricular systole, passive emptying of LA and LA contraction phases, respectively. LA senses pressure and volume changes from the left ventricle (LV) particularly early in the disease process where LV parameters are still normal. This invaluable information affects LAS which precedes LA volumetric parameters like LA volume and volume index and helps in early assessment of a variety of cardiac conditions like heart failure, atrial fibrillation, coronary artery disease, valvular heart diseases, hypertrophic cardiomyopathy, and early toxicity in cancer patients on potentially cardiotoxic medications and adds to the existing diagnostic criteria particularly in evaluating LV diastolic dysfunction. LAS, mostly measured as longitudinal strain, is also useful to predict outcomes like all-cause mortality, cardiac mortality, heart failure admissions, atrial fibrillation recurrence and stroke. Increase in LA fibrosis leads to an increase in LA stiffness and decreases LAS by impairing LA relaxation. LAS is measured by echocardiography and cardiac magnetic resonance, which also measures LA fibrosis. Of these two modalities, echocardiography techniques are feasible, easily available and cost effective. This includes two-dimensional echocardiography, tissue Doppler imaging, and velocity vector imaging. Two-dimensional echocardiography is the most used technique and with increasing evidence of clinical usefulness of LAS makes it a promising tool to use in mainstream echocardiography in the near future.

Key Words: Left atrial strain, Left atrial fibrosis, Two-dimensional speckle tracking echocardiography, Echocardiography

Introduction: Left atrial strain (LAS) is a measure of the change in the left atrial (LA) myocardial tissue size and deformation during the contraction and relaxation phases of the cardiac cycle. LAS is the functional component of LA which senses an increase in left ventricular (LV) pressure and consequently remodels LA by increasing left atrial volume (LAV) and LA pressures (1). Assessment of LV morphological and functional parameters are well studied and is of great importance in assessing cardiac function and outcomes as LV function directly translates into cardiac output. Along with the LV characteristics, assessment of LA characteristics is also important as it can provide more information in the earlier stages of cardiac conditions like left ventricular diastolic dysfunction (LVDD), heart failure (HF), and atrial fibrillation (AF) than LV parameters. LAV and left atrial volume indexed to body surface area (LAVI) are LA morphological parameters and better studied as they are easy to measure than the LAS.

During the last two decades, with advancements in the cardiac imaging, focus has now shifted more towards LAS. LAS has also emerged as an important prognostic indicator and in predicting outcomes better than LA volumetric parameters because it was shown in several studies that changes in LAS precede LAV and pressure changes and has an incremental value in the diagnosis of LVDD (2). Predicting and diagnosing these cardiac conditions early with LAS and strain rate (which is the rate of LA myocardial deformation) assessment helps in implementing necessary prevention and treatment strategies.

LAS is measured by different cardiac imaging modalities like echocardiography, cardiac computed tomography, and cardiac magnetic resonance (CMR).

Echocardiography is the most common, and cost-effective modality to measure the LAS. Echocardiography techniques include two-dimensional speckle tracking echocardiography (2D-STE), tissue Doppler imaging and velocity vector imaging. The latter two techniques are less commonly used as compared to 2D-STE for evaluating LA strain. 2D-STE is performed on routinely acquired apical 4 chamber or both apical 4 chamber and apical 2 camber views when performing an echocardiogram and is currently the most used technique to measure LAS (3). Tiny areas in the LA wall called speckles have a unique ultrasonic signature and hence can be individually tracked throughout the cardiac cycle by a computer program. The LA wall may also be divided into larger regions of interest or segments for assessing segmental strain (4-6). Generally, the LA wall is divided into 6 equal segments in each of the two apical views. LA wall is tracked starting from one side of the mitral annulus endocardial boarder to the other side of the mitral annulus without including the pulmonary vein orifices, LA appendage orifice and mitral orifice (5). LAS measurements have been shown to be vendor dependent and hence it is advisable to use the same equipment for follow up studies (7,8).

Types of Left Atrial Strain: LA longitudinal strain is the deformation of the LA myocardium along the long axis of the heart. LA radial strain is the deformation along the width and LA circumferential strain is the deformation around the perimeter of the myocardium (5). Of these, LA longitudinal strain is most commonly used in clinical practice (9).

Phasic Left Atrial Function: Left atrial strain (LAS) measures 3 distinct components of left atrial (LA) function: (i) LA filling during systole (LA reservoir strain), (ii) passive emptying of the LA during early diastole (LA conduit strain), and (iii) active emptying of the LA with atrial contraction (LA booster strain). (Figures 1 and 2) (10). Reservoir phase is the maximum LA relaxation during LV systole and the peak strain measured during reservoir phase is called peak atrial longitudinal strain (PALS). Conduit phase is the LA before LA contraction which correlates with the passive emptying of LA into LV and the LA strain measured during this phase is called LA conduit strain (LAS-cd). Booster phase is the contraction of LA and active emptying of blood into LV and the peak LA strain during booster phase is called LA contractile strain (PACS).

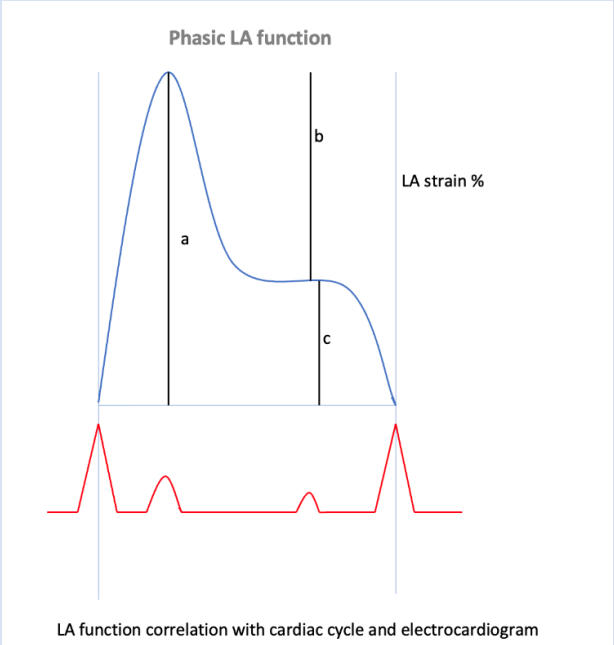

Figure 1: Schematic of phasic left atrial (LA) function. The X axis shows LA changes during phases of cardiac cycle and the Y axis LA strain percentage. a = LA reservoir strain, b= LA conduit strain, c= LA contractile (booster) strain.

In the literature, the terms left atrial reservoir strain (LAS-r) and PALS; left atrial contractile strain (LAS-ct) and PACS are used interchangeably. As per 2018 European Association of Cardiovascular Imaging recommendations (EACVI), LA reservoir strain gated with QRS complex (LV end diastole) is preferred over P wave (atrial contraction) as it is difficult to identify left atrial contraction with atrial arrythmias (5). Majority of studies used average LAS from both apical 2 chamber and 4 chamber views, EACVI recommended to use apical 4 chamber view to measure LAS to increase feasibility (5). Normal values are given in Table 1.

Table 1: Normal values of LA reservoir, conduit, and booster strain [9].

Phasic Left Atrial Strain	Mean with 95% CI
Left Atrial Reservoir Strain (PALS)	39.4% (38-40.8)
Left Atrial Conduit Strain (LAS-cd)	23% (20.7-25.2)
Left Atrial Booster or Contractile Strain (LAS-ct)	17.4% (16-19)

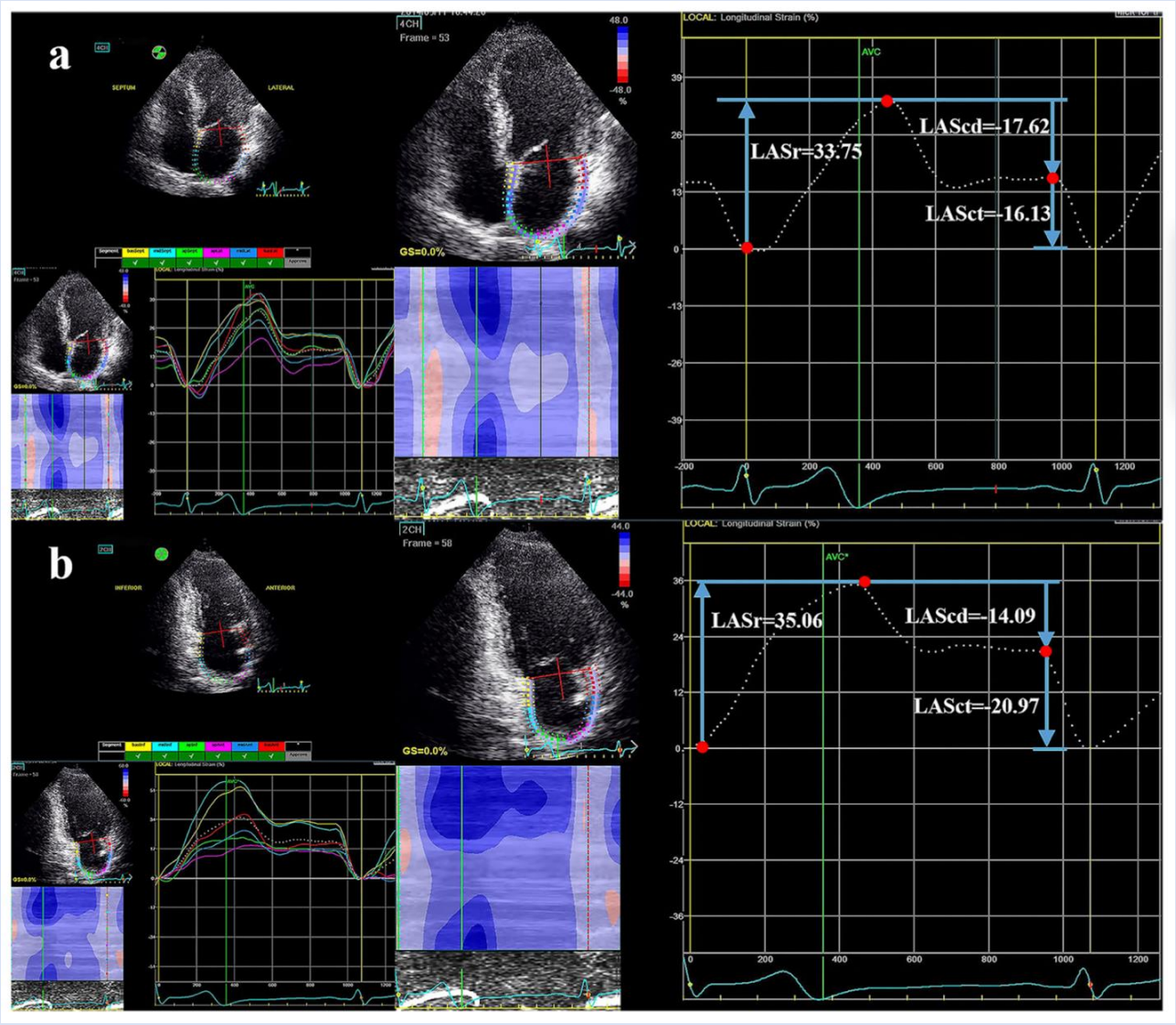

Figure 2: Two-dimensional transthoracic echocardiographic images showing measurement of phasic left atrial (LA) strain by speckle tracking echocardiography. a= Phasic LA strain measurement in apical 4 chamber view, b= Phasic LA strain measurement in apical 2 chamber view. LAS-r= LA reservoir strain, LAS-cd= LA conduit strain, LAS-ct= LA contractile strain. Reproduced from Lin et al, 2020 (reference # 10) with permission from Dr. Weichun Wu, corresponding author and no copyright permission required under CC BY 4 from publisher.

Table 2: Usefulness of Left Atrial Strain (LAS)

LAS is better than LV strain in diagnosis and/or predicting outcomes in	LAS adds benefit to LV strain, LAVI, E/E' in diagnosis and/or predicting outcomes in
LVDD and early stages of heart failure	Late stages of heart failure
AF, AF recurrence after catheter ablation	Cardiac resynchronization therapy-pacemaker
Post-operative AF, AF in HCM	Hypertrophic cardiomyopathy
Ischemic stroke, left atrial appendage thrombus	Mitral regurgitation
Cancer therapy-related cardiac dysfunction	Aortic stenosis

Heart Failure: PALS serves as a good predictor of left ventricular end diastolic pressure (LVEDP) and has a great diagnostic value for LVDD and HF with preserved ejection fraction (HFpEF). During the early stages of LVDD in HFpEF, increase in LV pressure conducts to LA and decreases LA relaxation and PALS. As the severity of LVDD increases, LAVI also increases which is used as one of the components to diagnose LVDD, along with other criteria-tricuspid regurgitation jet peak velocity, early transmitral Doppler velocity (E)/ tissue Doppler early diastolic mitral annular velocity (E') and septal or lateral wall velocities as per current ASE guidelines (11).

In 2018, Morris et al demonstrated that average PALS from both two chamber and four chamber views on 2D-STE was helpful in identifying patients at risk for LVDD before noticing changes in LA volumetric parameters (2). Studies by Morris et al and Singh et al showed that addition of PALS to LAVI and current ASE guidelines improves early diagnosis of LVDD and HFpEF as PALS declines steadily and earlier than other LVDD parameters (2, 12). PALS > 35% predicted normal diastolic function with greater than 90% sensitivity and specificity (12). This finding was further confirmed in a larger study by Fan et al in 2020 which showed PALS, LAS-cd, LAS-ct correlated with invasively measured LVEDP, and PALS was better than LAS-cd and LAS-ct (13). PALS is also useful in differentiating patients with LVDD and HFpEF. Another study by Morris et al showed PALS was worse in patients with HFpEF when compared to asymptomatic patients with LVDD (14).

PALS predicts New York Heart Association (NYHA) class, HF exacerbations, cardiovascular mortality, and sudden death. A subset from TOPCAT study evaluated the effect of PALS using 2D-STE in patients with HFpEF and showed PALS was useful in predicting HF admissions, cardiovascular (CV) mortality, and sudden death (15). LV strain and PALS were the only significant factors that predicted HF admissions and outcomes. This may be because PALS is independent of LV parameters earlier during LVDD or HF and as HF progresses, LV also remodels, and PALS is then no longer an independent predictor of outcomes.

PALS also predicts outcomes in heart failure with reduced ejection fraction (HFrEF). Decrease in LV ejection fraction in HFrEF causes an increase in LV pressure and volume, which decreases the PALS and increases PACS to be able to pump blood effectively to LV. Comparison of LA parameters in patients with HFrEF and HF with moderately reduced ejection fraction showed PALS and LA stiffness correlated with significant outcomes such as CV events, HF admissions, all-cause mortality, and cardiac death similar to earlier studies on HFrEF patients (16). LA stiffness which is the ratio of mitral E/E' and PALS correlated better with outcomes than PALS alone (16). PALS has a prognostic significance in both acute HF exacerbations and chronic HFrEF (17-18). PALS was also associated with worse NHYA class and an indicator of LV function, AF, mortality, and CV mortality in chronic HFrEF patients (18-19).

Cardiac resynchronization therapy pacemaker (CRT-P) improves LV systolic function and mortality in HFrEF. Improving LV systolic function with CRT-P, also improves LV global longitudinal strain (GLS), LAV, PALS and LA strain rate. In a retrospective study of 68 HFrEF patients who were CRT-P responders, less improvement in global PALS was correlated with CV outcomes and improvement in the coordinated LV function leads to low LV pressures and LA pressures and improves PALS (20). In a prospective study in 2021 by Galli et al, the effects of CRT-P in HFrEF on LV GLS and PALS showed PALS pre-CRT-P was correlated with LV GLS and is a good predictor of LV reverse remodeling post CRT-P in responders and this was further validated by a meta-analysis which showed that a significant improve-ment in PALS was correlated independently in responders compared to non-responders (21-22). PALS and peak LAS-ct were also useful in predicting new AF in patients who had received CRT-P and was better than other LA and LV reverse remodeling factors like LAVI, mitral annular velocity, LA ejection fraction, and LV volume (23).

Atrial Fibrillation: PALS predicts new onset AF and recurrence of AF after catheter ablation. Park et al also showed lower PALS in patients with AF compared to no AF group and in patients with AF and HTN (17 24). A large retrospective study in 2020 confirmed that PALS can predict new onset AF in patients with HF (25). In a retrospective study with patients without AF and with normal LA volumes, LAS-ct was better at predicting AF (26). This signifies the importance of earlier changes in the average PALS than LAV in AF and leads to better predicting and preventing complications of AF. PALS was higher in paroxysmal AF compared to persistent AF, this is probably due to more inflammation and fibrosis in LA as the disease progresses (27).

PALS pre ablation predicts recurrence of AF post ablation. Low PALS by 2D-STE predicts persistent AF

recurrence after catheter ablation and PALS also remained a significant predictor of persistent AF even after second catheter ablation (28). These findings were confirmed by two meta-analyses in 2016 and 2020 which showed that lower PALS before catheter ablation was significantly correlated with AF recurrence post ablation (29-30).

There is a complex interplay between LAV, PALS and LA fibrosis in patients with AF. While increased LAV acts as a substrate for initiation and propagation of AF, AF itself leads to inflammation, further LA remodeling and causes LA fibrosis. The amount of LA remodeling and fibrosis depends on the duration of AF and it increases from paroxysmal AF to permanent AF. Increase in LA fibrosis causes less deformation of LA and decrease in PALS. Paszkiet et al showed that noninvasive assessment of LA fibrosis through electro anatomical mapping correlated with PALS on 2D-STE (31). Low PALS also correlates with AF incidence and with the size of myocardial infarction on CMR which in turn increases scar tissue, and fibrosis (32).

Postoperative AF is common after cardiac surgery. In patients who had coronary artery bypass graft (CABG), preoperative LAV, and PALS on 2D-STE were shown as a predictor of postoperative AF. Along with other LA parameters, PALS can be used to predict AF postoperatively in CABG patients. The study also showed the relationship of LA fibrosis and AF and presence of greater amount of fibrosis prior to surgery was a risk factor for AF postoperatively in CABG (33).

It is well known that LAV has a great diagnostic value in AF patients. Often, LA volumes are evaluated in patients with AF to estimate the success of cardioversion and to diagnose the cause of stroke. A small prospective study by Kurzawski et al in 2020 showed that PALS independently predicts left atrial appendage thrombus (LAAT) and PACS was lower in LAAT group (34). Effect of LA appendage occlusion on LAS was studied by Ijun et al in their retrospective study which showed improvement in PALS and PACS in both AF and sinus rhythm groups after LA appendage occlusion with more improvement in sinus rhythm group during follow ups (35).

Atrial Fibrillation and Stroke: AF causes LA and LAA thrombus and is the most common cause of embolic stroke. The risk of embolic stroke in patients with AF can be modified by risk stratification with the traditional CHADS2VASc score and anticoagulation. In addition to risk stratification, PALS also predicts stroke in patients with AF. A retrospective study by Liao et al showed that AF patients with stroke had worse PALS measured while the patients were in AF (36). This study also showed improvement in the risk stratification of stroke with the addition of PALS to CHADS2VASC score (36). Other tools like CHARGE-AF, uses demographic and cardiac comorbidities without LAS to predict 5-year risk of AF (37). In contrast to the known findings, a small study showed no independent significance between PALS or LAAT with ischemic stroke (34).

Coronary Artery Disease (CAD): CAD causes LVDD and leads to HF. The severity and progression of LVDD, and changes in PALS in CAD depends on the coronary vessel, part of the vessel involved, and intervention received. Decrease in the global PALS correlates with the degree of LVDD in CAD, and PALS correlates more than LAS-ct (38). In addition to significant CAD, in patients with HFpEF and coronary microvascular dysfunction PALS correlates with coronary flow reserve measured by color Doppler (39). A study comparing PALS and LA strain rate in patients with CAD with stenosis ≥50% and normal subjects showed PALS was lower in patients with CAD and enlarged LAV than patients with CAD and normal LA volumes and comparison of CAD patients with normal LAV and normal subjects revealed that PALS was lower in patients with CAD (40). In 2017 Hosseinsabat et al studied LA phasic strain in patients with CAD with and without diabetes mellitus (DM). Interestingly, it showed significant correlation of LAS-cd and LAS-ct with the CAD rather than PALS which were shown in earlier studies (41). LA phasic strain also depends on the vessels involved in CAD as left anterior descending artery lesions cause greater LV systolic dysfunction and with more volume overload, LAS-ct increases whereas more proximal left circumflex lesions affect LA and decreases LAS-ct. A recent study showed that PALS correlated with LVEDP in patients with CAD >50% (10). This study also showed better correlation between PALS/E/E' to LVEDP than PALS alone. PALS or PALS /E' can be used in the future to diagnose LVDD early in CAD before LA enlargement occurs and necessary precautions can prolong the duration of disease-free state (10). PALS also correlates well with CAD in patients with myocardial infarction (MI). Both MI and DM are known to affect LAS and a study comparing PALS in patients with and without DM and MI showed PALS was low in patients with MI

independent of DM as the acute effect of MI is greater on PALS than DM [42].

Chronic Kidney Disease: Chronic kidney disease (CKD) causes volume overload, chronic inflammation and fibrosis and leads to an increase in LAV and decrease in LAS. PALS is independent of LAV in CKD but if patients have other comorbidities that increase LAV, then PALS is more dependent on LAV. Hassanin et al in a prospective study in 2016 demonstrated that PALS was decreased in patients with end stage renal disease on hemodialysis (43). In 2018 a study by Li et al with 59 CKD patients showed PALS correlated with low estimated glomerular filtration rate and hence predicted CKD. In contrast to previous studies, PALS combined with LAVI predicted LVDD than PALS alone in patients with CKD (44). Aksu et al in their small prospective study of 44 patients with CKD showed that higher PALS and LAS-ct were noted in the peritoneal dialysis group because peritoneal dialysis is a more physiological process and lower PALS was found in hemodialysis patients due to fluid shifts over a short period (45).

Valvular Heart Disease: LAS is useful in predicting the progression of valvular diseases, the success of interventional procedures, and post procedural complications. In patients with aortic stenosis (AS), impaired relaxation of LA from increased LV and LA pressures leads to lower PALS. Comparison of PALS pre and post transcatheter aortic valve replacement (TAVR) predicted HF and mortality after TAVR, and PALS can be used to risk stratify patients before TAVR with more aggressive medical management and follow ups to prevent complications (46). Meimoun et al showed that PALS in moderate to severe AS reduced and it predicted acute HF and mortality independent of other morphological and functional parameters (47). PALS was low in patients with severe AS with HFpEF and correlated with worse NYHA class and symptoms. PALS can be used along with the existing prognostic factors in severe AS and in predicting symptoms (48).

LAS is also useful in mitral regurgitation (MR) to predict outcomes. Garsee et al compared PALS and PALS strain rate in patients with undersized mitral ring annuloplasty and MR recurrence (49). Improved PALS was shown in patients without MR recurrence and PALS was unchanged in patients with MR recurrence. This signifies that in patients with recurrence in MR had more preload whereas patients without MR recurrence had less preload and more improvement in the PALS. PALS can be a parameter to identify patients who can benefit the most from mitral annuloplasty and patients at risk for recurrence (49). Studies comparing PALS in patients with severe MR and candidates for surgery showed significant correlation between PALS and LA fibrosis from biopsy, post MR surgery mortality and heart failure (50). Addition of PALS to current risk stratification methods helps to better triage patients for mitral valve procedures.

Hypertrophic Cardiomyopathy (HCM): HCM causes myocardial fiber disarray leading to abnormal LV contraction and an increase in LV and LA filling pressures. Increase in LA filling pressures leads to decrease in LA relaxation and PALS. Koayashi et al showed PALS, LAS-cd and LAS-ct were low in patients with HCM compared to healthy individuals (51). PALS and LAS-cd were found to be low in patients with obstructive HCM and who received septal ablation treatment compared to nonobstructive HCM, no difference in LAS-ct was found (51). This could be due to an increase in LAS-ct function and strain in all HCM patients despite HCM phenotype. This signifies the importance of PALS and LAS-cd in HCM, and these can predict obstructive HCM.

AF in HCM patients indicates for anticoagulation. HCM also causes LVDD and sudden cardiac death. AF in HCM causes changes in LAV and LA function. Vasquez et al showed the association of low PALS in patients with HCM and AF, PALS predicted outcomes like HF, stroke, and death in patients with HCM (52).

Effect of Chemotherapy on LAS: Anthracyclines like Doxorubicin, Daunorubicin and monoclonal antibody against human epidermal growth factor 2 receptor (HER-2) like Trastuzumab are cardiotoxic medications. These medications cause cancer therapy-related cardiac dysfunction (CTRCD) and are commonly used in HER-2 positive breast cancer. Because of increased cardiotoxicity these drugs are prescribed sequentially rather than simultaneously. LV ejection fraction is used to diagnose LV systolic dysfunction and as a prognostic indicator but there is increasing evidence of using LV GLS and PALS in predicting outcomes in CTRCD. A small retrospective analysis by Park et al in 2020 showed the significance of PALS in predicting cardiotoxicity in patients who received anthracycline chemotherapy and compared the change in the PALS after patients received Trastuzumab (53). PALS decreased in patients who received anthracycline chemotherapy and the decrease was more in patients who

were later diagnosed with CTRCD. PALS before Trastuzumab therapy also correlated with CTRCD after Trastuzumab, better than LV GLS. PALS can be used in predicting CTRCD and appears superior to LV GLS [53]. This finding was later confirmed by a prospective study which showed decrease in both PALS and LAS-cd in patients who received anthracyclines without Trastuzumab (54). In contrast to the earlier studies, a retrospective study in 2019 with 100 patients with chemotherapy with anthracyclines followed by Trastuzumab showed no difference in PALS after the treatment and age was the only significant factor that predicted LVDD (55).

Limitations: Even though LAS is easy to measure by echocardiography, it has its own limitations which include poor echocardiographic windows with difficulty in delineating fully the LA endocardial border when using 2D-STE. Age and gender differences in LAS parameters have not been fully delineated. Any 2D echocardiographic image represents a very thin slice through the heart and it is easy for a group of speckles to move outside the imaging plane during the cardiac cycle because of heart motion. Thus, the computer may not be tracking the same speckles during the cardiac cycle leading to potential inaccuracies in the assessment of LAS by 2D-STE. Many of the studies to date are small, retrospective and/or observational, and there is also intervendor variability which means the technique of LAS assessment by 2DSTE has not been standardized so far.

Future Direction: Compared to 2D echocardiography, 3D echocardiography acquires a large pyramidal section of the heart rather than a thin slice making it more difficult for speckles to move out of the 3D section thereby increasing accuracy. Also, 3D echocardiography tends to prevent foreshortening of LA which may lead to more accurate assessment of LAS parameters (56). Thus, in the future 3D-STE may replace or supplement 2D-STE in the assessment of LA strain parameters.

Conclusions: LAS is a functional component of the LA and many studies have shown its usefulness as an independent parameter or adjunct to other echocardiographic criteria in the assessment of many cardiac conditions. Assessment of LAS and LA strain rate is not yet mainstream echocardiography, but current evidence suggests it might soon become an essential part of clinical echocardiography.

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Case Report

Lactation Ketoacidosis:
A Rare Case of Metabolic Acidosis in Non-diabetic Female

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Abstract: Lactation Ketoacidosis is a rare, documented cause of high anion gap metabolic acidosis. Lactating women spend high energy and if they do not keep up with the calories spent, biochemical pathways initiate ketogenesis and eventual ketoacidosis. Prompt recognition by eliminating other etiologies is pivotal in treating lactation ketoacidosis. Treatment includes fluid resuscitation with dextrose, repletion of electrolytes as needed and resumption of well-balanced diet. We present a case of non-diabetic ketoacidosis in a breast-feeding mother, who has been on a low carbohydrate diet (GAPS Diet).

Key Words: ketoacidosis; lactation; low-carbohydrate/ketogenic diet; bovine ketoacidosis; anion-gap metabolic acidosis

Introduction: Metabolic acidosis is a severe and potentially life-threatening condition that requires timely identification and proper treatment. The most common causes include ketoacidosis, lactic acidosis, renal failure, and ingestion of substances such as salicylates, methanol, and ethylene glycol (1). Uncontrolled type-1 diabetes mellitus is, by far, the most common cause of ketoacidosis. Other common causes include alcoholism, starvation, and ketoacidosis can also occur during periods of extreme stress. We present a non-diabetic patient with ketoacidosis resulting from breastfeeding while on a low-carbohydrate diet. Lactation or 'Bovine' ketoacidosis is a well-documented condition that occurs in lactating cattle, but it is a condition rarely found in humans with only a few cases reported in literature (2).

Case Presentation: Written informed consent was obtained from the patients for this study. And no personally identifiable information is presented here. A 33-year-old Caucasian female presented to the Emergency Department with a one-day history of nausea, vomiting, abdominal pain and headache. Her abdominal pain is described as a constant and dull ache across her lower abdomen. She describes the headache as a migraine consisting of constant and throbbing pain in the frontal region bilaterally of moderate to severe intensity. She denied any dysuria, fever, urinary urgency or frequency, chest pain, and shortness of breath.

She is eight months post-partum from a complicated delivery consisting of a total placenta previa and uterine rupture, which required a Cesarean section. She has past medical history of ADHD (attention deficit hyperactivity disorder), non-medicated, and is currently breastfeeding. She was a social drinker but denied alcohol intake since she started breastfeeding. She notes that about one month ago she started a new diet called 'GAPS Diet' (Gut and Psychology Syndrome Diet), which consists mainly of protein and vegetables with no added sugar and no carbohydrates. She has been considerably constipated for the past 3 weeks, requiring daily enemas.

Upon initial assessment, the patient was slightly tachycardic and tachypneic with a heart rate of 103 and respiratory rate of 22 per min. She was mildly febrile with a temperature of 100.3 °F and her exam was relatively benign, except signs of dehydration. Initial investigative labs are notable for a venous blood gas with a pH of 7.180, pCO2 15.4 mmHg, pO2 61 mmHg, HCO3 5.7 mmol/L, and base excess of negative 23 mmol/L. Metabolic panel indicated mild hypernatremia with sodium of 147 mmol/L, low bicarbonate of 7 mmol/L, glucose of 83 mg/dL, and an elevated anion gap of 37 mEq/L. Analysis of the urine was positive for ketones and elevated hyaline casts. A CT scan of the abdomen and pelvis with intravenous contrast was obtained with incidental findings, but nothing to explain the patient's illness.

The cause of the high anion gap metabolic acidosis was thoroughly explored. Ethyl alcohol level, salicylate level, and serum lactate were all normal. The osmolar gap was calculated to rule out ingestion of other alcohols and was found to be normal. A urine drug screening was also normal. The serum beta-hydroxybutyrate level was 5.06 mmol/L (normal 0.02-0.27 mmol/L) and the diagnosis of ketoacidosis was made. Further evaluation exploring possible underlying issues that may predispose this patient to develop ketoacidosis, including thyroid, hepatic, renal function tests, lipase, creatine phosphokinase, C-reactive protein, erythrocyte sedimentation rate, vitamin B12 and hemoglobin A1c were all normal.

Final assessment was that the high anion gap metabolic acidosis was due to elevated ketones secondary to the low-carbohydrate diet possibly superimposed with dehydration and gastroenteritis as well as the increased energy demand of breastfeeding. The patient was initially given normal saline and then started on lactated ringers' solution with dextrose. Since the cause of low-grade temperature was initially unknown, the patient was started on broad spectrum antibiotics to cover any underlying infectious etiology which were discontinued once cultures were negative. This patient was admitted to the ICU for close monitoring of blood gases and electrolytes. Her condition quickly improved with intravenous fluids with dextrose. The anion gap and electrolytes normalized, and the patient was tolerating a normal diet without complications and was subsequently discharged after two days in the ICU with instructions to discontinue the GAPS diet while breastfeeding.

Discussion: The formation of ketone bodies is a physiologic mechanism by which many bodily tissues, particularly the brain, are supplied with an alternate metabolic fuel during times of low glucose availability (1, 3). The three main ketone bodies produced in humans are acetoacetate, β-hydroxybutyrate, and acetone. The process of fatty-acid metabolism and ketogenesis is regulated by a complex relationship of hormones and metabolic substrate availability. In a non-pathologic state, insulin levels increase with glucose availability, and inhibit the breakdown of fat stores. Conversely, counter-insulin and stress hormones including glucagon, catecholamines, cortisol, and growth hormone increase during low glucose availability and at times of stress. These all activate the biochemical pathways involved in increasing metabolic fuel when carbohydrate intake is inadequate. Initially glycolysis and gluconeogenesis keep glucose levels stable, but as the need for alternative energy sources persist, peripheral fat stores are then utilized. Triglycerides are broken down into glycerol and fatty acids and sent to the liver where they are metabolized through of β-oxidation, producing acetyl coenzyme A (acetyl-CoA). The oxidative capacity of the hepatocytes is eventually overwhelmed, and the accumulation of acetyl-CoA then goes into the ketogenic pathway for the formation of ketone bodies (1). The excessive formation of ketones leads to a high hydrogen load and consequently, a high anion-gap metabolic acidosis (4).

Ketosis, the formation of ketone bodies without significant acidemia, is a normal physiologic response. Fasting ketosis develops within a few hours and happens in most people while they sleep (3). Starvation ketosis occurs when caloric intake is limited for a prolonged period (days to weeks). In this situation, glycogen stores will become depleted within a few days and the body will become more dependent on ketone bodies for energy. The production of ketones will be maximal at about three days, but the serum levels of ketones will continue to increase for three or four weeks. However, starvation ketosis is usually benign unless superimposed with some other stressor such as infection, pregnancy, or alcohol

consumption, at which time could trigger a life-threatening ketoacidosis (1).

The physiology of ketosis is the basic principle underlying low carbohydrate diets (LCDs), which have increased in popularity over recent years due to their ability to induce weight loss, and as more recent studies may suggest, potentially offer therapeutic benefits in several other diseases as well (5). The efficacy of LCDs with weight loss has been well documented in literature (5-12). As Paoji, et al (5) discuss, ketogenic diets have established benefit in conditions such as cardiovascular disease, type 2 diabetes, and epilepsy besides weight loss. Their work also highlights emerging evidence of a therapeutic role of ketogenic diets with acne, cancer treatment, polycystic ovarian syndrome, and multiple neurological disorders aside from epilepsy, including headache, neurotrauma, Alzheimer's and Parkinson's disease, sleep disorders, autism, and multiple sclerosis (5). The acid-base safety of ketogenic diets has been explored in multiple studies, and while the conclusions can be debated, there are a few reported cases in the literature where LCDs induced a non-diabetic ketoacidosis (6, 9, 12). This paper does not intend to evaluate the efficacy or safety of low carbohydrate / ketogenic diets, so focused and in-depth literature reviews were not performed on these topics. It is rather our intent of this section to illustrate the popularity of these diets and to show that a state of 'dietary ketosis could potentiate the development of ketoacidosis in the presence of additional stressors.

Bovine or lactation ketosis occurs in lactating cattle that has been well described in veterinarian medical literature (13). This condition is characterized by ketonemia, ketonuria, and low levels of hepatic glycogen stores in postpartum lactating cattle (1). Bovine ketosis, as the name implies, is a ketosis that occurs because a negative energy balance exists, where the energy requirements of milk production and secretion exceed the amount of ingested carbohydrates and glycogen stores and the animal must rely almost exclusively on hepatic gluconeogenesis as a source of glucose for milk production (14, 15). Lactation ketoacidosis in humans is rare because the increased energy demand of galactopoiesis is normally met through diet, fat stores, and metabolic adaptions. Breastfeeding is a high-energy expenditure, requiring 500 kcal/day from birth to 6 months and 400 kcal/day through one year (15). Women with restricted dietary intake, such as with a LCD, have decreased glycogen stores that can be depleted rather quickly with such an increased energy demand. If intake and gluconeogenesis can't maintain the energy demand, fat metabolism and the formation of ketone bodies will be amplified and can lead to a life-threatening ketoacidosis.

A through literature review was performed, and we found less than 20 reported cases of non-diabetic, lactation induced ketoacidosis in the literature. All cases nearly presented similar to this patient with non-specific symptoms including headache, vomiting, abdominal pain, and malaise in a breastfeeding female. A high anion gap metabolic acidosis was found in all reported cases, with varying degrees of acidemia. Precipitating factors prompting the development of ketoacidosis were present usually with the most common being fasting and low-carbohydrate diets. The main stay of treatment includes providing dextrose and repletion of electrolytes as needed.

Conclusion: Lactation ketoacidosis is a rare but potentially serious condition that occurs when the diet of a lactating female doesn't provide the nutrients required for the increased energy demand. While more common causes of elevated anion gap metabolic acidosis need to be ruled out first, clinicians need to keep this diagnosis in mind when presented with a breastfeeding female with a high anion gap metabolic acidosis and non-specific symptoms (i.e., vomiting, abdominal pain / cramping, headaches, malaise). Non-diabetic ketoacidosis during lactation will be a condition that's diagnosed with increasing frequency as more emphasis is placed on post-partum weight loss in women of childbearing age with aid of ketogenic/low carbohydrate diet. It is a relatively benign condition, with full and quick recovery, if quickly identified and properly treated. The treatment of choice, as evident throughout the literature, is intravenous fluid and dextrose infusion. With a balanced diet, education, and treatment of any underlying conditions, there is little risk of reoccurrence and no reports of mortality to date (4).

Disclosure: Authors declare no competing interests.

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In Depth Review Article

Mechanism of Cytokine Storm in COVID-19:
How can Probiotics Combat It?

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Abstract: The COVID-19 pandemic caused by SARS-CoV-2 virus has infected about 250 million people causing about 5 million deaths worldwide as of October 2021. Despite introducing promising COVID-19 vaccines about a year ago, the pandemic is not yet contained. It is still spreading with viral mutations, making the prevention or treatment rather hard to achieve. The vaccine technology is aimed at producing antibodies to the viral spike proteins. Due to large single strand positive sense RNA, the SARS-CoV-2 virus has been mutating frequently. Recently several cases of infection have been reported in the vaccinated individuals, indicating that the antibodies generated due to vaccines have either short life or not able to neutralize fully and effectively the newly mutated viral particles. These issues dictate the need to understand in depth the cellular and molecular mechanisms of infection by SARS-CoV-2 virus, and the cytokine storm induced by it. Such understanding will aid in developing both preventive and therapeutic strategies to conquer the COVID-19 pandemic. Thus, the main emphasis of this article is to present a comprehensive picture of the genesis and pathophysiology of lethal cytokine storm during COVID-19 infection, causing deaths due to ARDS (acute respiratory distress syndrome). Second, this article will elucidate the molecular basis of preventive or therapeutic mechanisms exerted by probiotics along with their growth end products (immunomodulins) to overcome COVID-19 pandemic

Key Words: Multiple Mixed Strain Probiotics, Immunomodulins, Immune Checkpoints, CTLA-4, PD-1/PD-L1, Checkpoint Inhibitors, SARS-CoV-2, Acute Respiratory Distress Syndrome

Introduction: Death due to SARS-CoV-2 infection is predominantly attributed to coronavirus induced cytokine storm, causing acute respiratory distress syndrome (ARDS) (1, 2, 3). Cytokine storm is a systemic response to infections and drugs leading to excessive activation of immune cells and the generation of proinflammatory cytokines (4, 5). According to Cron and Behrens definition, cytokine storm is an activation cascade of auto-amplifying cytokine production due to unregulated host immune response to different triggers, such as infections, malignancy, and rheumatoid disorders etc. (6).

Before we proceed with the pathophysiology of COVID-19 induced cytokine storm, it is beneficial to refresh the reader with the basics of immune cells involved in the human immune system, to eliminate confusion regarding their identification with English and Greek alphabets, numerals etc. As we know the immune system has two main divisions, one is innate immunity, more like local police, and second one is adaptive immunity, comparable to specialized national guard (7, 8). They both protect the human body from un-invited invaders, such as pathogenic bacteria, viruses such as pandemic creating SARS-CoV-2, pathogenic molds causing mucormycosis (during or after COVID-19 infection), and pathogenic yeasts such as *Candida albicans* etc. Evolutionarily, the innate immune system is primitive one, being present in invertebrate animals also. The innate immune system is represented by these immune cells, which perform non-specific functions of inhibiting or destroying the foreign invaders, and communicating and activating the adaptive immune system: Macrophages, Neutrophils, Natural killer cells (NK

cells), Basophiles, and Eosinophiles etc. The adaptive immune system present in vertebrate animals only performs specialized immune functions, and its immune cells are T-cells and B-cells, which predominantly control both cell-mediated (to destroy the intracellular pathogens including viruses, without involving antibodies), and humoral immunity (involving antibody production by B-cells to inactivate or destroy pathogens). The T-cells are further divided into CD4+ T-helper (Th) cells with several subsets represented as Th-1, Th-2, Th-3, Th-17 etc. CD stands for Cluster Differentiation, a nomenclature to identify and classify leucocytes based on the cell surface antigens. The other set of T-cells are CD8+ T-cells (also known as T-killer cells), which are mainly involved in cytotoxic effect by directly killing the virus infected cell. The regulatory T-cells (T-regs) are a specialized subpopulation of T-cells that act to suppress the immune response, maintaining homeostasis and self-tolerance. T-regs can inhibit T-cell proliferation and cytokine production and play a critical role in preventing cytokine storms. The CD8+ effector T-cells display predominantly perforin-dependent cytolysis of the virus infected cells or tissue. For more details refer to the review by Marshall et al (9).

What are Cytokines? The term cytokine is Greek word - "cyto" means cell and "kinos" meaning moving. The cytokines are cell signaling molecules which aid in cell-to-cell communications in immune response. They stimulate and control the movement of immune cells towards the site of inflammation. The cytokines are generated in the initial phase of the infection by the innate immune cells, upon stimulation by invading pathogens (10, 11). They in turn induce the activation and proliferation of adaptive immune cells to perform a joint immune function, through production of more cytokines including various interleukins and interferons (12, 13). Structurally the cytokines exist as proteins, peptides, and glycoproteins. They are produced by endothelial cells, epithelial cells, and macrophages, T-cells, and to put in simpler terms, any cell in the human body with intact nucleus capable of producing IL-1, IL-6, and TNFα (tumor necrosis factor-alpha). The cytokines under normal circumstances function even at minute picomolar concentration. However, during acute infection or severe inflammation their concentrations can increase by thousand-fold (nanomolar) or even more (micromolar or millimolar) causing severe cytokine storm (14, 15). The cytokines include interleukins (IL), interferons (IFN), and chemokines, which regulate the immune systems in response to infection and inflammation. For more details about cytokines, refer to the review article by Lin and Leonard (16).

What is the Difference between Interferons and Interleukins? The term interleukin denotes "inter" between and "leukins" leukocytes or white blood cells. They serve as messenger molecules between immune cells, and they are denoted by IL-number such as IL-6, IL-10 etc. Whereas interferons (IFN) are mainly involved in inhibiting viruses by making cells non-permeable to viral infections. They are produced by the virus infected cells to signal the neighboring cells regarding viral infection threat. The examples for interferons are IFN-α and IFN-γ etc. They also activate macrophages and promote T-helper cell responses. Tumor Necrosis Factors (TNF), for example TNF-α, which is involved in maturation of macrophages also categorized as cytokine.

Chemokines also come under cytokines. The term "chemo" means chemical and "kinos" means moving. The difference between chemokines and cytokines is that cytokines are the general category of messenger molecules, whereas chemokines are special cytokines that direct specifically the migration of white blood cells to infected or damaged tissue. The chemokines are produced in thymus and lymphoid tissue. The inflammatory chemokines include: CCL2, CCL3, CCL5, CXCL1, CXCL2, CXCL8, and CXCL10 (17, 18).

The Cytokines are Divided into Three Types: Proinflammatory Cytokines, Anti-inflammatory Cytokines, and Regulatory Cytokines.

- ***Pro-inflammatory Cytokines*** are produced mainly by T-helper cells (Th) and macrophages. They include interleukin-1 (IL-1), IL-6, IL-8, IL-12, IL-18, TNF-α (Tumor Necrosis Factor-alpha), Interferon Gamma (IFN-γ), and Granulocyte-macrophage Colony Stimulating Factor (GM-CSF). They are involved in the upregulation of inflammatory responses. The IL-6 plays a major role as pro-inflammatory cytokine.
- ***Anti-inflammatory Cytokines*** include: IL-4, IL-10, IL-11, IL-13, IFN-α, and TGF-β (Transforming Growth Factor-beta) etc. The IL-10 predominantly plays a major role as anti-inflammatory cytokine.
- ***Regulatory Cytokines*** include: IL-35, IL-27 and diverse functional TGF-β and IL-10. They orchestrate the optimum modulation of the immune system.

Although the above explanations sound like basics of immunology, it is required as a refresher to understand multiple immunological game play in the COVID-19 infection, with the least confusion.

How does the SARS-CoV-2 Virus Induce Cytokine Storm? If we analyze the genesis of the cytokine storm, it starts with innate immune cells attempting to kill the virus through phagocytosis and then producing cytokines to activate the adaptive immune cells. The adaptive CD4+ T-cells upon recognizing the SARS-CoV-2 peptides attached to MHC-2 (a major histocompatibility complex) on the antigen presenting cells, activate Th-17 cells, which recruit and send excess numbers of neutrophils and macrophages to the infection site, aided by interleukins and chemokines. CD4+ T-cells also produce more T-killer cells to attack the SARS-CoV-2 virus in the lung tissue leading to further damage to the lung. In addition, the CD8+ T-cells, after recognizing the SARS-CoV-2 viral peptides in the infected cell (in cooperation with MHC-1), release excess cytotoxic molecules that kill the infected cell to stop SARS-CoV-2 virus from spreading. SARS-CoV-2 virus also selectively induces macrophages to produce IL-6, which will cause the lymphocyte necrosis. In addition, IL-6 significantly upregulates PD-1 (Programmed Cell Death Protein-1), and CTLA-4 immune checkpoint receptors on T-cells, which may cause more chances to make the T-cells ineffective, due to interaction with their ligands produced on the virus infected cells. COVID-19 infection causes a decrease in lymphocyte count and increase the C-reactive protein (CRP), which is an indication of severe systemic inflammation due to the cytokine storm. Although the normal lymphocyte count in the adult human should be around 3000 or over, per microliter, during severe COVID-19 infection it may drop down to below 1000 per microliter causing lymphocytopenia. The major sets of T-lymphocytes CD3+ CD4+, CD3+ CD8+ T-cells, are reduced during the COVID-19 infection, resulting in lympho-cytopenia. The COVID-19 infection finally leads to cytotoxic lymphocyte exhaustion due to cytokine storm. For more detailed description of cytokine storm, refer to Fajgenbaum and June (19).

The severely ill patients due to COVID-19 have higher pro-inflammatory cytokines, especially IL-6, and chemokines such as CXCL-10 and CCL-2 (20-23). Postmortem examination of lungs from patients died of COVID-19 revealed the existence of ARDS (acute respiratory distress syndrome) and evidence of pathological T-cell overactivation (24). This phenomenon is due to an increased activity of Th-17 cells and the abnormally high toxicity exerted by CD8+ T-cells. The innate and adaptive immune responses activated by SARS-CoV-2 viral infection lead to uncontrolled inflammatory responses and cause the cytokine storm. This cytokine storm ultimately leads to apoptosis (programmed cell death) of pulmonary epithelial and endothelial cells, with extensive vascular leakage resulting in acute respiratory distress syndrome (ARDS) and death. These immunological reactions collectively are called cytokine storm. The COVID-19 disease progression due to SARS-CoV-2 virus infection is depicted in Figure 1. Figure-2 depicts schematically the genesis of cytokine storm during lethal COVID-19 disease with resultant ARDS, and the effect of multiple mixed strain probiotics and their immuno-modulins to prevent or cure COVID-19 by controlling the cytokine storm.

It is interesting to note at this stage that unlike other viral infections, SARS-CoV-2 virus, has multiple paths of pathogenesis depending on the level of immunity, age, and comorbid conditions of the host. The progression and multiple infection patterns of COVID-19 disease are:

If the immunity of the host is good, the SARS-CoV-2 will be inactivated even at nasopharyngeal orifice due to the action of defensins, produced by host epithelial cells.

If the victim has previous antibodies to recognize the spike protein antigens, the SARS-CoV-2 will be inactivated aided by specific antibodies, thus the COVID-19 disease won't progress any further.

If the immunity is modest (not optimum), the virus will penetrate the human cell using the ACE-2 (Angiotensin Converting Enzyme-2) receptor, within 15 minutes after contacting the cell surface, aided by spike proteins (roughly 90 spikes per virus particle) thus integrating with the host cell membrane to inject its RNA. Using the host machinery, viral RNA will replicate to produce active virus particles in large numbers. The time it takes from interaction of viral RNA to the time the new virus particles come out of the infected cell is called "eclipse period", which varies from 12 to 36 hours. The number of virus particles generated from one eukaryotic cell from a single virus infection is called "burst size". The burst size of SARS-CoV-2 is around 700 particles. Due to high infective rate having 90 spikes, the viral infection is fast and continuous to infect neighboring cells. However, at this stage the

innate immune system's macrophages and neutrophils will try to inactivate the virus. If the viral load is more, the adaptive immune system is activated to call for effector T-cells and T-killer cells to inactivate the virus. Hypothetically, if the eclipse period of the virus is 36 hours or longer and the burst size is lower, the spread of the virus is slower, and the patient will recover faster from COVID-19. But if the eclipse period is short, 12-hours with a high burst size, the spread of the virus is high. Then it will take the patient longer time to recover. Thus, the patient may experience mild symptoms for up to 3 to 4 days and get over the infection.

If the immune system of the host is sluggish, the SARS-CoV-2 will multiply at a much faster pace by inactivating the T-effector cells, perhaps through interaction of virus induced PD-L1 like ligands with the checkpoint PD-1 receptors of the T-cells. Once the T-effector cells are inactivated, SARS-CoV-2 will multiply at a rapid pace. Here once again, the SARS-CoV-2 virus will impact the Type-II pneumocytes in alveoli, thus causing collapse of the lungs. In addition, the neutrophils will pour in excessively into lungs causing oxidative damage to the lung tissue, besides inducing excess fluid buildup in the lungs, thus causing death, due to respiratory failure.

The immune system of the host, in a massive viral infection, is excessively activated aided by T-effector cells, T-killer cells, neutrophils, macrophages, and native killer cells etc. The over-active immune system, due to cytokine storm induced by excess production of inflammation provoking interleukins and interferons, will do more damage to the host, even after clearance of the virus, thus killing the host. This situation arises due to the lack of or insufficiently low numbers of T-regulatory cells, and inflammation reducing interleukins such as IL-10 etc. Perhaps under these circumstances, the SARS-CoV-2 virus may also inactivate T-regulatory cells since they also have PD-1 and CTLA-4 checkpoint receptors.

If the host has comorbid conditions, with a low number of T-effector cells, T-killer cells, and depressed innate immune system, the SARS-CoV-2 will be virulent and kill the host. This could be the reason the COVID-19 disease is lethal in people with comorbid conditions such as diabetes, hypertension, cancer, obesity, and cardiac diseases etc.

If the host is elderly, naturally due to immuno-senescence, their T-cells and T-killer cells etc. are very weak and slow to respond, thus SARS-CoV-2 will be virulent and kill the host, due to lack of proper immune response (25). This could be the reason for the high infectivity and death rate in people over the age 60.

Even if the host has a good level of antibodies due to a prior infection, SARS-CoV-2 can mutate to overcome or override the antibody inhibition. This is one difficulty vaccine companies are facing to develop an effective and universally acceptable single dose vaccine, since SARS-CoV-2 can mutate to change its spike antigenic structure. The new viral mutants involving spike mutation have been detected in South Africa, Brazil, and the United Kingdom. The mutants have 50 to 70% higher infectivity rate.

Now one can appreciate why SARS-CoV-2 created such a chaos throughout the world due to its varied pathophysiology, and its pathogenesis depends on the level and immunity and age of the host, and due to its mutation rate to deceive the humoral immune system. Thus, even the vaccines, antibody therapies and other specific drug therapies may not function fully and effectively to protect the victim (26, 27, 28).

A Novel Strategy to Prevent or Treat COVID-19 Disease Using Multiple Mixed Strain Probiotics (U.S. Patent # 11,077,052 B1): In August 2021, the United States Patent and Trademark Office (USPTO) has issued a new patent (US patent #11,077,052 B1) to the author to prevent SARS-CoV-2 infection, or treat COVID-19 disease, by using multiple mixed strain probiotics along with their immunomodulins, antioxidants, surfactants, and other microbial stimulants and protectants (1). The patent outlines these multiple ways SARS-CoV-2 infection can be prevented or treated, as stated in the abstract of the Patent: *A multi-phase treatment of a respiratory disease in which one treatment is a lysing defense by applying inhibitory agents to respiratory passages. Application may be by nasal irrigant, oral gargling mouthwash, or smelling salt. Another treatment is an immune system suppressing defense by providing a liposome-based countermeasure to excess activity of the host immune system during COVID-19 infection. The liposome increases bio-activity of probiotics, probiotics-produced therapeutic peptides, bio-peptides, and antioxidant level in the blood with a sustained massive dose of antioxidants to counteract excess oxidation produced by excess activity of the host immune system, when administered through oral route (29,30,31). The liposomal preparation with multiple mixed*

strain probiotics and their immunomodulins might have also corrected the dysbiosis, to confer immunity to prevent or treat SAR-CoV-2 infection. Further details of this patented approach will be published elsewhere and beyond the scope of this focused review on mechanism of cytokine storm in COVID-19. To explain it briefly, as presented in Figure 2 (pointed by red arrows), the immunomodulins of multiple mixed strain probiotics stimulate (up-regulate) the production of anti-inflammatory cytokines (IL-10 etc.), regulatory cytokines, T-Reg cells, T-Effector cells, and B-cells. Simultaneously they also reduce (down-regulate) the production of proinflammatory cytokines (IL-6 etc.) and chemokines to suppress the cytokine storm, and thus protect the patient through immunomodulation. For more details on this therapeutic aspect, the reader is referred to the published U.S. Patent #11,077,052 B1 (1).

How does SARS-CoV-2 virus inactivate T-cells, and how does the immunomodulins produced by multiple mixed strain probiotics activate T-cells to prevent the SARS-CoV-2 viral multiplication, to prevent the COVID-19 disease?

Figure-2 schematically outlines the mechanism of probiotics and their immunomodulins in reducing cytokine storm by SARS-CoV-2 virus. On a separate note, our immune system is constantly on lookout for pathogens including viruses to attack and inactivate them through immune response to protect the host from infection. To reduce the chances of immune system attacking the healthy cells and tissues, it must be tune down. This function is accomplished by the immune checkpoints on the T-cells and the antigen presenting cells, which maintain the self-tolerance and limit the tissue damage by recognizing the ligands expressed on self-tissues. Unfortunately, immune checkpoints also expressed on many viral infected cells allowing them to cleverly evade the host immune response, unless the checkpoints are inhibited. Although there are many immune checkpoint molecules, two such identified and extensively studied molecules are CTLA-4 and PD-1, which serve as negative regulators of T-cell activation, thus preventing unwanted immune response.

The immune checkpoints PD-1 and CTLA-4 present on T-cells serve as the negative regulators of T-cells activation, to prevent our own (self) tissues getting attacked by our own T-cells. However, if the PD-L1, B7-1 / B7-2 ligands are produced (under the influence of SARS-CoV-2), then the SARS-CoV-2 virus infected cells interact with PD-1 and CTLA-4 receptors on T-cells, the T-cell gets inactivated. Thus SARS-CoV-2 cannot be inhibited or destroyed by the T-cells and COVID-19 disease progresses at a rapid pace. But immunomodulins produced by the multiple mixed strain probiotics may act as check point inhibitors to block the interaction of PD-L1 and B7-1/B7-2 ligands (produced by the virus infected cells) with PD-1 and CTLA-4 check points (on T-cells), to activate the T-cells. Thus, the SARS-CoV-2 virus can be destroyed, and the host can be protected from the COVID-19 disease, by the activated T-Cells. The explicit details of these mechanisms are presented in Figures 3 and 4. Thus, one can hypothesize that the immunomodulins of multiple mixed strain probiotics, besides simmering the cytokine storm through immunomodulation (32, 33, 34), might also act as check point inhibitors, more like specific monoclonal antibodies in cancer immune checkpoint therapy (35, 36, 37), inducing no adverse side effects, to prevent or treat SARC-CoV-2 viral infection.

Similar mechanism was observed and published by the author, regarding prevention or treatment of bacterial nosocomial infections, using the multiple mixed strain probiotics and their immunomodulins successfully as therapeutic agents (38). In a different context, multiple mixed strain probiotics along with their immunomodulins were used as adjuvant therapeutic agents along with the immune checkpoint cancer therapy and other standard cancer therapies to prevent or treat cancer successfully by the author (39, 40, 41). Due to the space constraints, and in an attempt not to deviate from the current focus on cytokine storm, except for a few articles referenced (42-45), minute details regarding comprehensive role of probiotics, at the molecular level, to prevent the SARS-CoV-2 infection could not be presented.

Conclusion: In this article I have comprehensively reviewed cytokines, cytokine storm, and how it makes the COVID-19 disease fatal. I have also presented the effect of multiple mixed strain probiotics and their immunomodulins to prevent or cure the cytokine storm, and also their importance of acting as checkpoint inhibitors to control the SARS-CoV-2 infection through activation of effector T-Cells.

Disclosure: Author is a scientist heavily involved in probiotic research and holds over 150 US and International Patents. His company (IMAC, Inc.) is involved in research,

development, and manufacturing food grade microbial cultures and high-tech essential enzyme fortified functional products that go into manufacturing cheese and other functional dairy food products in the United States, Canada, Europe, Asia, and South America.

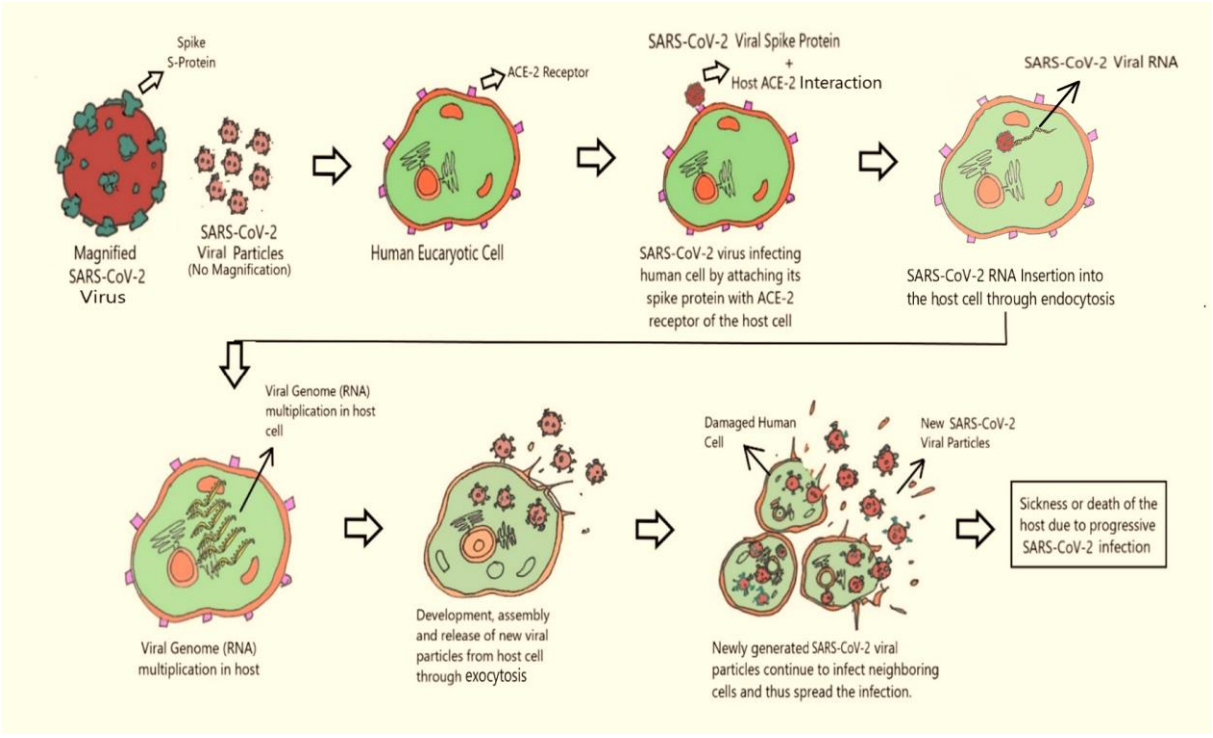

Figure 1: Pictorial presentation of SARS-CoV-2 viral infection

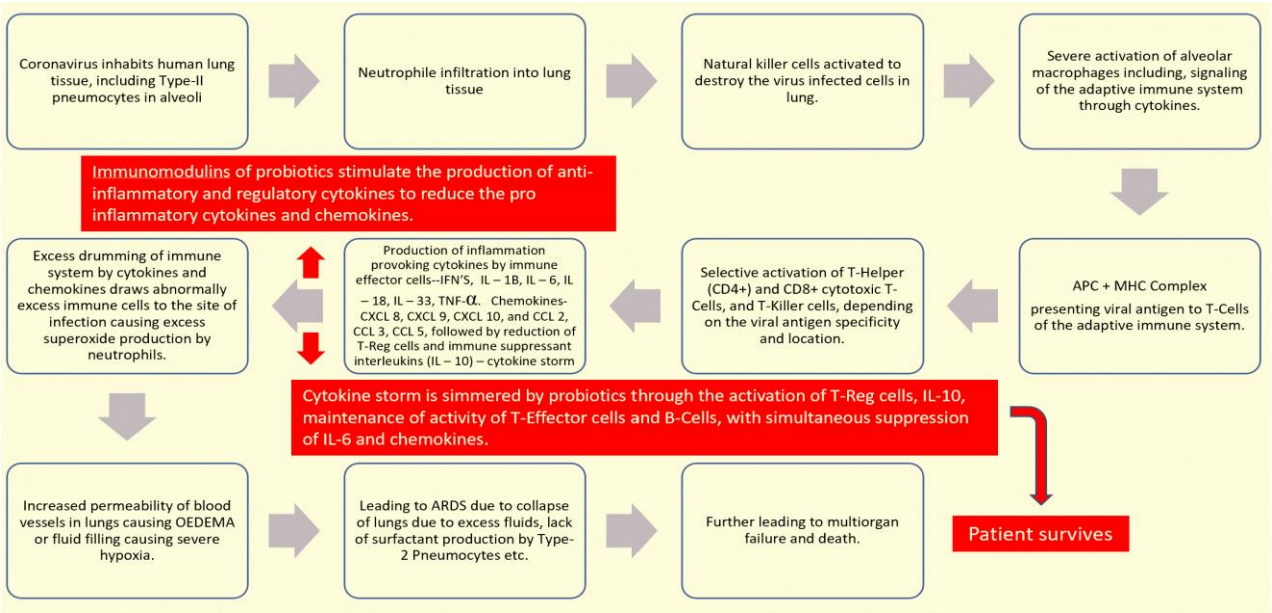

Figure 2: Schematic presentation showing COVID-19 induced cytokine storm, and its potential prevention with the aid of multiple mixed strain probiotics and their immunomodulins.

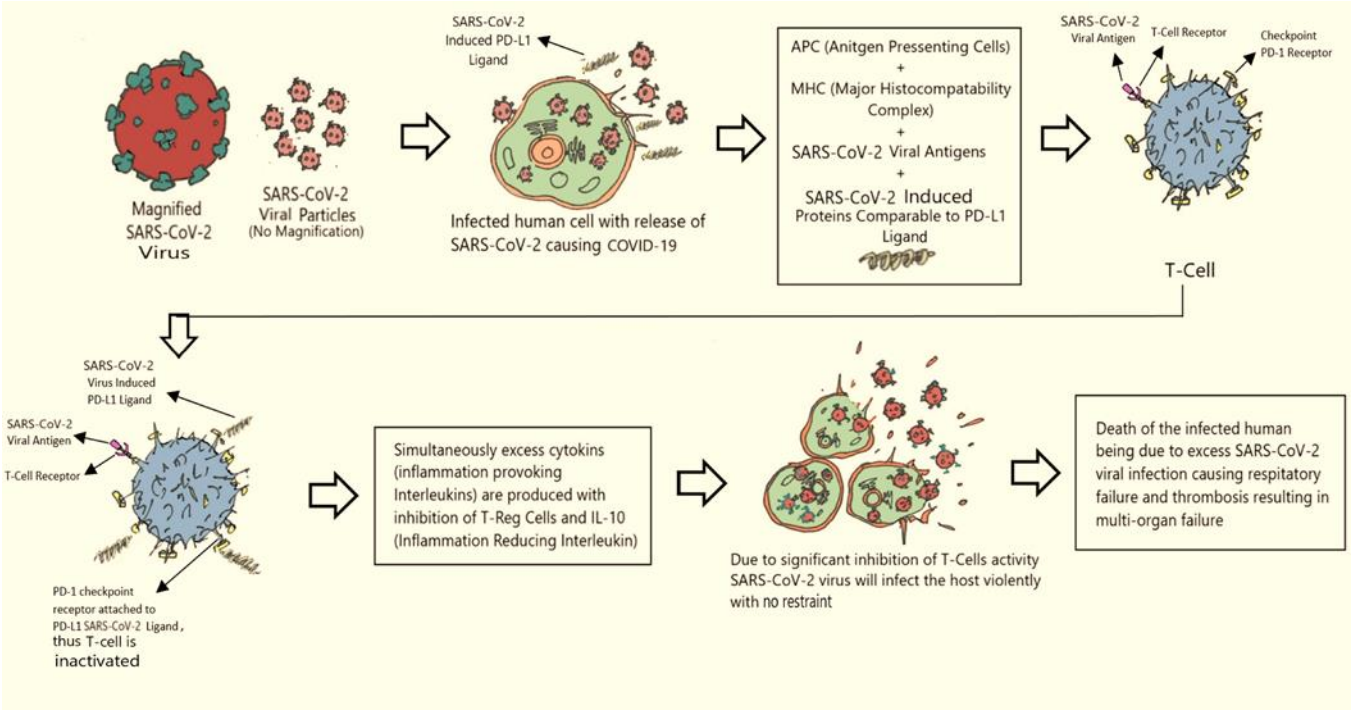

Figure 3: Pictorial presentation of COVID-19 disease pathogenesis through dysfunction of T-cells due to interaction of SARS-CoV-2-induced PD-L1 ligands with PD-1 immune checkpoint receptors on T-cells

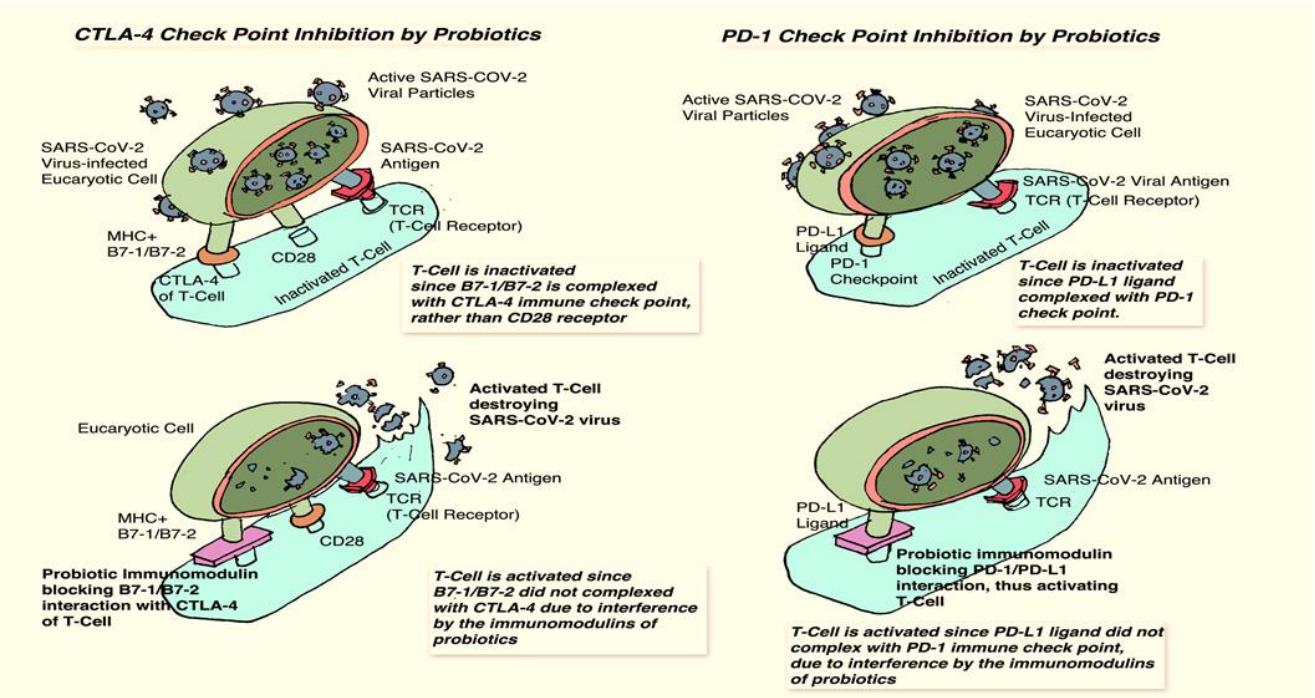

Figure 4: Hypothetical pictorial presentation showing immunomodulins of the multiple-mixed strain probiotics acting as checkpoint inhibitors to block CTLA-4 and PD-1 immune checkpoints on T-cells from interacting with B7-1/B-2, and PD-L1 ligands, thus activating T-cells to inhibit the SARS-CoV-2 virus.

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Diagnostic Update

Updates in Lung Cancer Screening

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Highlights:

- Two randomized controlled trials have shown mortality benefit from lung cancer screening with low-dose chest CT (LDCT).
- The most recent guidelines from USPSTF recommend annual LDCT in patients meeting the following criteria.
  - Age: 50-80 years
  - Tobacco exposure ≥ 20 pack-years
  - Ex-smoker: Less than 15 years since quitting
- Shared decision making with patients and multi-disciplinary approach to positive findings is mandated by CMS.
- Risk stratification of nodules detected on LDCT with Brock University Cancer Prediction Equation is recommended.
- Lung-RADS or NCCN recommendations should be followed for management of nodules detected on LDCT.

Key Words: Lung cancer screening, Low-dose chest CT, Screening-detected lung nodules.

Introduction: Lung cancer is the leading cause of cancer-related mortality both in the United States and worldwide (1). Following diagnosis, only 15% of patients survive for 5 years or more (2). The relative lack of symptoms during the early stages of lung cancer results in delayed diagnosis; more than half of patients already have metastatic disease at the time of noticing symptoms (3). Given a 60% 5-year survival rate for stage I disease, compared with a <5% 5-year survival rate for stage IV disease, early detection can have a significant impact on lung-cancer mortality (4). This has led to considerable interest in screening methods for early detection of lung cancer. Beginning in the 1960s, screening was attempted with chest x-rays, sputum cytology, or a combination of both. Numerous trials were conducted but these options showed no evidence of mortality benefit (5-13).

Clinical Trials Data: The Early Lung Cancer Action Project (ELCAP) was formed in 1992 to assess the usefulness of annual chest CT screening for lung cancer. A prospective trial of 1000 patients screened with low-dose chest CT (LDCT) was published by ELCAP in 1999. It showed that LDCT detected more early-stage lung cancers than a standard chest x-ray (14). Of note, the radiation exposure from LDCT is 1.5 mSv when compared to 8 mSv for a standard chest CT.

Subsequently, 2 randomized, controlled studies have been conducted to prove the mortality benefit from LDCT screening –

A. **National Lung Cancer Screening trial (NLST):** Enrollment for the trial began in 2002 and the follow-up concluded in 2009. The main inclusion criteria for the study were age between 55 and 74 years, more than or equal to 30 pack-year tobacco exposure, and if ex-smokers, less than 15 years since quitting. The study randomized 53,454 patients to either LDCT or chest x-ray. The imaging studies were performed annually for 3 years. The results showed a 20% relative reduction in mortality (95% CI 6.8 to 26.7; p=0.004) given the earlier diagnosis with LDCT (15).

B. **Nederlands-Leuvens Longkanker Screenings Onderzoek (NELSON):** A Dutch and Belgian study that randomized 13,975 men to LDCT or no screening. For the LDCT group, the protocol involved four rounds of LDCT screening with increasing intervals (at baseline, 1 year, 3 years, and 5.5 years). The study included current and former smokers aged 50 to 74 years. The results showed a 24% risk-reduction in lung cancer mortality for the screened group (16).

In 2013, based on the results of NLST, various professional organizations recommended lung cancer screening with LDCT for high-risk populations. The population to be screened mirrored the inclusion criteria for NLST. The National Comprehensive Cancer Network (NCCN), a consortium of 31 cancer centers that provides guidelines for cancer management, published guidelines for lung cancer screening that were more inclusive and took additional risk factors such as occupational exposures, radon exposure, personal history of cancer, family history of lung cancer, and presence of chronic obstructive pulmonary disease or pulmonary fibrosis into consideration. Based on the high quality of both randomized trials (level 1), the grade of recommendation is either A (strong recommendation) or B (recommend-dation) for most professional organizations that recommend lung cancer screening. The number needed to screen (NNS) to prevent one lung cancer death was 323 over 6.5 years of follow up (NLST) and 130 over 10 years of follow up (NELSON). In comparison, the NNS to prevent one breast cancer death is 781 over 8 years and one colorectal cancer death is 1250 over 8 years (17) In 2021, United States Preventive Services Task Force (USPSTF) updated the recommendations based on expert reviews to expand the screening age (50 to 80 years) and include patients with 20 or more pack-year tobacco exposure (18).

Recommendations for 2022:

Based on USPSTF guidelines –

Annual LDCT screening is recommended for patients between the ages of 50 and 80, with more than or equal to 20 pack-year tobacco exposure, current smokers, or have quit within the past 15 years.

The Centers for Medicare & Medicaid Services (CMS) requires the patient to visit with the healthcare provider and have the following documented-

1. Determine eligibility
2. Use one or more decision aids to cover benefits, harms, follow-up diagnostic testing, over-diagnosis, false positive rate, total radiation exposure (shared decision-making component). Example: http://www.shouldiscreen.com/benefits-and-harms-screening
3. Counsel on adherence to annual LDCT, impact of comorbidities, ability, or willingness to undergo diagnosis and treatment
4. Counsel on tobacco cessation and maintenance of abstinence.

Guidelines for Management of Nodules Detected on LDCT:

In NLST, 18,146 of the 75,126 scans had a positive finding (non-calcified nodule >/= 4 mm) and lung cancer was confirmed in only 649 (3.6%). The high false positive rate can cause unnecessary invasive tests. Therefore, CMS has recommended a multi-disciplinary approach to managing lung nodules detected on screening. Mayo Pulmonary Nodule Risk Classifier and Fleischer Society guidelines are often used to assess the risk of malignancy and plan subsequent work-up. However, both of the tools were developed for incidentally found lung nodules and not screen detected nodules. For risk assessment, Brock University cancer prediction equation is recommended (https://www.uptodate.com/contents/calculator-solitary-pulmonary-nodule-malignancy-risk-in-adults-brock-university-cancer-prediction-equation).

For management of the nodules, Lung-RADS assessment (https://www.acr.org/-/media/ACR/Files/RADS/Lung-RADS/LungRADSAssessmentCategoriesv1-1.pdf) or

NCCN guidelines on lung cancer screening are recommended. To simplify decision making, most reports of LDCT now have the Lung-RADS score with recommendations included.

Despite supportive evidence and recommendations for lung cancer screening by multiple professional societies, uptake has been low in clinical practice. In 2018, 5% of all eligible patients underwent lung cancer screening (19). Efforts are underway to improve compliance by incorporating lung cancer screening tools into electronic health record systems and simplifying the shared decision-making process for physicians.

Conclusion: Two randomized control trials have shown mortality benefit with LDCT for lung cancer screening in

high-risk populations. However, compliance with screening remains low. Guidelines and management recommendations from professional societies have simplified the screening process and the management of patients with nodules detected during the screening.

Disclosure: Author declares no competing interests.

Resources for Additional Information:

For Patients:

https://www.nccn.org/patients/guidelines/content/PDF/lung_screening-patient.pdf

For Healthcare Providers:

https://www.uspreventiveservicestaskforce.org/uspstf/recommendation/lung-cancer-screening

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Update of Guidelines

Estimated Glomerular Filtration Rate (eGFR):

An Update of Recent Recommendations from a Joint ASN-NKF Task Force Reassessing the Inclusion of Race

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Highlights:

- eGFR is an estimate of mGFR using a mathematical prediction formula that requires inputs of easily obtainable clinical information and laboratory data.
- Serum creatinine and cystatin C are commonly used blood markers of glomerular filtration.
- After extensive study the eGFR formulae have recently been revised to exclude race as a clinical parameter input.
- Care must be taken to utilize and interpret the eGFR correctly in the patients.

Key Words: Glomerular filtration rate; eGFR; mGFR; Renal clearance; Serum creatinine; Serum cystatin; Chronic kidney disease

Background: A simplistic view of the function of the kidneys is to filter the blood of metabolic waste. One can therefore approach the measurement of kidney function from two perspectives which are essentially two sides of the same coin – clearance or removal of waste by measuring/estimating its appearance in the urine or the rate of glomerular filtration itself. Each approach has utility but also pose challenges. Assessment of clearance requires a marker filtered by the nephron but neither secreted nor reabsorbed by the tubules. The marker's clearance is measured in a timed urine collection. Twenty four-hour urine collections are unfortunately cumbersome and are prone to errors. Thus, we have focused on measuring the rate of filtration at the glomerulus or the "GFR".

Inulin is a polysaccharide that meets these criteria and is the gold-standard marker for GFR measurement. Inulin requires intravenous infusion to establish steady-state serum levels and bladder catherization to measure GFR (1-3). Iothalamate coupled to a nuclear tracer has also been employed for GFR measurement and is delivered by a subcutaneous injection (4, 5). Although these and other markers of GFR are accurate, they are time consuming, cumbersome, and are not easily available outside of research protocols. This led to the desire to develop methods/formulae to estimate GFR from easily obtainable clinical information and laboratory data.

Estimated GFR (eGFR): Creatinine and cystatin C are relatively easy to measure in the serum and although not ideal (creatinine is secreted into the renal tubules), they come close to meeting the criteria for markers for assessing GFR. The Modification of Diet in Renal Disease (MDRD) study GFR estimating equation was developed using data from the MDRD study in which subjects with chronic kidney disease had two measurements each of serum creatinine and iothalamate-GFR performed within a short period of time (6). The iothalamate measured-GFRs (mGFR) were entered into a statistical prediction model to elucidate and generate predictions or estimated GFRs (eGFR) using easily obtainable clinical information (7). The modeling process tested many urinary, demographic and serum factors but ultimately yielded the well-known key parameters of serum creatinine, age, sex (male or female) and race (African American vs. non-African American) (7). Since the cohort used to generate the model was specific (GFR 25-55 ml/min, mostly white and non-diabetic), the

MDRD eGFR equations are best and valid for prediction in patients who meet the demographic and clinical parameters of the MDRD study (8). Subsequently, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was developed from subjects of many different research studies including those with diabetes, younger age and with lesser degrees of kidney dysfunction (9). This model has GFR-estimating equations employing both creatinine and cystatin C while requiring the same demographic parameters (age, sex, race) as the MDRD equation. Over the past 10-15 years, due to utility most clinical laboratories reported eGFR based on one of these equations (CKD-EPI or MDRD) and indicated on the results which formula was employed.

Updated Equations Excluding Race: The last couple of years have brought attention to using race in all types of clinical algorithms as the American society has grappled with differentiating biological variables and social constructs and the consequences of their use or disuse in advancing equity in health care and justice in society. This led to the National Kidney Foundation (NKF) and American Society of Nephrology (ASN) to form a joint task force to re-evaluate the inclusion of race as a key clinical parameter in GFR-estimating equations and the implications for the diagnosis and management of patients with or at risk for kidney diseases (10, 11). The task force recently published its analysis of the issues, which is a product of extensive study. This involved modeling/simulation of potential changes to GFR-estimating equations and their effect on patient diagnosis, clinical care, transplantation and clinical trial eligibility and analysis (12, 13). The task force recommended the clinical deployment in the United States of a new eGFR equation developed without race as a key clinical parameter (14). These equations using serum creatinine alone and both serum creatinine and cystatin C were developed from two large developmental data sets that contained 23 studies and nearly 14,000 participants. The equations were then validated using a data set containing 12 studies with approximately 4,000 total participants and were judged to have "acceptable performance characteristics and potential consequences that do not disproportionately affect any one group of individuals" (12, 13). The task force went further to recommend increased efforts to make cystatin C more easily available in a prompt fashion for clinical use (12, 13). The updated race-free eGFR calculator can be found on the NKF website for immediate use (15).

Key Points to Remember when Utilizing eGFR: The eGFR was developed as tool to quickly assess kidney function using easily obtainable clinical and laboratory values and patient demographic variables. The data sets used to develop the eGFR employed mGFRs done on an outpatient basis with steady-state serum creatinine. Thus, these equations and the resultant eGFR are best used in the outpatient setting. The eGFR is *not* valid in the setting of acute kidney injury when the kidney function is changing rapidly and thus the serum creatinine is not at steady state. Next, before using the eGFR one must be certain the serum creatinine measurement is an accurate reflection of the patient's kidney status. All the study populations used to derive the estimating equations enrolled subjects who were largely free of other systemic disease that would reduce muscle mass and included average muscle mass subjects. Thus, the clinician must consider this when interpreting the eGFR values. For example, low (as in patients with other severe chronic illnesses such as cirrhosis or cancer) or high muscle mass (as in an elderly patient who exercises enough to have a higher muscle mass than the average patient his/her age or an elite athlete) can lead to overestimates or underestimates of true GFR respectively. Additionally, medications such as cimetidine and trimethoprim decrease the tubular secretion of creatinine thereby increasing the creatinine without a corresponding decrease in the GFR. In these situations, obtaining a cystatin C level and utilizing an eGFR equation based on it may be helpful. Also, the equations were all developed as cross-sectional measurements. Therefore, they represent the average muscle mass and creatinine generation at a given age. This reflects that the average older person has less muscle mass to generate creatinine than the average young person. The mathematics of the formulae lead to an interesting issue: regardless of clinical status and even if a given patient's muscle mass may not have changed with age, one always loses kidney function utilizing the eGFR equations on one's birthday. Lastly, as researchers test the performance of these eGFR formulae in other populations, the P10 and P30 metrics are usually reported. These refer to the proportion of eGFRs within ± 10 and 30% of the mGFR, respectively (16). An important role for the clinician is to understand all these nuances of the eGFR and recognize when the eGFR needs to be interpreted considering other clinical factors.

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Review Article

Autoimmune Pancreatitis: A Review for Non-gastroenterologist

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Abstract: Autoimmune Pancreatitis (AIP) is an uncommon, however treatable disease on the differential alongside pancreatic malignancy for individuals presenting with obstructive jaundice with or without a pancreatic mass. AIP has been classified into two types. Serologic testing and imaging modalities are helpful in the initial investigation; however, histology is ultimately essential for securing the diagnosis and type of AIP. Type I or lymphoplasmacytic sclerosing pancreatitis (LPSP) can be part of the constellation for IgG4 related disease and has a higher degree of relapse. Type 2 or idiopathic duct centric chronic pancreatitis (IDCP) has been associated with inflammatory bowel disease, does not have elevated IgG4, and carries a lower rate of relapse. Steroids are the mainstay of management for both types of AIP and, in the majority of patients, are effective in inducing remission and preventing long term complications such as pancreatic dysfunction, biliary strictures, and extra-intestinal involvement. The aim of this review is to guide the general practitioner through the recommended approach to the diagnosis and management of AIP while exposing knowledge gaps.

Key Words: Autoimmune pancreatitis, Chronic pancreatitis, IgG4, Lymphoplasmacytic sclerosing pancreatitis, Idiopathic duct centric pancreatitis, HISORt

Glossary of Abbreviations: See at the end of text of the article

Introduction: Autoimmune Pancreatitis (AIP) is a relatively uncommon entity in general medical practice. However, it has generated increased recognition and elucidation over the last two decades. Our improved understanding of AIP is evidenced by its renaming, previously having been classified under many names including non-alcoholic destructive pancreatitis, tume factive pancreatitis, sclerosing pancreatitis, and intra-biliary pancreatitis (1). Further understanding of AIP and the role of Immunoglobulin G4 (IgG4) as a diagnostic marker has also led to the discovery of AIP as a part of a multi-organ process known as IgG4-related disease (2).

The incidence of AIP is low, representing the etiology for chronic pancreatitis in up to 6% of cases (3). Broadly, AIP is a predominantly benign disease characterized by obstructive jaundice with and without a pancreatic mass, histological findings consistent with lymphoplasmacytic infiltrate and fibrosis, and a dramatic therapeutic response to steroids. Because of the similarities of clinical presentation with pancreatic malignancy, misdiagnosis is common and the distinction between the two remains difficult without definitive tissue sampling. The timely and appropriate diagnosis of AIP versus pancreatic adenocarcinoma (PDAC) is essential to avoid unnecessary operative resection (4) and morbidity and mortality from inappropriate management.

In this review, we summarize our latest understanding of AIP; from its clinical presentation to the criteria for securing its diagnosis and therapeutic options available to prevent complications. The two types of AIP

accepted by international consensus—Type 1, or lymphoplasmacytic sclerosing pancreatitis (LPSP), and Type 2, or idiopathic duct centric chronic pancreatitis (IDCP)—are discussed and differences between them are highlighted particularly compared to PDAC. We maintain a particular emphasis on what the general practitioner needs to know to care for patients with AIP.

Epidemiology: AIP is a rare disorder though true population-based studies are lacking to confirm its prevalence. In countries such as Japan (5), Italy (6), and Germany (7), studies have shown an increasing incidence of AIP, likely attributed to advances in classification and awareness as a disease (8). It is estimated that 4.6 to 6% of patients with chronic pancreatitis have AIP (3). In the United States, the largest epidemiologic study was the Mayo Clinic cohort which, published in 2010, showed that only 4% of patients suspected to have pancreatitis had AIP. However, > 33% of AIP cases had features consistent with acute or chronic pancreatitis at presentation (9).

The age of onset with AIP is variable and confounded by diagnostic delay. The majority of patients present in the fifth to sixth decades of life (6), though reports have confirmed cases much younger and older (10). Men appear to be two to three times more likely to develop AIP than women (11), which is notably different from most autoimmune conditions which disproportionately affect women.

Pathogenesis: The pathophysiology of AIP remains poorly understood. Similar to other autoimmune conditions, AIP is likely a result of complex immune-regulatory dysfunction with additional multi-factorial contributions from genetic and environmental factors (12). Large scale genome wide association studies for AIP are needed to confirm smaller population-based studies that have recognized increased susceptibility with certain HLA serotypes (DRB1*0405 and DQB1*0401) and single-nucleotide polymorphisms (encoding tumor necrosis factor alpha, Fc receptor-like 3, and cationic trypsinogen) (11). Components of the innate and adaptive immune systems have been implicated in AIP. At present, more evidence is available for Type 1 AIP with repeated demonstration that inflammatory cells, namely CD3 T cells and IgG4 cells, invade pancreatic tissue in this subtype (11). This mechanism also drives other extra-pancreatic manifestations seen in "IgG4-related disease", a broad entity under which Type 1 AIP exists (Figure 1).

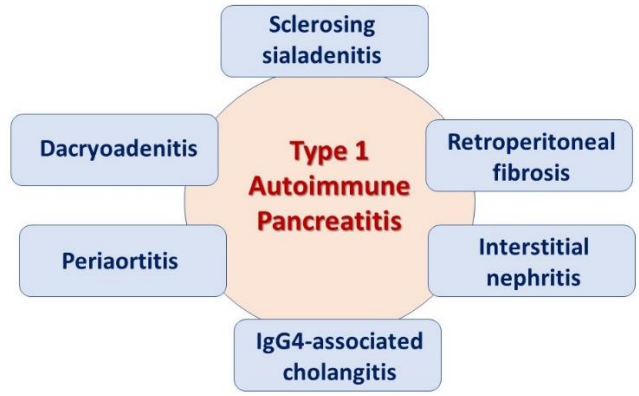

Figure 1: Extra-pancreatic manifestations of Type 1 AIP. Modified from Figure 12D.1 in *Muniraj et al 2017 (16)*.

Clinical Manifestations:

Pancreatic Manifestations: Classically, patients with AIP present with painless obstructive jaundice. This is often the result of biliary ductal compression from pancreatic head enlargement or from biliary stricture. Abdominal pain can be associated with AIP, though typically is not persistent or severe. Other clinical symptoms include weight loss and endocrine complications such as new-onset diabetes and steatorrhea. Even though AIP may be part of the differential, in any patient with a persistent pancreatic mass and painless jaundice, the primary objective should be to investigate further for malignancy (11, 13). As a result, diagnostic delay after symptom onset can be common given the overlap seen with PDAC, along with pancreatic masses, sclerosing cholangitis, and cholangiocarcinoma (11, 14, 15). It is also important to note that AIP can be a silent disease. In one study addressing this, 30% of asymptomatic patients were incidentally diagnosed with IgG4 related disease during routine medical visits (16).

In comparison with Type 1 AIP, individuals with Type 2 AIP are more likely to present with acute pancreatitis which can be noted in up to 50% of cases (17). In Type 2 AIP, early presentation can mimic acute pancreatitis while late manifestations appear like chronic pancreatitis with pancreatic duct strictures (18). The presence of inflammatory bowel disease (IBD), ulcerative colitis (UC) in particular, can also be a clue for Type 2 AIP as there is a strong association between the two (17).

We recommend patients with painless obstructive jaundice and acute pancreatitis with unclear etiology, or a medical history of other autoimmune conditions be worked up for AIP.

Extra-pancreatic Manifestations: The extra-pancreatic structures involved with Type 1 AIP include the salivary glands, thyroid gland, lymph nodes, kidneys, gallbladder, and the aorta (Figure 1) (16, 19). A study by Inoue et al. found that, in males with Type 1 AIP, there was more evidence of associated peri-aortitis, whereas in females, there were higher rates of sialadenitis and dacryoadenitis (16).

Diagnosis: The Mayo HISORt Classification is used to describe, categorize, and assess AIP. The basic tenets of this classification revolve around histology, characteristic imaging, serologic testing (IgG4 levels specifically), other organ involvement (OOI) and response to mainstay treatment (steroids) which are further detailed to the right. Other diagnostic classifications including the Japanese and International Consensus Diagnostic Criteria (ICDC) (20) have used similar criteria, however the inclusion of glucocorticoid response has made the Mayo HISORt more widely accepted.

Mayo Clinic HISORt Criteria (21)

Histology
Lymphoplasmacytic infiltrate, storiform fibrosis, IgG4+ cells ≥10 per high power field (HPF)

Imaging
1) Diffusely enlarged gland with rim enhancement or irregular, attenuated main pancreatic duct (MPD)
2) Focal pancreatic duct mass, stricture, atrophy, calcifications, or signs of pancreatitis

Serology
Elevated serum IgG4 levels

Other Organ Involvement (OOI)
Hilar/intrahepatic biliary strictures, parotid or lacrimal gland involvement, mediastinal lymphadenopathy, retroperitoneal fibrosis (Figure 1)

Response to **S**teroid **t**herapy
Resolution or marked improvement of pancreatic/extra-pancreatic manifestations after initiation of steroid therapy (Figure 2, Figure 3)

While serology and clinical manifestations can be helpful as screening tools, these markers are nonspecific and cannot substitute for pancreatic biopsy. Currently, AIP remains a histologic diagnosis.

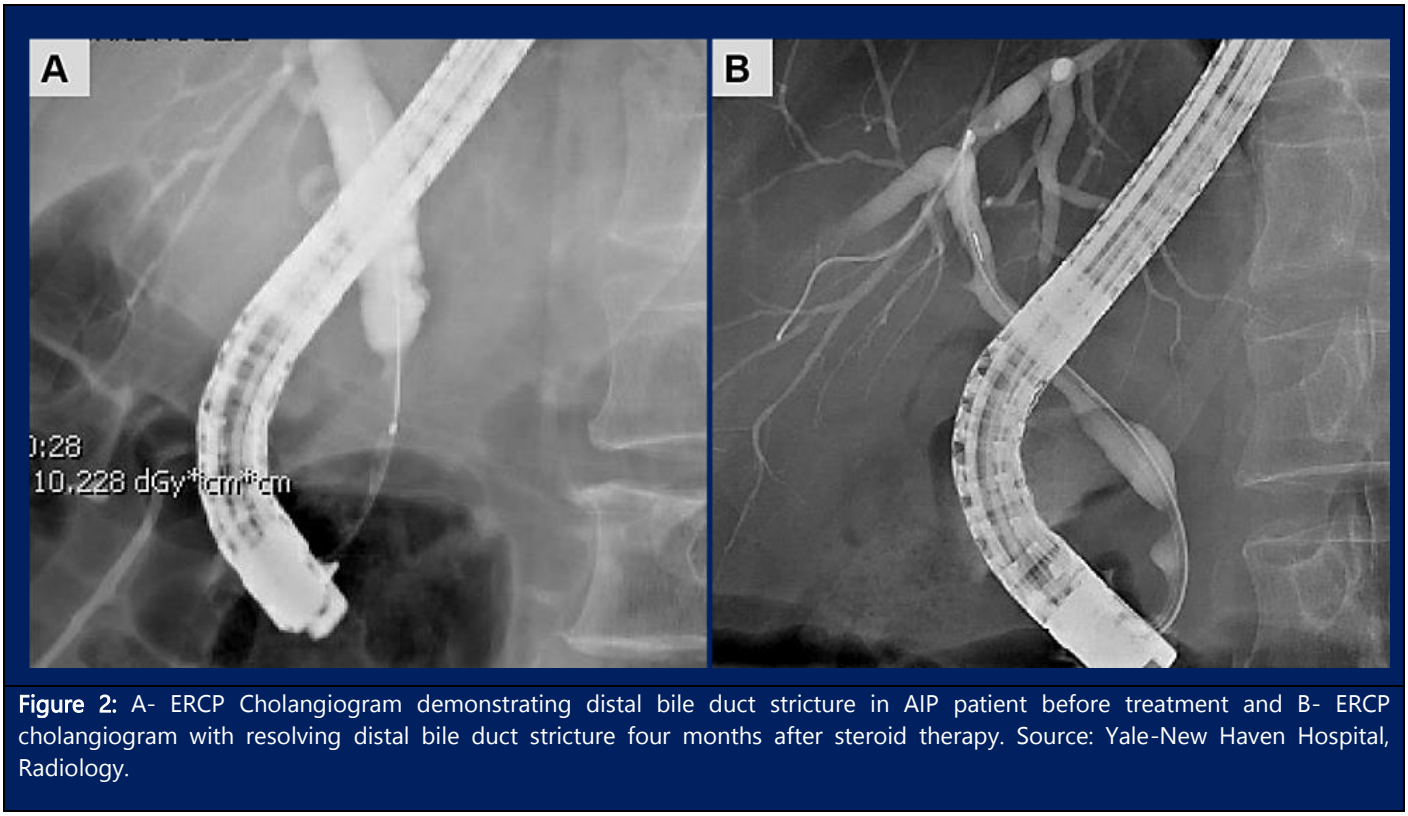

Figure 2: A- ERCP Cholangiogram demonstrating distal bile duct stricture in AIP patient before treatment and B- ERCP cholangiogram with resolving distal bile duct stricture four months after steroid therapy. Source: Yale-New Haven Hospital, Radiology.

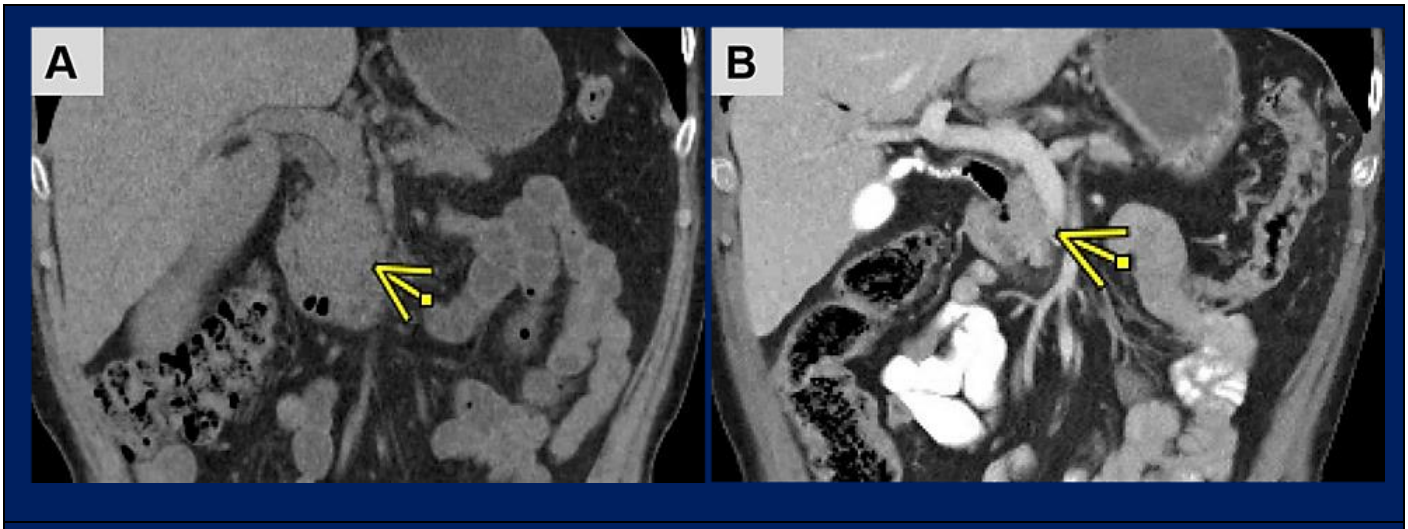

Figure 3: A- CT demonstrating pancreatic head mass in an AIP patient before treatment and B- CT after three months of steroids with resolution of pancreatic enlargement as indicated by arrows. Source: Yale-New Haven Hospital, Radiology.

Histology: Endoscopic ultrasound (EUS) guided fine-needle biopsy (FNB) using a 19G or 22G needle is the current standard procedure to guarantee a sufficient sample size to diagnose AIP by histopathology. Efforts to achieve diagnosis with EUS guided fine-needle aspirate (FNA) without a core biopsy have been studied and discouraged by a consensus panel of pathologists (Hawaii Consensus) mainly based on the lack of tissue architecture with cytology alone (22). Although there is concern of slightly increased post-procedure complications with EUS-FNB, it has been shown that EUS-FNB with core biopsy samples preserves the gland architecture and is essential for tissue diagnosis (23). In particular, the ICDC recommends using FNB core biopsy when the diagnosis remains unclear in an individual with a focal pancreatic mass and/or obstructive jaundice (24).

Endoscopic retrograde cholangiopancreato-graphy (ERCP) can be pursued with or without adding EUS, though typically is indicated in the setting of biliary stricture or potential therapeutic management of obstructive jaundice. In comparison studies, both EUS and ERCP have been found to increase the diagnostic sensitivity of AIP, however without a statistical difference between the two (25). One benefit of ERCP is that it can allow for a mucosal biopsy of the peri-ampullary area, which can then be stained for IgG4. Positive IgG4 immunostaining of the pancreatic duct is extremely specific for AIP and can confirm the diagnosis in a patient who is otherwise asymptomatic with normal serum IgG4 levels (26). However, it should be noted that both celiac disease and PDAC can also show > IgG4 per HPF on biopsy (26). Performing cholangiogram with ERCP can further assist in identifying a long strictured segment of the main pancreatic duct (MPD) with side branches along with bile duct stricture (11), a distinctive feature of AIP (Figure 2). This is compared to primary sclerosing cholangitis (PSC) in which the only the bile ducts are affected and are usually seen in a beaded or pruned-tree appearance (27).

The biopsy, once obtained, allows for definitive diagnosis of AIP and determination of the subtype–Type 1 versus Type 2 AIP (Table 1). In patients with Type 1 AIP (LPSP), histopathologic samples demonstrate dense lymphoplasmacytic infiltrate with storiform fibrosis, obliterative phlebitis, and abundant (>10 cells/HPF) IgG4-positive cells. In patients with Type 2 AIP (IDCP), histopathologic samples demonstrate granulocytic epithelial lesions (GEL) with or without granulocytic acinar inflammation as well as absent or scant (0-10 cells/HPF) IgG4-positive cells (28).

Imaging: Available imaging modalities for the diagnosis of AIP include conventional ultrasonography (US), computed tomography (CT) often with pancreatic protocol (28), magnetic resonance imaging (MRI), and magnetic resonance cholangiopancreatography (MRCP). These imaging methods are not only useful for diagnosis, but also in monitoring response to therapies. US and CT are generally the first imaging tests performed for workup of presenting abdominal complaints.

In AIP, imaging can show focal, multifocal, or diffuse involvement in the pancreas and biliary system (19). Supportive US findings are a diffusely enlarged and hypoechoic pancreatic parenchyma and when doppler is utilized, the presence of hypervascularity (13, 30). On CT imaging, the hallmark finding suggestive for AIP is an enlarged, hypodense sausage-shaped pancreas (11, 30). Other imaging findings for AIP include a hypodense capsule-like rim around the pancreas (30, 31) and multiple narrowing of the MPD or an irregularly narrowed MPD (>3 cm) [30]. MRCP, in comparison, has advantages as it is non-invasive and provides for detailed evaluation of the MPD without the use of a contrast agent (29).

A key role of imaging is in distinguishing AIP from PDAC by evaluating pancreatic mass morphology, patterns of enhancement and distribution of pancreatic duct strictures (12). While MRI and CT appear to have similar specificities (97% versus 99%, respectively) for differentiating AIP from PDAC, MRI has emerged as superior to CT in its sensitivity (84% versus 59%) (12). The absence of up-stream marked MPD dilatation on MRI is highly sensitive against PDAC as it is more common in AIP to see tapered narrowing of ducts rather than abrupt occlusion. Other validated findings on MRI consistent with AIP include the presence of a peripancreatic rim, duct penetration sign (when normal looking MPD penetrates mass), and multiple pancreatic duct strictures (32). In general, multiplicity of parenchymal and ductal involvements clues towards AIP.

Serology: Historically, elevated IgG4 levels (normal levels 8-140 mg/dL) have been the only accepted serological marker characteristic of Type 1 AIP. Ghazale et al. found that the positive predictive value for serum IgG4 > 140 mg/dL was 36% and serum IgG4>280 was 75% (32). IgG4 levels may also be elevated in other pancreatic diseases such as PDAC, though often to a lesser degree (under 2-fold the upper limit of normal). Given the lack of specificity, the utility of elevated IgG4 levels holds more value as a screening and surveillance measure instead of a diagnostic tool (33, 34). The tumor marker Carbohydrate antigen (CA) 19-9 carries high specificity for PDAC at high values (>1,000U/mL), though also can be measured at elevated levels in AIP. A study by van Heerde et al. found that a combination of high IgG4 > 1 g/L and low CA 19-9 <74 U/mL identified Type 1 AIP with 94% sensitivity and 100% specificity (35).

Other Laboratory Testing: Liver enzymes such as bilirubin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) are commonly elevated in AIP, and often in a cholestatic dominant pattern, though are widely variable (33). Lipase, a marker typically elevated in pancreatic diseases, is not useful in the diagnosis of AIP (33).

Classification: Table 1 below summarizes the characteristics of Type 1 versus Type 2 AIP that have been discussed above with a comparison to PDAC.

	Type 1 AIP	Type 2 AIP	Pancreatic Adenocarcinoma (PDAC)
Epidemiology	Male>Female >50 years old	Male = Female 30-50 years old	Male > Female >50-60 years old
Clinical Manifestations	• Obstructive jaundice • Mild abdominal pain	• Less jaundice • More acute pancreatitis	• Obstructive jaundice • Cachexia, weight loss
Histology	• Lymphoplasmacytic sclerosing pancreatitis (LPSP) • Storiform fibrosis • Obliterative phlebitis • Abundant (>10 cells/HPF) IgG4-positive cells	• Idiopathic duct centric pancreatitis (IDCP) • Granulocytic epithelial lesions • Absent or scant (0-10 cells/HPF) IgG4-positive cells	• Malignant cells
Imaging	• Multiple parenchymal and ductal involvements including MPD strictures and duct penetration sign • Enlarged hypodense sausage shaped pancreas • Homogenous stiffness • Hypervascularity		• Focal pancreatic lesion with MPD dilation • Heterogenous stiffness • Hypovascularity
Serology	Elevated IgG4	Normal IgG4	Elevated CA 19-9
OOI/Associations	IgG4-related disease (Figure 1)	Inflammatory bowel disease (IBD)	Metastases (liver, lungs, bones, etc.)
Response to Steroids	Yes High rate of relapse	Yes Low rate of relapse	Maybe Definitive therapy is surgery or chemotherapy

Management

Initial Episode: A trial of corticosteroids is included in most diagnostic algorithms for AIP (Figure 4). Ideally, however, initiation of steroids occurs in a patient with a supportive clinical presentation including obstructive jaundice, correlating imaging findings and histologic confirmation for LPSP or IDCP. In asymptomatic patients, the presence of a persistent pancreatic mass, persistent liver test abnormalities, or OOI indicate for treatment (36). The goal of therapy is to achieve remission while preventing long term complications which include progression to pancreatic fibrosis, exocrine and endocrine pancreatic dysfunction, proximal biliary strictures and OOI (37). Treatment response is defined by resolution of pancreas mass and the bile duct stricture. Despite 10-25% of patients experiencing a spontaneous improvement without treatment, the benefits of therapy to avoid relapse and irreversible organ dysfunction are often worth risks of adverse treatment related effects. Oral prednisone or prednisolone has become the standard starting treatment regimen at a dose of 0.6 mg to 1mg/kg/day (or standard dose 40 mg) for 2-4 weeks followed by a prolonger taper by 5 mg per week for a minimum induction duration of 12 weeks. Low-dose maintenance therapy is then continued at a dose of 2.5-5 mg/day for 6 months up to 3 years (36). In patients who are intolerant to steroids, rituximab is an acceptable alternative induction agent given as two separate infusions, 15 days apart. Azathioprine can also be considered as a steroid-sparing maintenance therapy (38). At 2 weeks into therapy, reassessment is strongly recommended and a lack of clinical or radiologic improvement should prompt re-investigation of overlooked PDAC. Response to steroids can also be seen in PDAC which can be deceiving, though often it is less robust. Reassuringly, there is a high rate of steroid response in >90% of patients both Type 1 and Type 2 AIP. Due to the success of steroids and complications of invasive interventions, endoscopic biliary drainage or stenting is not utilized unless severe acute pancreatitis or cholangitis is present. Surgical therapies are reserved for refractory cases that require palliative pancreatectomy.

Relapse: Recurrence of AIP is typically a consequence of premature cessation of steroids. Management begins with re-initiation at same initial dose, though with a longer induction and tapering duration. If repeat steroid courses are ineffective, immunomodulators such as azathioprine, 6-mercaptopurine (6-MP), and mycophenolate mofetil (MMF) are used (39) (Figure 4). In comparison studies for relapse of AIP, rituximab outperformed immunomodulators with an efficacy of 94% versus 65% (40). It may be appropriate in circumstances to involve steroid-sparing regimens (rituximab or immunomodulators) early, even as maintenance therapy, in cases where risk of relapse is high. Features found to be predicative for relapse include serum IgG4 levels > 4 times the upper limit of normal before therapy with persistent elevations after steroid treatment, diffuse enlargement of the pancreas, proximal IgG4 sclerosing cholangitis, and involvement of over 2 extra-pancreatic organs (40). Rates of relapse are higher in Type 1 AIP, occurring in up to 30% of cases, with majority in the first 3 years from diagnosis (40).

Inconclusive Diagnosis: There are situations where initial testing is discordant or unable to reach a definitive diagnosis of AIP. If there remains a clinical suspicion for AIP or PDAC, it is prudent to repeat the cross-sectional imaging in a short interval (4 to 6 weeks) followed by a repeat EUS core biopsy. If the diagnosis of AIP is still uncertain, but cancer can be confidently ruled out, a 2-week course of steroid therapy can be initiated, and response to steroids should be assessed with follow-up cross-sectional imaging and laboratory tests.

Monitoring: Although the same imaging modalities discussed for diagnosis can be used for monitoring of response and surveillance after treatment, enhanced CT is most widely used followed by MRCP which may be preferred to avoid the repeated administration of contrast and radiation. Rapid regression of enlarged pancreas and disappearance of capsule like rim, pancreatic cysts, and extra-pancreatic lesions are all radiologic hallmarks of effective therapy (42) (Figure 2, Figure 3). Histologic re-examination is not recommended and thus, routine EUS or ERCP are not advised for monitoring (36). The use and interpretation of IgG4 levels following treatment is a controversial topic. It should not be used in isolation though is likely beneficial in conjunction with other objective data (37). Biochemical parameters such as liver enzymes should be routinely measured every 3-6 months with an expected decline to normalization of their values.

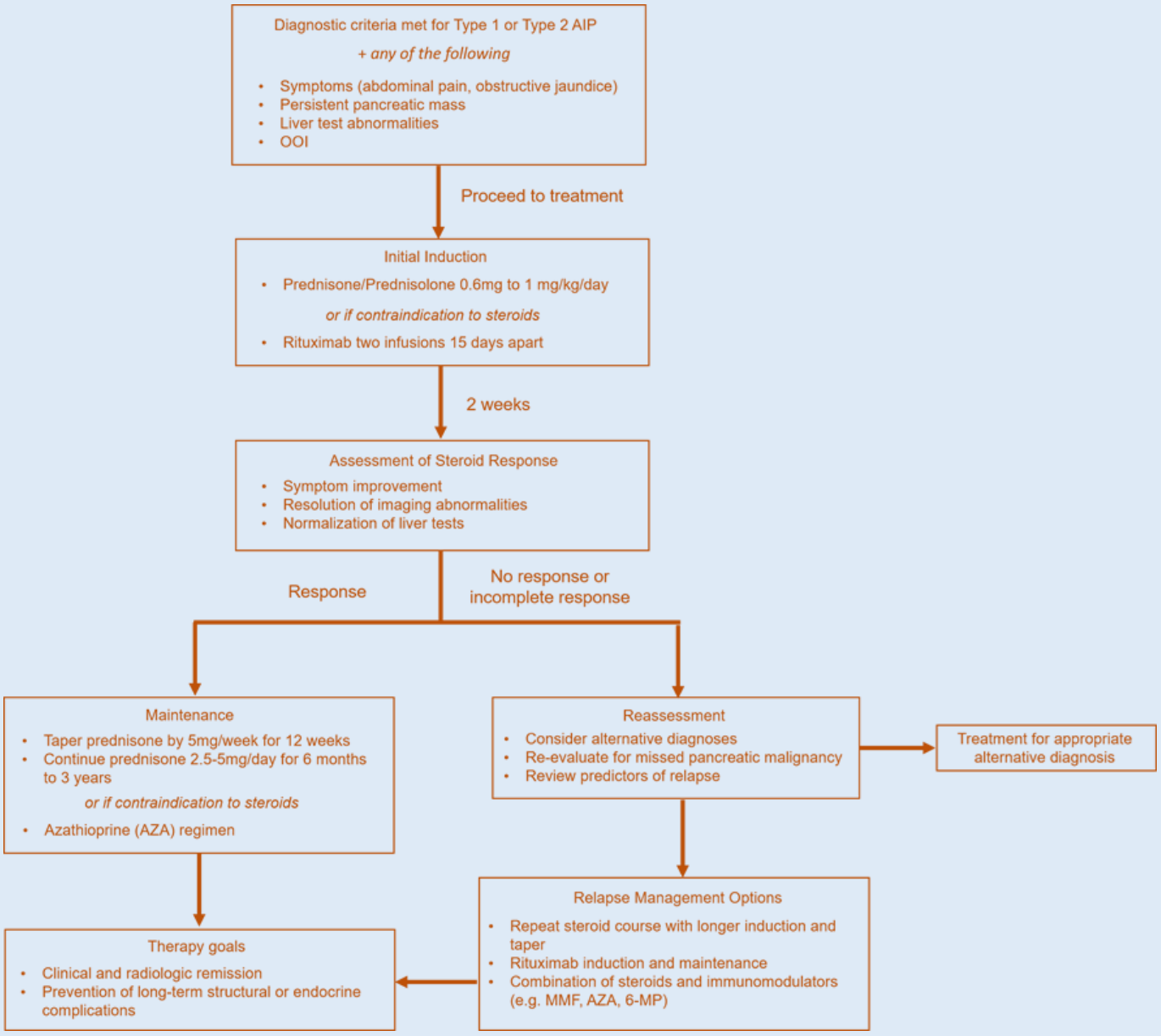

Figure 4: Accepted algorithm for management of initial presentation and relapse of AIP.

Role for the Primary Care Clinician: Given the complexities of diagnosing AIP and non-trivial rates of relapse, management of AIP requires the involvement of the advanced gastroenterologist and/or pancreatologist. That said, the primary clinician is invaluable in helping manage many aspects of post-treatment cares as patients remain on therapy for months to years. Prior to onset of steroid treatment, it is helpful to screen and optimize management for diabetes or impaired glucose tolerance. Frequent glucose monitoring is essential for both diabetic and non-diabetic patients while on steroids. Close follow-up is also advised to ensure appropriate steroid prophylaxis and monitoring for the systemic steroid related complications that can develop. Osteoporosis screening is recommended, with addition of calcium and vitamin D supplementation. Regarding use of rituximab and immunomodulators, it is imperative that all patients be tested prior for hepatitis B to avoid reactivation and be up to date on age-appropriate vaccinations. Patients should also be counseled on specific side effects such as the potential of drug-induced pancreatitis with azathioprine. Smoking cessation should be strongly

encouraged. In conjunction with gastroenterology, additional workup should be pursued in patients with evidence of exocrine pancreatic insufficiency which can present despite adequate therapy and often with fat-soluble vitamin deficiencies. Stool pancreatic elastase levels and empiric use of pancreatic enzymes can aid in diagnosis and management, respectively.

Role of the Primary Care Clinician in AIP

Diagnosis:

- Order enhanced CT or MRCP in all patients with obstructive jaundice and if there are any suggestions of pancreatic mass on other imaging
- Early referral to advanced gastroenterologist/ pancreatologist

Pre-treatment:

- Identify barriers to steroid therapy
- Hepatitis B serology (particularly for rituximab)
- Ensure up to date, age-appropriate vaccinations
- Encourage smoking cessation

During and Post-treatment:

- Liver chemistries every 3-6 months
- Osteoporosis screening (particularly when using steroids)
- Glucose monitoring (particularly when using steroids)
- Monitoring for therapy specific side effects (azathioprine induced pancreatitis, infections, etc.)
- Screening for exocrine pancreatic insufficiency

Emerging Areas in AIP: Since 1995 when Yoshida et al. first identified AIP as a distinct disease process (43), our understanding of AIP has greatly improved, along with the tools we have to diagnose and manage it. There remains opportunity to further our progress in AIP particularly to minimize diagnostic delay, avoid misclassification from pancreatic malignancy, and improve outcomes for steroid refractory cases. Innovations seen in radiology and immunotherapy, in particular, hold promise in what they can offer in AIP.

Integrating artificial intelligence and machine learning is one exciting avenue that could standardize the process of identification of AIP and ensure high diagnostic accuracy. Software has been developed using over 1 million unique images to differentiate normal pancreatic findings from AIP and pancreatic malignancy. Initial results suggest the algorithm is 99% sensitive and 98% specific in distinguishing AIP from a normal pancreas, and 90% sensitive and 93% specific in distinguishing AIP from pancreatic malignancy (44). This a significant improvement compared to human interpretations. Additionally, increasing expertise in EUS may prove beneficial to determining biliary involvement, histological sampling, and ultimately disease classification. EUS elastography, which allows for the quantification of tissue stiffness, can help distinguish AIP from acute pancreatitis by features of diffuse parenchymal stiffness and hypervascularity (45). Another imaging modality in investigation is the use of 18 F-fluorodeoxyglucose positron emission tomography (PET), in which limited studies have shown statistically relevant differences between AIP and chronic pancreatitis (46, 47).

While corticosteroids are an effective treatment for the majority of AIP case, there are limited options for refractory cases. Besides rituximab, combinations of other biologic agents and immunomodulators are being studied as possible therapeutic agents for AIP. An ongoing Phase I clinical trial is assessing the effectiveness of daily lenalidomide to rituximab to treat Type 1 AIP and other IgG4 related disorders (National Library of Medicine [NLM], NCT02705638 (48). Given the association between Type 2 AIP and inflammatory bowel disease, joint treatment strategies have also been proposed (49).

Other novel therapeutic approaches are being derived in the form of stem cell therapy, theorizing the use of mesenchymal stromal cells as possible interventional agents. These remain in a preliminary stage with significant regulatory, cost, and procurement limitations (50). As our understanding of the pathogenesis of AIP improves, disease specific autoantibody targets are likely to offer individualized treatment strategies (51).

Conclusion: AIP is a distinct chronic fibro-inflammatory disease that should be considered especially in patients presenting with obstructive jaundice or chronic pancreatitis of unclear etiology. The priority in diagnosis remains to exclude pancreatic malignancy after which prompt initiation and prolonged taper of steroids can often induce durable remission. Advances in non-invasive imaging modalities and identification of serum biomarkers will hopefully absolve the need for pancreatic biopsy in the future diagnosis of AIP.

Disclosure: The authors declare no competing interests.

Abbreviations Used

IBD, inflammatory bowel disease
UC, ulcerative colitis
OOI, other organ involvement
ICDC, International Consensus Diagnostic Criteria
HPF, high powered field
MPD, main pancreatic duct
EUS, endoscopic ultrasound
FNB, fine needle biopsy
FNA, fine needle aspiration
ERCP, endoscopic retrograde cholangiopancreatography
PSC, primary sclerosing cholangitis
GEL, granulocytic epithelial lesions
US, ultrasonography
CT, computed tomography
MRI, magnetic resonance imaging
MRCP, magnetic resonance cholangiopancreatography
CA 19-9, Carbohydrate Antigen 19-9
ALP, alkaline phosphatase
ALT, alanine aminotransferase
AST, aspartate aminotransferase
6-MP, 6-Mercaptopurine
MMF, mycophenolate mofetil
PET, positron emission tomography

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Commentary

Spectrum of Kidney Pathology in COVID-19

Insights from a Multi-Center Retrospective Cohort Study

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Highlights:

- Acute kidney injury (AKI) among COVID-19 patients admitted into ICU often requires renal replacement therapy and/or contributes to mortality.
- Pathophysiologically, AKI among COVID-19 patients is not due to direct effect of SARS-CoV-2 but appears to be the result of systemic and intrarenal factors.
- AKI in COVID-19 disease disproportionately affects African Americans and has strong association with *APOL1* high-risk genotypes in this group.
- Histopathologically, collapsing glomerulopathy has a higher frequency in COVID-19-induced AKI, leading to the designation of this entity as COVAN (COVID-19 associated nephropathy).
- Prevalence of myoglobin cast nephropathy due to rhabdomyolysis in COVID-19 can be an early marker for impending AKI by determining the creatin kinase levels in plasma.

Key Words: COVID-19, SARS-CoV-2, Acute kidney injury, Kidney biopsy, APOLI1, COVAN

Introduction: Although the pathophysiology of COVID-19 mostly involves the respiratory system, and death is often due to severe acute respiratory syndrome (SARS) associated with cytokine storm, other organs, such as heart and kidney contribute to the overall mortality of COVID-19. Pre-existing conditions, such as obesity, cardiovascular disease, hypertension, chronic respiratory diseases, and cancers have been documented to be associated with higher case fatality rates in COVID-19 (1). However, with the kidney, the impact of COVID-19 burden, including mortality rate, is underrecognized, because kidney disease causes no symptoms. But COVID-19 disease can significantly affect kidney both acutely and chronically.

Acutely, SARS-CoV-2 infection can injure the kidney, causing patients to manifest signs of kidney disease ranging from proteinuria to acute kidney failure that requires dialysis therapy (2, 3). The prevalence of acute kidney injury (AKI) in the United States and Europe was about 20-40% among COVID-19 patients admitted into intensive care unit (ICU) (4, 5). Up to 40% of patients requiring mechanical ventilation need concurrent renal replacement therapy (6). Thus, AKI can markedly affect case fatality rate in COVID-19.

But what alarms is patients who survived COVID-19 disease are at higher risk of post-acute sequelae, which is generally called "long COVID". Long COVID involves kidney also, as evidenced by higher risks for AKI, decline in eGFR (estimated glomerular filtration rate) including steeper longitudinal decline, and development of end-stage renal disease (ESRD) (7). In a study conducted in over 1.7 million healthy and coronavirus-infected US Veterans from March 2020 to March 2021, it was found that relative to non-infected patients, in non-hospitalized COVID-19 patients there was 15% higher risk of a major adverse

kidney event, (e.g., chronic kidney disease, CKD). The risk for AKI was 30% higher and for ESRD, it was 215% higher (8). Surprisingly, the risk factor is not zero in the patients with milder disease. These findings dictate the need for post-acute COVID-19 kidney care and monitoring because the health implications for long haulers are not known.

Pathophysiology of COVID-19-associated Kidney Injury: Although clinical observations and laboratory tests showed higher prevalence of AKI in COVID-19 patients, the exact mechanism by which SARS-CoV-2 virus injures kidney acutely is not clear. Interestingly, despite description of COVID-19 as a cytokine storm syndrome, levels of circulating cytokines are often lower in patients with COVID-19 than in patients with acute respiratory distress syndrome due to causes other than COVID-19 (9). AKI observed in COVID-19 disease can be multifactorial, with predisposing conditions such as sepsis, hypovolemia, and nephrotoxins. Cardiorenal syndrome secondary to COVID-19 pneumonia, causing right ventricular failure might lead to kidney congestion and AKI. Left ventricular dysfunction might lead to low cardiac output and kidney hypoperfusion due to atrial underfilling. Thus, systemic hemodynamic instability coupled with tissue inflammation and local immune cell filtration might contribute to renal tubular injury, as might endothelial injury and microvascular thrombi (9). In this context, a recently published multi-center retrospective cohort study defined the spectrum of kidney pathology in COVID-19 disease as described below (10).

Clinical Study under Focus: An international collaborative multi-center retrospective cohort study was conducted in 30 centers, of which two are in Switzerland, one each in Australia and India, and the rest are in the United States. A total 284 kidney biopsies were evaluated to improve understanding of kidney disease in COVID-19 (10). About 63,575 native biopsies before the pandemic and 13,955 allograft biopsies were used to compare diagnoses with the above biopsies from kidney disease in COVID-19 to identify diseases that have increased due to COVID-19. In parallel, 107 African American and Hispanic patients were genotyped for *APOL1* (apolipoprotein L1) alleles G1 and G2. The presence of SARS-CoV-2 virus in 273 kidney biopsy specimens was detected by immunohisto-chemistry. Clinical information was collected at the time of biopsy. The leading indication for native biopsy was AKI (45.4%), followed by proteinuria with or without concurrent AKI (42.6%). African American patients constituted a major proportion (44.6%) as compared to other ethnicities. The pie charts in Figure 1 show frequencies of diagnosis in COVID-19 kidney biopsies compared to the pre-pandemic biopsied population.

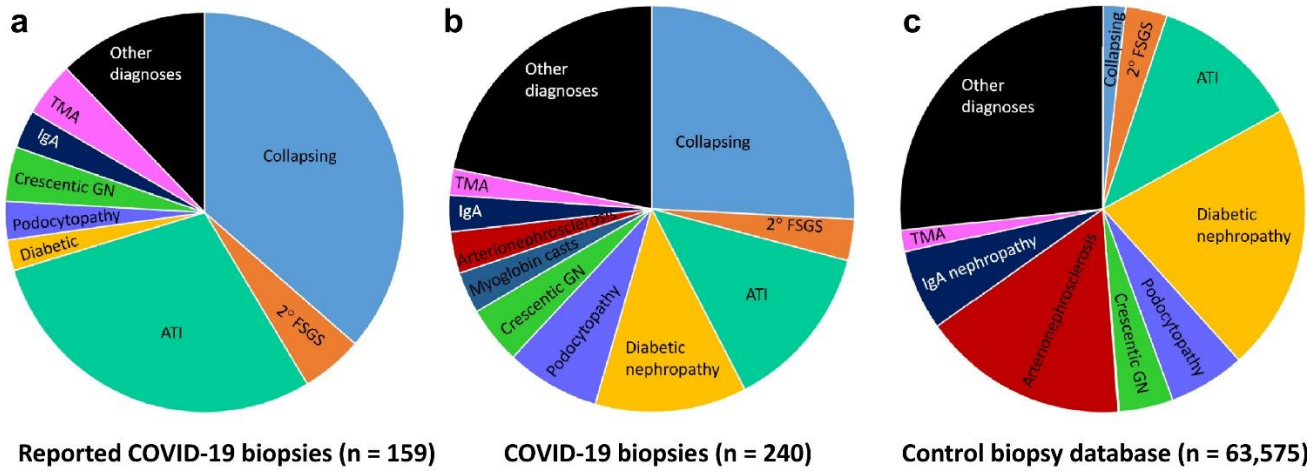

Figure 1: Frequencies of diagnosis in COVID-19 disease kidney biopsies compared to the pre-pandemic biopsied population. **(a)** Frequencies of diagnosis of COVID-19 kidney biopsies reported in the literature (n = 159 patients). **(b)** Frequencies of diagnosis of COVID-19 kidney biopsies in the multi-center cohort study (n = 240 patients). **(c)** Comparison of diagnostic frequencies in the 5-year pre-pandemic biopsy cohort (n = 63,575 patients). ATI, acute tubular injury; FSGS, focal segmental glomerular sclerosis; GN, glomerulonephritis; TMA, thrombotic microangiopathy. Reproduced with permission from May et al, Kidney Int 100:1303-1315, 2021 (10).

As depicted in Figure 1, the most common diagnosis in native biopsies was collapsing glomerulopathy (25.8%), which was associated with high-risk *APOL1* genotype in 91.7% of cases. Compared to the control biopsy data, the frequency of myoglobin cast nephropathy and proliferative glomerulonephritis with monoclonal IgG deposits was increased in patients with COVID-19 (3.3% and 1.7%, respectively). Interestingly, there was a reduced frequency of chronic conditions known to contribute to kidney disease, such as diabetes mellitus, IgA nephropathy, and arterionephrosclerosis as the primary diagnosis. In transplants, the leading indication was AKI (86.4%) for which rejection was the predominant diagnosis (61.4%). Direct SARS-CoV-2 viral infection was not identified. Thus, the multi-center cohort study identified kidney diseases that disproportionately affect COVID-19 patients, and their association with *APOL1* high-risk. But no direct evidence of SARS-CoV-2 infecting the kidney was found.

Discussion: The multi-center retrospective cohort study, which brought out new observations, provided insights into how SARS-CoV-2 viral infection can affect the kidney. We discuss these salient features briefly here.

No Evidence of SARS-CoV-2 Directly Infecting the Kidney: ACE2 (angiotensin converting enzyme-2), the cell surface receptor for SARS-CoV and SARS-CoV-2 viruses is heavily expressed on the brush border of kidney proximal tubules and to a lesser extent on the podocytes of glomeruli (11, 12). ACE2 has physiological roles in the kidney that are beneficial for vascular function and salt and water homeostasis. Previous data from autopsies showed endothelial damage in the kidney with presence of viral-like particles in the endothelium (13). Autopsies also revealed diffuse proximal tubule injury with loss of brush border, non-isometric vacuolar degeneration, and even frank necrosis (14), and collapsing glomerulopathy with leakage of protein into Bowman's capsule (15). Based on these autopsy observations, it has been postulated that SARS-CoV-2 may induce AKI through direct viral infection. However, in the current multi-center cohort study evaluation of biopsies for direct viral infection using immunohistochemistry backed by in situ hybridization as a reflex test did not reveal the presence of virus in the kidney samples. The previously reported virus-particles in electron microscopic examination of autopsy specimens of kidney might be due to morphologic mimics of various organelles, such as secretory vesicles, multivesicular bodies, exosomes, coatomer-coated vesicles, and clathrin-coated vesicles. Thus, it appears the renal pathology observed in biopsy specimens from COVID-19 patients is not due to direct infection with the virus.

Association of AKI with APOL1 Genomic Risk Alleles: The multi-center cohort study showed 2.9-fold increase in the proportion of African Americans as compared to representation of this group in general population within the United States (44.6% vs. 15.4%). This is in line with the known fact that African Americans face a disprop ortionately higher burden of COVID-19 infection leading to AKI as compared to other ethnic populations. The incidence of SARS-CoV-2 infection in African Americans is nearly 3 times that in Caucasians (16). There are several factors for this higher incidence, such as geographic location of dwellings in urban areas with hotspots for SARS-CoV-2 infection, higher prevalence of comorbid conditions contributing to kidney dysfunction (diabetes mellitus, hypertension, obesity etc.). However, the multi-center cohort study revealed that the most important factor contributing to disproportionately higher prevalence of AKI in African Americans is likely related to *APOL1* gene risk alleles (17, 18). Since 14% of all African Americans and 1% of Hispanics carry a high-risk *APOL1* genotype (19), genotyping was done for these two populations in the multi-center cohort study. Genotyping revealed that a whopping 60.7% of kidney biopsies were from African Americans with a high-risk *APOL1* genotype, i.e., greater than 4 times as many as in the general African American population. Thus, it appears that the *APOL1* genomic risk allele is responsible for higher incidence of AKI in African Americans with COVID-19 disease. Our understanding of susceptibility to non-diabetic kidney disease in African Americans advanced substantially with the discovery of *APOL1* gene association (18, 20). Two renal risk variants of *APOL1* (G1 and G2) coding regions are strongly associated with several progressive kidney diseases in African Americans than in patients of other races. Apparently, these genetic variants arose 10,000 years ago in Sub-Saharan Africa, where they afforded protection against *Trypanosoma brucei rhodesiense*, known to cause African sleeping sickness.

Emergence of COVAN: Similar to the observation in HIV patients, the frequency of diagnosis of collapsing glomerulopathy in COVID-19 patients was markedly increased from that in the general population undergoing kidney biopsy (Figure 1). Reports of increased prevalence of collapsing glomeruopathy in patients of African ancestry

associated with high-risk *APOL1* genotype infected with SARS-CoV-2 has been termed COVAN (COVID-19 associated nephropathy) (21). This new entity may particularly affect individuals in certain regions of the world, and the physician community needs to know this potential complication of COVID-19. Similar kidney condition in HIV patients was previous named as HIVAN (HIV-associated nephropathy).

Increase in Myoglobin Cast Nephropathy: Biopsy data from the multi-center cohort study also revealed a small, but significant increase in the diagnosis of myoglobin cast nephropathy in patients with COVID-19. Rhabdomyolysis in COVID-19 can be due to necrotizing myopathy. Since rhabdomyolysis is often a late complication of SARS-CoV-2 infection (22), but can present before the development of AKI, measurement of plasma creatine kinase level on admission could lead to early recognition of rhabdomyolysis, so hydration therapy can be initiated to prevent AKI in such patients.

Conclusion: Despite some limitations in the study, such as lack of longitudinal clinical follow up, and detailed ultrastructural evaluation, which the authors acknowledged, this multi-center retrospective cohort study provided significant insights into the kidney disease due to COVID-19. Besides providing histopathological diagnoses of the kidney disease, this study pointed to the association of *APOL1* gene variant with higher incidence of kidney disease in COVID-19 in African Americans. The study also identified the emerging COVAN entity of kidney disease in COVID-19 and provided a window of opportunity to prevent AKI in COVID-19 due to rhabdomyolysis. Further studies should unravel more valuable clinico-pathological data which will help to prevent or reduce deaths due kidney disease in COVID-19.

Disclosure: One center participated in the multi-center retrospective cohort study described in this commentary is Division of Nephrology and Hypertension, University of Utah Health, Salt Lake City, Utah, in which the author is an Adjunct Professor. However, the author did not participate in the cohort study. The author is Co-Founder, President, CEO and CSO of ePurines, Inc., a drug development startup working on obesity, metabolic syndrome, and primary liver and kidney diseases. Author declares this commentary has been prepared with no industry or commercial support, and in the capacity of Adjunct Faculty at the University of Utah Health.

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SANKARA NETHRALAYA USA

Sankara Nethralaya OM Trust Inc., was established in June 1988 in Rockville, Maryland, USA as a 501 (c) (3) non-profit organization. This organization d/b/a 'Sankara Nethralaya USA' –is A 30-year-old Indian American saga of bringing back vision to those who cannot afford to 'See' the World. The office is located at 9710 Traville Gateway Drive No.392, Rockville, MD 20850, (855) 4NETHRA - Toll Free. Tax ID # 52-1611548

Visit our website: www.sankaranethralayausa.org

Sankara Nethralaya – Temple of Eye

Inspired by a speech by Pontiff of Kanchi Kamakoti Mutt he attended in 1974, Dr. S S Badrinath founded Sankara Nethralaya in Chennai in 1978 with the sole objective of providing world class eye care free to the poor and affordable price to others. Nethralaya also holds eye camps, school screenings and eye bank. One of the unique service is Mobile Eye Surgical Unit (MESU), only one of its kind approved by Government of India. These are operation theatres on wheels. MESU consists of two buses serving as operation theatre, go out remote villages, perform cataract surgeries on 150 to 200 patients over a two-week period and return to main campus after post-operative care.

Nethralaya has five branches in Tamil Nadu, one in Andhra Pradesh and three in West Bengal. Over the years, Nethralaya treated over a million patients from all over India, and neighboring countries such as Bangladesh, Sri Lanka, Nepal, Pakistan, Oman, Mauritius, and Saudi Arabia. Daily, they treat 3,000 outpatients and perform 300 surgeries. Fifty percent of outpatients are treated free, and thirty-five percent of the surgeries are performed at no cost to indigent patients.

Sankara Nethralaya USA (SN USA), our fund-raising arm in US was established in 1988 with the sole objective of raising funds for Nethralaya to provide free services to indigent patients. SN USA is managed by thirty-five honorary trustees spread across fourteen chapters in US and has been raising over a million dollars during the past several years.

Nethralaya professionals were awarded with numerous awards for their outstanding accomplishments. Our founder Dr. S S Badrinath was given the prestigious awards of Living Legend and Padma Bhushan for his outstanding public service. Newsweek named Sankara Nethralaya as one of the four Best Ophthalmic institutions and one of the Best one hundred hospitals in the World. SN USA, our fund-raising arm in US was given the given the highest rating of four stars by prestigious rating organization Charity Navigator.

For additional information and to support our cause, please visit us at www.sankaranethralaya.org and www.sankaranethralayausa.org.

With best regards,

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Portrait of Hippocrates (1787), by the Majorat of Setúbal.
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Science is the father of knowledge, but opinion breeds ignorance.
- Hippocrates