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On the Cover

"Cells at Work"
by Abeeshan Selvabaskaran.

This cover represents the vastness that encompasses this field of study and how a single identifier: the cell, can unify so many different areas of study. In the background are icons representing several disciplines (microbiology, cell and systems biology, oncology, physiology, neuroscience, etc.) to showcase the diversity within the life sciences.

Staff List

Letter *from the* Editors

Dear readers,

It is our great pleasure that we present to you the 13th issue of the Journal of Undergraduate Life Sciences (JULS). As a discipline, the life sciences encompass a broad scope of basic, applied, and interdisciplinary fields. While these specialties differ significantly in direction, they are unified by a common rigor and curiosity towards the study of life. In our 13th issue, we are proud to continue our publication of outstanding undergraduate research reflective of this common pursuit.

This year, our cover features original artwork by UofT Physiology and Immunology student Abeeshan Selvabaskaran. In a piece entitled "Cells at Work", Selvabaskaran depicts the humble cell, a single identifier which unifies many areas of scientific inquiry. From microbiology to neuroscience, the study of the cell showcases diversity and variety with the life sciences. For us at JULS, this variety also applies to the undergraduate community, with a range of interests, experiences, and career paths. Thus, JULS continues its commitment as a research platform made and accessible for students of all scientific backgrounds.

In this issue, we highlight such diversity through interviews with five members of the UofT biomedical community. In particular, three of them discuss the growth of AI in medicine, and their own research regarding its uses and implications. We also feature the conference proceeding from the 2018 Medicine by Design Symposium, a collaborative initiative of regenerative medicine and cell therapy researchers. Finally, we present original primary research and review articles by UofT undergraduate students, with topics ranging from medical education to microbubble gene therapy.

Like any modern scientific study, JULS is the product of extensive collaboration. As the 2018-2019 cycle comes to a close, we first extend our thanks to the JULS editorial staff, whose tireless contributions made this issue possible. We also thank our faculty reviewers and departmental contacts, who provided scientific and academic support for our operation. Most of all, we present our utmost gratitude to the undergraduate research community, who continue to publish and provide readership for the journal. Thank you – JULS would not be possible without your continued support.

Sincerely,

Michael Lee and Yun Kim
Co-Editors-in-Chief, 2018-2019

More than just the flu: Influenza-associated myocardial infarction and its recent revelations.

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[1]University of Toronto, Canada

Approximately 12,200 Canadians are hospitalized with seasonal influenza virus annually, posing a mounting public health issue that has become the epicentre of community-based vaccination campaigns. However, this public health issue has become exacerbated by the recent emergence of literature investigating the association between influenza infection and myocardial infarction, commonly known as heart attack. In other words, does contracting the flu make one more likely to experience a heart attack? This is the question at the heart of previous epidemiological and correlational studies due to the far-reaching clinical implications with respect to vaccination programs. However, previous research on this subject presented a common limitation regarding the lack of standardized assessment of influenza infection. With methodologies and selection criteria differing between studies, the presence and extent of any association between influenza infection and myocardial infarction has been tentative at best. In recognizing these limitations and appreciating the clinical importance of this issue, Dr. Jeffrey C. Kwong and colleagues set out to characterize the precise nature of this association by employing a methodologically robust design. Their findings were included in the article "Acute Myocardial Infarction after Laboratory-Confirmed Influenza Infection", published in The New England Journal of Medicine in January 2018.

The authors utilized a self-controlled case-series design to investigate the association between influenza infection and myocardial infarction. The patients' respiratory specimens were tested for influenza and other viruses, while the data for acute myocardial infarction were included based on the diagnosis outlined in the International Classification of Disease (ICD-10). The observation period ranged from one year before to one year after the respiratory samples were obtained (termed the index date), and patients who had at least one instance of acute myocardial infarction during this period were included in the data. A risk interval and control interval were established, with the former extending to the first week following the index date and the latter extending to the remaining fifty-one weeks.

Dr. Kwong and colleagues found that the incidence of hospital admissions for acute myocardial infarction was six times higher during the week that followed a laboratory-confirmed diagnosis of influenza than it was during the control interval. This result demonstrated a significant association between influenza infection and subsequent myocardial infarction.

In deciding to delve deeper into the topics discussed, we spoke directly with the authors of the paper. We wanted to understand the article with regards to its potential impact on clinical practice as well as parse out the ideas of the researchers in their own words. Here we interview a few notable experts in the field. First, we spoke with Dr. Jeffrey C. Kwong, a senior scientist at the Institute for Clinical Evaluative Sciences (ICES), family physician, and first author of the article in question.

Q: What was the motivation for studying and researching the subject?

JK: For some time, it has been known that influenza is linked with cardiovascular outcomes, for example, during the influenza season, more people die from heart attacks. So, we just wanted to confirm this association in order to show that influenza truly is associated with myocardial infarction—the idea being that if influenza infections truly are associated with heart attacks, [we would be able to prevent these] heart attacks with flu shots. [Specifically], we used serology to [identify] influenza infection and also used a self-controlled case series design, which greatly reduces bias.

Q: We were wondering whether you thought that influenza will display similar associations with diseases other than cardiovascular disease (CVD). Or do you think this association is specific to CVD due to underlying mechanisms?

JK: That's a very good question! Outcomes we were planning to look at include stroke, renal disease, and others. We're planning to conduct studies on these in the future; we just haven't gotten to that yet.

Q: So, why do you think that influenza may be associated with these other diseases, is there a particular mechanism you're interested in investigating?

JK: You know, I think influenza can cause a lot of different problems in the body such as making people sick, causing inflammation and can even lead to death for people who have [pre-existing] chronic diseases. We know people with chronic conditions are at higher risk for complications if they do become infected with influenza, and so it might be a good idea just to show how strong this association is with other diseases.

Q: Do you think further research would be warranted in terms of examining specific CVD risk factors (e.g. diabetes) so that we could tease apart the individual associations between influenza and these risk factors?

JK: Yeah! I think it's a good idea because people would be able to understand who is at higher risk for complications if they are infected with influenza. Hopefully this would give patients more motivation to get vaccinated against influenza.

Q: It was also noted that there was increased incidence of myocardial infarction after influenza infection despite vaccination and it was stated that this was probably because the vaccinations were only 40-60% effective for adults in preventing laboratory-confirmed influenza. On this note, we found another article suggesting high-dose vaccinations [as a remedy to this problem]. Do you think high-dose vaccines would be effective for the CVD patient population that may be older and may exhibit decreased immune responsiveness to regular-dose vaccines?

JK: Yeah, so there's this big randomized controlled trial going on right now asking this very question! So what they are doing is they are recruiting patients who are at risk for myocardial infarction and who have already had a cardiovascular event, and then they've been randomizing [these patients] to high-dose versus standard-dose vaccines. We do have good evidence that the high-dose vaccine is more effective than the standard-dose vaccines in preventing influenza infection, with the idea being that if we can prevent infection, we can prevent myocardial infarctions from occurring.

Q: In terms of tailoring this information to patient population in clinical practice, what are your thoughts on using SQL (Structured Query Language) computerized queries to create a database of patients with CVD risk factors and follow up with these high-risk patients for vaccinations?

JK: Yeah, I think that's a great idea and I think that's definitely possible. Nowadays, many physicians use electronic medical records, and I think it would be smart to use this in order to notify and contact patients.

Q: What are your thoughts on community-based outreach programs that are organized to raise awareness and encourage patients with CVD and CVD risk factors to get their influenza vaccinations? Would these programs be effective?

JK: It's really hard to say. It is incredibly difficult to convince people to get their flu shots in the first place let alone every year. There's this huge misperception out there that influenza vaccines don't work, that it's not a big deal, and concerns about the safety of the vaccine. There are many misperceptions to overcome. We only vaccinate about one-third of our population and even in high-risk groups we're not doing as well as we'd ideally want to be.

Q: Based on your research findings, do you think we should take more care in treating patients with CVD or CVD risk factors when they are in a hospital setting due to an elevated risk of catching the flu while in the hospital environment?

JK: That's a huge problem. We haven't been able to get high coverage of healthcare workers [with respect to their vaccination]. We have patients who are in the hospital for one reason, and then they get influenza and end up with something else on top of whatever else they came in with, so this is definitely an issue that we have not been entirely successful in addressing. [But] that's a whole other can of worms!

Q: Aside from seasonal influenza, we know that pandemic influenza can be a potentially devastating public health crisis. Do you think a similar relationship would hold between pandemic influenza and myocardial infarction?

JK: It wouldn't surprise me at all if we saw the same association. Pandemic influenza is usually seen more in younger adults, and as we saw in the 2009 pandemic, the virus did not seem to infect as many older people. This would be something known as a 'Signature Pandemic' where we see more disease in younger adults when normally for influenza we'd expect the majority of the burden to be with very young children and older adults.

Q: We understand that there is likely to be numerous confounding factors (e.g. comorbidities) when conducting research with patients, which can make it hard to isolate any effect and can limit the generalizability of such studies. How have you mitigated this issue?

JK: The nice thing about the self-controlled case series design is that it eliminates confounders. In this design we use each person as their own control; everyone in the study has had laboratory-confirmed influenza and myocardial infarction and so what we did was look at the timing between the myocardial infarction event relative to the laboratory confirmation of their influenza infection.

Q: Do you have any final comments regarding future research endeavours on this subject?

JK: The question I have is whether milder infections are worth looking into. [I'm interested in looking at] the association between milder influenza infections and myocardial infarction with the hopes of determining whether [someone with the average symptom profile] would also have an increased [risk] of myocardial infarction after infection.

We also got in touch with Dr. Kevin L. Schwartz, a paediatric infectious diseases physician, ICES adjunct scientist, and second author of the article, and he provided his thoughts on the implications of this research.

Q: What are the implications of your research findings?

KS: They demonstrated a strong association between confirmed

influenza infection and acute myocardial infarction. This association has important implications for the benefits of preventing influenza infection in vulnerable individuals which should include annual immunization, hand washing, and other important infection control measures that can reduce transmission.

Q: In your experience, has the management of this issue changed since your research has been published? Do you believe it is being addressed appropriately?

KS: I am not aware of data on immunization coverage this year, but this will be important to look at going forward. There are multiple factors that motivate the public to get vaccinated (or not) for influenza, and [it] would be hard to attribute any change directly to our study. However, I think it has answered an important research question using a more robust design.

Q: How might your research be used to inform physicians and their training? What changes would you like to see in clinical practice?

KS: It is important for physicians-in-training to consider the pathophysiology of diseases and this is a good example of how understanding the effects of influenza infection on a patient can manifest in a variety of ways including acute myocardial infarction. Physicians should be aware of the risks associated with influenza infection, beyond a respiratory illness. All physicians should be recommending influenza immunization yearly to their patients, particularly those at risk of cardiovascular events.

Q: In your opinion, is further research on this issue warranted? What direction would you like to see this research take in the future?

KS: I think there is need for future research evaluating the effects of both influenza infection and influenza immunization on downstream health outcomes in patients. Innovative ways for knowledge translation are needed to optimize vaccination rates. The self-controlled design is a potentially powerful design for answering questions related to rare events and those susceptible to time-invariant confounding. In this study we observed a relationship with other non-influenza respiratory infections and acute myocardial infarction as well, which deserves further attention and consideration for vaccine development for other respiratory viruses.

With the knowledge of there being an association between influenza infection and myocardial infarction, it becomes clinically relevant to investigate the role of influenza vaccination in preventing major cardiovascular events like myocardial infarction. Furthermore, for patients that are particularly vulnerable in terms of a decreased immune response to vaccines, it would be important to examine the potential advantages concerning the efficacy of high-dose vaccines. For more information on this related topic, we spoke with Dr. Jacob A. Udell who is a cardiologist, a scientist at Women's College Research Institute, and an author of a recent article on the efficacy of high-dose influenza vaccines for high-risk CVD patients.

Q: From your perspective, what was the motivation for studying this subject regarding your most recent article on the use of high-dose influenza vaccines to improve clinical outcomes in high-risk CVD patients?

JU: I thought it would be interesting to interject into any clinical study, the opportunity to study any public health intervention, in this case vaccination, to reduce the impact of heart disease in Canadians and internationally. We were looking for the right fit, and it ended up at the time when I was training in cardiology and looking at opportunities, a lot of the research was focusing on small studies that were starting to report on the role of flu shots as a cardioprotective mechanism in patients with coronary artery disease. So that was the motivation for it, trying to do something big and important that could have a lot of impact at a very low cost.

Q: After reviewing other articles on the subject, we found that vaccination isn't a complete safeguard with statistics indicating only 40-60% efficacy for adults in preventing laboratory-confirmed influenza infection. Was this the rationale behind studying the potential benefits of high-dose vaccines?

JU: Yes, so that's correct, the flu shot in any one year may not be effective for vulnerable patient populations including those who are older, those afflicted with chronic conditions such as heart disease or exhibit risk factors for heart disease, kidney disease and/or diabetes. However, there is this one vaccine on the market [called the Fluzone High-Dose vaccine] that is four times more concentrated than the flu shot, covering three strains of flu. [This vaccine] has been tested in healthy community adults (some of which have a history of heart disease but not active heart disease), and it was shown that the higher dose flu shot boosted the antiviral response. Now in our study, we're targeting [CVD] patients who [are at greatest risk of decreased response to a flu shot], maybe because they're 65 and older or have a number of comorbidities for heart and lung outcomes, [and as such, these patients] might be better off with a higher dosage flu shot.

With research indicating a link between acute influenza infection and increased incidence of cardiovascular events for people with CVD risk factors, it is imperative that the implications of such findings are discussed in both clinical and community-based contexts. Specifically, it may be wise for primary care physicians to implement computerized queries that efficiently generate lists of patients with CVD risk factors who have yet to receive a flu vaccination. Following up with these patients to set an appointment for vaccination would then serve as a preventative measure for both influenza infection and myocardial infarction. Furthermore, the use of high-dose vaccines for these high-risk patients, as explored in Dr. Udell's research, has proved to be effective since their

immune response to regular-dose vaccines is often compromised. Another concern is that patients with cardiovascular conditions, while receiving care in hospitals, would have to be carefully monitored to prevent contraction of hospital-sourced influenza. This scenario could otherwise spiral out of control by increasing the risk of myocardial infarction and potentially exacerbating these patients' conditions.

Despite the insights gleaned from Dr. Kwong, Dr. Schwartz, and Dr. Udell, further research is still warranted in the realm of investigating the association between milder influenza infection and myocardial infarction as well as the interaction between influenza infection and different CVD risk factors. Presently, the current literature provides an impetus for promoting awareness of the intimate link between influenza and myocardial infarction for patients at elevated risk of CVD through community-based programs. These programs would ideally advocate for patients by promoting accessible, preventative strategies to combat contraction of influenza and by advancing efforts directed towards increasing vaccination rates more broadly.

The authors would like to acknowledge the contributions of: Dr. Larry Sawchuk, PhD; Dr. Jeff Kwong, MD MSc CCFP FRCPC; Dr. Kevin Schwartz, MD MSc FRCPC; Dr. Jacob A. Udell, MD MPH FRCPC.

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Innovations in Cell & Gene Therapy, 3rd Medicine by Design Symposium

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Medicine by Design, a group of regenerative medicine and cell therapy researchers at the University of Toronto (U of T), hosted its 3rd annual symposium at the MaRS Discovery District Auditorium on December 4th, 2018. Twelve researchers from local and international institutions presented their research on muscle, gene, neural, and immune engineering.

Commencing the session on muscle engineering, Dr. Bénédicte Chazaud (Université Claude Bernard Lyon) discussed the role of macrophages in skeletal muscle repair and disease. Macrophages are most notably involved in destroying pathogens, but are also crucial in regeneration, stimulating muscle stem cell proliferation and differentiation. Dr. Chauzaud highlighted recent work on Duchenne muscular dystrophy (DMD) drug treatments, where macrophage behaviour was manipulated to prevent fibrosis and improve muscle function in DMD models. Regarding heart function, Dr. Stephanie Protze (University Health Network – UHN) introduced the use of human pluripotent stem cells (hPSC) to generate sinoatrial node pacemaker cells for patients with abnormal heart rhythms. This technique exposes hPSC-derived cardiomyocytes to specific signaling molecules, triggering their differentiation to sinoatrial node pacemaker cells. If clinical trials are successful, this personalized approach may evade complications of electronic pacemaker implantation. For patients experiencing heart failure, Dr. Michael Laflamme (UHN) presented his work on myocardial regeneration. When a heart attack occurs, healthy myocardium is replaced with non-contractile scar tissue, limiting the heart's contractility and leading to eventual failure. Dr. Laflamme's research explores two therapeutic possibilities: synthesizing new myocardium from pluripotent stem cells and converting scar tissue into healthy myocardium. Both options aim to improve heart function after a heart attack without the need for a transplant.

Transitioning into gene engineering, Dr. Rudolf Jaenisch (Massachusetts Institute of Technology) discussed epigenetic regulation in development, aging and disease. Since epigenetics affect gene expression through covalent modification of DNA or its histones, Dr. Jaenisch examined the need for a dynamic reporter to track individual cells' DNA methylation status. He additionally presented his work employing a modified CRISPR/Cas9 system to treat epigenetic disorders like Fragile X Syndrome. Dr. Laura Prochazka (U of T) presented work

on the development of synthetic gene circuits to direct hPSC differentiation. She utilizes miRNA-based gene circuits which combine sensory input, computational processing, and physiological output to alter gene expression. Compared to traditional approaches, gene circuits are inexpensive and provide an internal control system that is less dependent on the environment. Dr. Prochazka is currently working on implementing multi-inputs/outputs circuits to optimize the efficiency of induced stem cell differentiation. Closing the session, Dr. Jennifer Mitchell (U of T) discussed her research examining stem cell gene regulation by identifying transcription enhancers. Her work is focused on *Sox2*, a gene known to regulate neural stem cell differentiation and maintenance. *Sox2* was recently found to be regulated by the *Sox2* Control Region (SCR) enhancer, where mutations in the SCR impair neuronal differentiation. Her research may elucidate stem cell manipulations for medical applications.

Following a break, Dr. Lorenz Studer (Memorial Sloan Kettering Cancer Centre) introduced the theme of neural engineering, presenting his studies on neural crest development and hPSC therapy for treating nervous system diseases. He explained how genetically-manipulated cells derived from stem cell precursors can be used to treat Hirschsprung's disease, which causes impaired neural cell migration. Dr. Studer demonstrated the efficacy of this therapy in chick and mouse

models, where migratory behaviour was restored with direct cell injections. Dr. Yun Li (SickKids Hospital) discussed brain organoids (3D cell cultures) as a model for in vivo human-specific cortical development. Using organoids, her studies of ventricular formation and neuronal emergence involve genetic manipulation to enhance cortical growth and folding. For example, Dr. Li demonstrated Zika virus infections result in microcephaly and prevent normal cortical development. To conclude the session, Dr. Alain Dabdoub (Sunnybrook Research Institute) addressed auditory neuron regeneration. Currently, auditory neuron damage leads to permanent hearing loss. However, cochlear cells can be endogenously reprogrammed with transcriptional activators that turn on genes expressed in auditory neurons. *In vivo*, adenoviruses were applied to mouse models to introduce neuronal differentiation genes. Ultimately, Dr. Dabdoub aims to apply these techniques to human models.

As an interlude, Dr. Alán Aspuru-Guzik (U of T) highlighted the use of artificial intelligence and automation in current science. He discussed two major developments from his lab: 1) machine learning algorithms that can accelerate the process of drug candidate generation; and 2) a software, ChemOS, that can be combined with robotic platforms to create self-driven laboratories. Overall, Dr. Aspuru-Guzik hinted that these technologies can revolutionize the technique and efficiency of scientific discovery.

Opening the final session on immune engineering, Dr. Yvonne Chen (University of California, Los Angeles) presented her work on immune cell therapy for cancer treatment. This involves manipulating existing immune cell receptors and engineering new ones to improve tumour targeting. Although this technique allows for personalized treatments, it may miss some tumour cells or target healthy cells. To prevent the former, Dr. Chen described a specifically engineered immune receptor that recognizes a wider range of tumour cells. To address the latter, Dr. Chen described another engineered receptor which activates only under specific multi-component target recognition. Dr. Sarah Crome (UHN) concluded the conference with a discussion on characterizing novel innate immune cells for immunotherapy. In tumours, these cells negatively impact immune response and shorten relapse time. As demonstrated in mouse models for autoimmune disease, these cells can control harmful inflammation and may stimulate tissue regeneration. Dr. Crome highlighted the importance of cell characterizations for future clinical applications, such as targeting immune cells to enhance immune tolerance, which could reduce transplant rejection and autoimmune disease risks.

In all, the 3rd Medicine by Design Symposium on cell and gene therapy served as a platform to share innovative ideas among the regenerative medicine community and to inspire the next generation of scientists. Congratulations to all the speakers for presenting their exciting findings, and to Medicine by Design for the hosting of a successful conference.

Acknowledgements

A special thank you to Dr. Ashley Bruce and Dr. Jennifer Mitchell from the University of Toronto for organizing the Disciplinary Focus in Stem Cells and Developmental Biology, which allowed us to attend this conference. Additional thanks to the other members in the focus who helped us with background research and preparation for attending the conference: Taylor Barbieri, Phoebe Bhagoutie, Yi Peng Chang, Laili Jing, Felicia Liu, Miranda Li, Olivia Mazzurco, Erika McCartney, Iyeh Mohammadi, Jevithen Nehru, Komal Parmar, Karinna Pe, Foram Vyas, Samantha Yang.

JULS REVIEWS

Integrin $\alpha_{IIb}\beta_3$ in Cardiovascular Thrombotic Events

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Abstract

Integrin signalling mediates several intercellular events in order to maintain hemostasis, including platelet adhesion and aggregation during the process of coagulation. Evolutionary pressures have directed the mechanisms which underpin coagulation to develop in a highly-regulated way, as both hypo- and hyper-coagulable states are incompatible with life. This review focuses on our current understanding of the "inside-out" and "outside-in" signalling events that mediate integrin activation and suppression in order to allow for appropriate aggregation of platelets following vascular trauma. In addition, it characterizes the most abundant integrin receptor, $\alpha_{IIb}\beta_3$, and the important biological roles this receptor plays in the context of cardiovascular thrombotic events.

Hemostatic Mechanisms

The process of hemostasis is carefully regulated by a variety of factors in the blood which become active following vascular trauma1. Exposed extracellular matrix molecules beneath vessel walls, in addition to factors released directly from the damaged endothelial cells, initiate a coagulation cascade that results in the activation of the protease thrombin. Activated thrombin acts enzymatically on circulating fibrinogen to produce polymerized fibrin [2, 3]. This fibrous protein forms crosslinks by transglutaminase factor XIII to provide a scaffold for blood clotting [4, 5]. Simultaneously, circulating platelets produced from megakaryocytes in bone marrow become chemically activated through exposure to markers of tissue damage not encountered in healthy vessels, e.g. collagen proteins, and both adhere to the site of damage and aggregate with each other [6, 7]. The interaction of activated platelets and fibrin initiates a further cascade of signalling events within platelets, encouraging further hemostatic function, and a blood clot or thrombus is formed to repair the damaged vessel wall [8, 9].

While platelet adhesion and aggregation are essential for hemostatic damage repair, excessive aggregation is considered pathological when a 'mural' thrombus binds to a vessel and decreases blood flow or an 'occlusive' thrombus entirely occludes a vessel [10, 11]. A thrombus can remain wedged in the vessel in which it formed, or it can detach from the vessel wall to become a free-flowing embolus. Emboli travel within the blood stream until they lodge within narrower vessels where they can cause ischemia, similar to mural or occlusive thrombi [12]. This can result in tissue damage and can lead to serious cardiovascular complications such as myocardial infarction or stroke [13, 14].

Platelet adhesion and aggregation events are mediated by integrins, heterodimeric adhesion receptors composed of one alpha (α) and one beta (β) subunit which each have unique cellular roles [15]. Platelets first form weak interactions with a damaged site via glycoprotein surface receptors, and then integrin $\alpha_{II}\beta_1$ binds collagen (largely type I and type III collagen) and other molecules in the vessel wall to mediate more robust adhesion [6]. Next, integrin $\alpha_{IIb}\beta_3$ makes connections with fibrinogen to initiate platelet aggregation [2]. Integrins are therefore promising targets for the pharmacological regulation of coagulation in clinical circumstances where it may be beneficial to up- or down-regulate platelet adhesion. This is particularly relevant in the context of patients suffering from trauma requiring massive blood transfusion, or those in genetic or iatrogenic hypercoagulable states.

The process of hemostasis must be tightly-controlled to allow for blood clotting in response to tissue damage while simultaneously preventing thrombus formation in healthy vessels. Integrin activity is therefore carefully regulated by a series of complex signalling events. Integrins act as bi-directional signalling molecules, first becoming activated by "inside-out" signalling events where intracellular signalling cascades initiated by other cellular receptors evoke changes in integrin structure, and then mediating "outside-in" signalling where the binding of extracellular matrix factors to activated integrin receptors stimulates intracellular signal transduction pathways that can regulate intracellular activity [16, 17]. Integrin receptor binding partners include a wide variety of both cytosolic and membrane-bound proteins with several structural and cytoskeletal roles [18]. $\alpha_{IIb}\beta_3$ is abundant in the platelet membrane and crucial in the aggregation events that govern both vascular damage repair and thrombus formation [19]. For this reason, an understanding of the precise activities of $\alpha_{IIb}\beta_3$ in activated platelets is critical in the study and treatment of cardiovascular thrombotic events.

Platelet Activation

Vascular injury exposures molecules in the subendothelium, such as von Willebrand factor (vWF) and collagen fibres, allowing platelets travelling in the bloodstream to bind to these proteins with membrane surface receptors GPIb and GPVI, respectively [10]. In addition to these two binding events, platelet adhesion is also regulated by the collagen-binding integrin $\alpha_{II}\beta_I$ [6, 20]. These adhesion events initiate platelet activation, during which intracellular molecular switches including small GTPases, regulate a variety of cellular activities [2]. Specifically, the GTP-binding proteins RhoA and Rac1 regulate cytoskeletal reorganization leading to platelet shape change, Rab27 and Ral control the secretion of platelet granules, and Rap1B and RhoA regulate platelet aggregation [2, 21]. Atomic force microscopy has allowed for detailed illustration of these morphological changes accompanying platelet activation, primarily characterized by the surrogate measure of filopodial growth which represents remodelling of the actin cytoskeleton [3, 22].

Adherent platelets undergo a process of exocytosis wherein dense intracellular granules merge with the cell membrane in order to expose transmembrane P-Selectin proteins to the extracellular space and release several cell products, including adenosine disphosphate (ADP), α-granules containing factor V, fibrinogen, vWF, thrombospondin, and α_2-antiplasmin, into the extracellular matrix [2, 4, 23]. These soluble factors, in concert with thrombin and thromboxane A2 produced by activated platelets, act through their receptors to increase platelet activity through a positive feedback loop and initiate an intracellular signalling cascade leading to the activation of integrin $\alpha_{IIb}\beta_3$ [24]. The precise characterization of activated platelets has traditionally been ambiguous, but attempts have been made to divide them into more well-defined subpopulations. Recently, a novel transglutaminase peptide substrate was used to identify and characterize such a subpopulation of transglutaminase-active platelets [4, 25]. While the precise roles of each subpopulation of platelets have yet to be elucidated, activated platelets as a whole are credited with integrin $\alpha_{IIb}\beta_3$-mediated aggregation in vascular repair and thrombosis. Future work in this area can be expected to elucidate specific hemostatic activities that subpopulations of platelets are responsible for, creating novel treatments of coagulopathic diseases.

Integrin αIIbβ3

The integrin $\alpha_{IIb}\beta_3$ is a heterodimer composed of transmembrane subunits α_{IIb} and β_3 subunit, which each traverse the cell membrane once. It is the most abundant receptor on the platelet surface at 50,000-80,000 copies per cell19,24. The literature has been controversial on the structure of $\alpha_{IIb}\beta_3$ in its inactive state, but many discussions of structure focus on a "switchblade hypothesis" with three core tenants: the inactive receptor is bent and extends with activation, the head region points towards to platelet membrane, and extension is achieved when the α_{IIb} and β_3 subunits' cytosolic domains separate and destabilize the interfaces of the extracellular domains in order to expose a ligand binding site [19, 26]. Recent three-dimensional reconstructions of $\alpha_{IIb}\beta_3$ using synthetic nanodisc lipid bilayers have refined this model through observation of the head region of inactive $\alpha_{IIb}\beta_3$ pointing outwards from the platelet surface [27]. The cytosolic legs of α_{IIb} are bent, while those of β_3 are freely coiled [27].

$\alpha_{IIb}\beta_3$ has high affinity for fibrinogen, as well as Arg-Gly-Asp (RGD) peptide sequences [3]. Fibrinogen has binding sites for $\alpha_{IIb}\beta_3$ in both its α and β chains, and so it is expected to link aggregating platelets via their $\alpha_{IIb}\beta_3$ integrins, which can each bind only one fibrinogen molecule [24]. Giant Unilamellar Vesicles (GUVs) are cell-sized lipid vesicles that are a useful model for the study of reconstituted integrin because, unlike comparable methods, they do not leave a space between the substrate and the lower bilayer leaflet that could impact protein diffusion rates. GUVs were used to determine that the diffusion of active $\alpha_{IIb}\beta_3$ bound by fibrinogen is slowed [24]. Based on the magnitude of diffusion diminution, active $\alpha_{IIb}\beta_3$ likely undergoes intracellular clustering induced by ligand binding24. This report of $\alpha_{IIb}\beta_3$ clustering is supported by new studies exploring integrin activity using immunohistochemical staining techniques [17, 28]. The aggregation of platelets in the blood stream in the absence of injury would threaten hemostasis, and for that reason integrins exist in a low-affinity basal state and shift into a high-affinity active state (called integrin activation, priming, or "inside-out" signalling) when contact with a wounded vessel is made [10, 17].

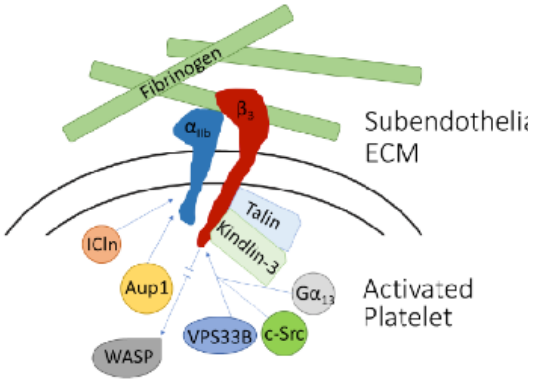

Figure 1. Summary of αIIbβ3 interactions. A representation of the binding interactions between platelet integrin αIIbβ3 and fibrinogen, ICln, Aup1, WASP, VPS33B, c-Src, Gα13, Talin, and Kindlin-3. ICln, Aup1, Talin, and Kindlin-3 have demonstrable roles in the inside-out signalling activation of αIIbβ3. Talin, Kindlin-3, Gα13, c-Src, and VPS33B have demonstrable roles in outside-in signalling initiated by fibrinogen binding to αIIbβ3. Together, this complicated set of interactions contributes to the ability of integrin αIIbβ3 in mediating the activation of platelets and their subsequent adhesion to sites of vascular tissue damage in the endogenous coagulation response.

Integrin αIIbβ3 Inside-Out Signalling

Integrin $\alpha_{IIb}\beta_3$ activation, via inside-out signalling, begins with the actions of molecules like thrombin, ADP, collagen, and thromboxane A_2 on their platelet surface receptors [6, 24]. These agonists increase intracellular levels of cytosolic calcium, activate kinases like PKC and PI3K, and result in the direct engagement of molecules with the cytosolic tail sequences of $\alpha_{IIb}\beta_3$ [24]. A vast array of intermediate signalling molecules exist within these signalling cascades, several of which are shown in Figure 1. Partial deletions of the αIIb cytoplasmic tail, mutations in a conserved N-terminal sequence (KVGFFKR) of this subunit, or mutations in the cytoplasmic tail of β_3 enhance the affinity of $\alpha_{IIb}\beta_3$ for its ligands [29]. Thus, these membrane proximal regions seem to have a negative regulatory function, locking $\alpha_{IIb}\beta_3$ in its low-affinity basal state. Transmembrane helix-helix interactions are critical in maintaining $\alpha_{IIb}\beta_3$ in an inactive state, and resting αIIbβ3 is poised to undergo conformational changes to expose its ligand-binding site [28]. In fact, synthetic peptides that bind this transmembrane domain and

interrupt these interactions have comparable effects to thrombin treatment [28, 30].

Inside-out signalling reaches its conclusion in a similar fashion when molecules bind the integrin's β subunit cytoplasmic tail, disrupting the helical interactions of inactive $\alpha_{IIb}\beta_3$. One such molecule is Talin, which has a head domain containing many integrin binding sites and a tail domain with binding sites for a host of other molecules such as vinculin and F-actin [6, 10]. Talin binds the integrin subunit β_3 between residues 722 and 738 [31]. Talin-null megakaryocytes were demonstrated to produce platelets with normal morphology, but impaired hemostatic function [6]. Another molecule that binds and activates the integrin β_3 subunit cytoplasmic tail, though at a site distinct (slightly more membrane-distal) from Talin, is Kindlin-3 [10]. The Kindlin family has three members, which all localize to integrin adhesion sites [10]. Kindlin-3 homozygous null mice had no change in the number of platelets, but developed hemorrhages within one week from birth [10]. In addition to their role as the final regulatory molecules of $\alpha_{IIb}\beta_3$ inside-out signalling, Talin and Kindlin-3 are also players in the subsequent outside-in integrin signalling events [10, 32].

A membrane-proximal KVGFFKR sequence on the α_{IIb} subunit is also involved in the regulation of integrin $\alpha_{IIb}\beta_3$ activity [18]. ICln, a chloride channel regulatory protein, is highly expressed in platelets and has been shown to bind α_{IIb} at this sequence and inhibit its activity [18]. Similarly, the binding of Ancient Ubiquitous Protein 1 (Aup1) to this sequence in α_{IIb} negatively modulates $\alpha_{IIb}\beta_3$ signalling in platelets [29]. Previous reports suggest that as much as 40% of α_{IIb} in cultured megakaryocytes is complexed with Aup1 [29]. Thus, numerous binding sites within the cytoplasmic domains of both α_{IIb} and β_3 are implicated in the regulation and activation of $\alpha_{IIb}\beta_3$ via inside-out signalling.

Integrin $\alpha_{IIb}\beta_3$ Outside-In Signalling

The binding of active $\alpha_{IIb}\beta_3$ to fibrinogen leads to platelet shape change, aggregation, and release of α-granules, mediated by calcium mobilization, an increase in cytosolic pH, generation of thromboxane A_2, and the tyrosine phosphorylation of many intracellular proteins like Focal Adhesion Kinase (FAK) and Src family members which complex with the actin cytoskeleton and are recruited to focal contacts [29, 33]. It is possible that initial binding of fibrinogen to activated $\alpha_{IIb}\beta_3$ is followed by secondary binding of the growing fibrin fiber to other platelet surface proteins by means of transglutaminase activity [4]. Integrin-mediated cytoskeletal reorganization causes platelet cell spreading, stabilization of cell adhesions and aggregation, secretion, and clot retraction [31]. The aggregatory effects of activated $\alpha_{IIb}\beta_3$ are due to its affinity for multivalent fibrinogen, but its many intracellular signalling outputs are mediated by a wide variety of signalling molecules (Figure 1) [34].

c-Src binds the β_3 subunit of active integrins via an SH3 domain, and itself becomes active [35]. c-Src binding appears to involve an "unlatching" of its own structure via the dephosphorylation of pTyr530, enabling Tyr419 autophosphorylation and c-Src activation [35]. The signal transduction of c-Src culminates in cytoskeletal reorganization and platelet spreading via a clustering of the $\alpha_{IIb}\beta_3$ integrin receptors [28]. Clustering is an essential step because it brings c-Src molecules together, required for Tyr419 autophosphorylation [28].

The protein WASP is mobilized in $\alpha_{IIb}\beta_3$ outside-in signalling, and localizes to the membrane skeleton of platelets [36]. WASP is a scaffolding protein that integrates cellular activation and cytoskeletal rearrangements by binding actin and actin-related protein complex 2/3 (Arp2/3) in order to bring about the polymerization and cross-linking of the actin cytoskeleton [36]. WASP knockout mice have no change in platelet size, integrin quantity, or fibrinogen binding, but fewer platelets and a marked reduction in the spreading of platelets on immobilized fibrinogen [36]. While WASP impairs the retraction of fibrin clots and the stabilization of the primary platelet plug, it does not effect inside-out signalling [36].

Ligand binding to $\alpha_{IIb}\beta_3$ also promotes the binding of the heterotrimeric G protein $G\alpha_{13}$ to integrin subunit β337. Ordinarily, $G\alpha_{13}$ is activated by G Protein-Coupled Receptors (GPCRs) and then employs RhoGEF to activate RhoA and cause morphological changes [37,38]. In contrast, the interaction of Gα13 with $\alpha_{IIb}\beta_3$appears to inhibit RhoA, and so $\alpha_{IIb}\beta_3$ has been established as a non-canonical $G\alpha_{13}$-coupled receptor that dynamically regulates RhoA [37]. This was demonstrated through the interference of $G\alpha_{13}$'s interaction with $\alpha_{IIb}\beta_3$ in mice, resulting in diminished c-Src activity and a stimulation of RhoA [37].

VPS33B has been recently identified as a binding partner of β_3 integrin using receptor pulldown methods [31]. VPS33B is a member of the Sec1/Munc18 (SM) family with a well-characterized involvement in granule biogenesis [31]. VPS33B binds integrin β_3 between residues 716 and 730, which overlaps the Talin binding site [31]. VPS33B knockout mice have normal platelet morphology but reduced platelet activation, longer bleeding times, and impaired fibrinogen spreading and clot retraction [31]. The latter two phenotypes have been explained by VPS33B's actions upstream of the RhoA-ROCK-MLC and Rac1 dependant pathways that lead to clot retraction and cell spreading, respectively [31]. Given the phenotypic associations of VPS33B alterations and the proximity of its binding site in relation to that of Talin, a promising future direction for research in the mechanisms of coagulation will be investigating the possibility of VPS33B binding to the integrin subunit β_3 preceding and if this can potentiate the binding of Kindlin-3 and Talin.

In summary, a wide variety of molecules interact with the β_3 subunit of $\alpha_{IIb}\beta_3$ in order to elicit diverse downstream effects. Outside-in signalling has an involvement in the spreading of activated platelets, the stability of adhesions (both between platelets and to the subendothelium), granule secretion, and clot retraction. The regulation of each of these events is critical to proper hemostatic function, and each step can potentially be error-prone leading to thrombotic events. The complexity of this pathway also illustrates several unique and potentially actionable targets for medical intervention.

Clinical Perspective

Integrin $\alpha_{IIb}\beta_3$ plays a crucial role in the aggregation of platelets during the healing response of damaged vessels. However, dysregulated aggregation can lead to the formation of a pathological thrombus and leading to further cardiovascular diseases [38]. Integrin αIIbβ3 and its numerous regulatory factors are therefore potential therapeutic targets in the treatment of thrombosis or embolisms, in order to prevent further vascular complications. However, over-inhibition of the mechanisms of hemostasis also poses a variety of risks, like intracranial or gastrointestinal bleeding [39]. An obstacle therefore implied in pharmacological targeting of

these molecules is striking the proper balance of $\alpha_{IIb}\beta_3$ activity so as to halt pathological thrombotic events without increasing patient morbidity and mortality related to iatrogenic hypo-coagulation. Several levels of the complex signalling pathway of integrin $\alpha_{IIb}\beta_3$ may hold promising therapeutic targets in the context of clot prevention and dissolution, and future work is expected to explore potential the clinical benefits of antagonizing $\alpha_{IIb}\beta_3$ itself or disrupting its interactions with molecules like fibrin and Kindlin-340-42.

Conclusion

The integrin $\alpha_{IIb}\beta_3$ plays a crucial role in the aggregation of activated platelets during the vascular repair process as well as in thrombosis. $\alpha_{IIb}\beta_3$, like other integrins, is activated through inside-out signalling initiated through binding of platelet agonists during the adhesion of platelets to the subendothelium at sites of vascular damage, as well as the binding of various molecules to the cytosolic α_{IIb} and β_3 subunit sequences which regulates $\alpha_{IIb}\beta_3$'s propensity to become active by modulating the integrin receptor's conformation. Following activation, $\alpha_{IIb}\beta_3$ binds to multivalent fibrinogen resulting in the aggregation of activated platelets; simultaneously, $\alpha_{IIb}\beta_3$ performs outside-in signalling by binding a diverse suite of molecules, and perhaps many more still undiscovered, at the cytosolic face of the membrane.

Some questions remain unanswered regarding $\alpha_{IIb}\beta_3$ signalling, such as the precise mechanism by which receptor clustering is mediated, and how Talin and Kindlin-3, two major players in integrin signalling, might interact with each other to mediate $\alpha_{IIb}\beta_3$ activation. These molecules and their downstream binding partners are known to cause changes in cell conformation via actin cytoskeletal reorganization, as well as granule secretion, further aggregation, and clot retraction. The pathways mediating these processes are complex, and future studies are anticipated to offer a deeper understanding of their control over, and relevance to, the process of hemostasis. The proper regulation of platelet integrin $\alpha_{IIb}\beta_3$ and its many partners is critical to cardiovascular health, and understanding the full mechanisms underpinning the integrin's activity in this domain promises to be an important area of research.

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JULS REVIEWS

The Role of Gut Microbiota in Neurodegenerative Diseases

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Abstract

Background:

The human gut microbiota comprises trillions of microorganisms that live in the gastrointestinal system and interact extensively with the host. The diversity and stability of the gut microbiota have been linked to not only intestinal health but also brain function. Recent studies suggest that the gut microbiota communicates with the brain through a variety of mechanisms, in what is termed the "gut-brain axis." The neural, endocrine, immune and humoral means of signaling from the gut microbiota to the brain provide a link between gut microbiota and neurodegenerative diseases.

Purpose:

This review summarizes the current evidence that gut microbiota affects brain function through the vagus nerve, hormone signaling, the immune system and microbial metabolites. The relevance of these interactions is discussed in the context of neurodegenerative diseases, focusing particularly on Alzheimer's disease and Parkinson's disease. Furthermore, the implications to therapeutic interventions for the onset and progression of neurodegenerative diseases are highlighted.

Findings:

Preclinical studies have established the significance of the gut-brain axis and identified various mechanisms in which the gut microbiota could affect brain development and function. Several animal studies link neurodegenerative diseases to altered microbial compositions, suggesting the potential of identifying novel microbes as biomarkers. Moreover, a few animal studies use novel strategies to alter microbial compositions and thereby rescue disease phenotypes, which reveal the therapeutic potential. Research in humans largely relies on an analysis of the patient populations, with an emphasis on the association of altered microbial compositions with the onset and progression of brain pathologies. Hence, high-quality clinical studies are needed to elucidate the relative impact and causal contribution of the human microbiota to neurodegenerative diseases. Indeed, such clinical investigations may pave the way for more feasible and readily accessible means of interventions, in the forms of diet, prebiotics, probiotics and fecal transplantation.

1. Human Gut Microbiota

1.1 Gut Microbial Composition

The human gut microbiota consists of 100 trillion microorganisms that reside in the gastrointestinal (GI) system [1]. These microorganisms include bacteria, viruses, fungi, protozoa and archaea, whose collective genome is termed the gut microbiome. There is increasing evidence that the gut microbiota resembles a densely populated and diverse microbial community [2]. In particular, recent advances in DNA sequencing technology and bioinformatics tools have enabled profiling of the microorganisms inhabiting in the healthy human GI tract [3]. The gut microbiome has been found to contain four million distinct bacterial genes4, reaching 22 different phyla, 1000 different species, and 1000 viable bacteria per gram of luminal content [5, 6]. The density of microbiome is highest in the large intestine, where Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria are the most abundant organisms and constitute the core microbiome in healthy adults [5, 7].

1.2 Gut Microbial Colonization and Alterations

The human microbiome changes with age, following a certain pattern of microbial colonization of the GI tract [8]. Although bacteria have recently been found in the placenta and amniotic fluid8, the fetal GI tract is considered sterile prior to birth, with the first major microbial colonization occurring at delivery [9]. The earliest gut microbiota primarily consists of bacteria that can metabolize the lactose absorbed from milk; its composition is influenced by factors such as the mode of delivery (vaginal birth vs. cesarean section), source of nutrients (breast milk vs, formula), geography and exposure to antibiotics [10-16]. The infant microbiota continues to evolve and begins to resemble that of the adult at 2-3 years of age [17]. With the introduction of solid food, the gut becomes dominated by bacterial species associated with carbohydrate, protein and fat utilization and vitamin synthesis [18]. As such, the early development of the gut microbiota is vulnerable and perturbations

may have far-reaching impacts on health, including brain development and disease [19-22].

Although healthy adults share a core microbiome [5, 7], the gut microbiota exhibits inter-individual differences and may undergo changes throughout life [23]. The taxonomic variability within the GI tract depends on many factors, including host genetics, living environment, drug and antibiotic use, stress, infection and diet, some of which can dramatically influence the microbial composition over a relatively short period of time [23-28]. Since the relative abundances of gut microbes depend on the energy source available, diet is particularly influential to the gut microbiome [29]. In fact, short-term consumption of diets composed entirely of animal or plant products rapidly changes the microbial community and decreases inter-individual differences in microbial gene expression [30].

1.3 Gut microbiota in health and brain function

The gut microbiota interacts with the host environment and plays a critical role in maintaining human health [31]. For instance, the gut microbiota provides antimicrobial protection against pathogenic bacteria, aids in digestion and the production of essential metabolites, and regulates immune and nervous system development [32-35]. The gut microbiota also synthesizes molecules able to modify host epigenome, maintain the integrity of the gut barrier, and regulate host metabolism [36-45].

The interaction between the microbiota and the host is bidirectional, involving feedback from the host environment that affects the gut microbiota, which in turns influence host development [29]. In fact, the host stress response may induce widespread changes in the gut microbial composition. Mice experiencing social stress were found to have significantly altered bacterial community structure in the cecum and elevated levels of inflammatory cytokines [46]. Moreover, human subjects undergoing a stressful event were found to have a decrease in the relative fecal concentration of lactic acid bacteria [47], which have immunomodulating effects and may influence the gut microbial composition [48-50].

Given the roles of gut microbes in human health, dysbiosis, or the imbalance of the gut microbiota, has been implicated in numerous diseases, such as intestinal and metabolic disorders [32]. Importantly, recent research has also linked dysbiosis to neurological disorders, including Alzheimer's disease (AD), Parkinson's diseases (PD), multiple sclerosis and autism [51]. Due to the emerging significance of the relationship between the gut microbiota and brain function, the gut microbiota is perceived as a critical player in the "gut-brain axis," which describes the bidirectional communication between the gut and the central nervous system (CNS) [52]. The gut microbiota interacts with the gut-brain axis through neural, endocrine, immune and humoral means of communication [53].

2. Mechanisms of Interactions in the Gut-Brain Axis

2.1 Vagus Nerve

The CNS connects with the enteric nervous system (ENS) through sympathetic and parasympathetic nerves, linking cognitive and emotional centres in the brain with peripheral intestinal functions. Gut bacteria and their secretions influence neuronal excitation in the ENS, regulating both gut motility and sensory afferent signaling to the brain [54]. Intrinsic primary afferent neurons are the cellular targets of neuroactive bacteria and transmit microbial messages to the brain via the vagus nerve, which serves as a critical route of communication between gut microbes and the CNS [55, 56].

2.2 Neuroendocrine Signaling

Bacterial products are known to stimulate enteroendocrine cells to produce neuropeptides such as peptide YY, neuropeptide Y (NPY), cholecystokinin, glucagon-like peptide-1 and -2, and substance P57. These neuropeptides may enter the bloodstream or directly influence the ENS. Bacteria may also interfere with tryptophan metabolism in gut mucosal enterochromaffin cells58. Tryptophan is the precursor of serotonin, which functions to regulate GI secretion and motility, and mood and cognition in the brain.

In addition, gut microbes can synthesize and respond to hormones and neurotransmitters identical to those produced by humans [59]. For instance, Lactobacillus species produce acetylcholine and gamma-amino butyrate (GABA); Bifidobacterium produce GABA; Escherichia produce norepinephrine, serotonin and dopamine; Streptococcus and Enterococcus produce serotonin; and Bacillus produce norepinephrine and dopamine [59]. While bacterial use these compounds for inter-bacterial communication and microbial gene regulation, these molecules also serve as the main excitatory and inhibitory neurotransmitters of the brain, influencing its metabolism and function [60].

The bidirectionality of host-microbiota signaling is evident in this route of communication, as the growth and virulence of Escherichia coli were found to be greatly enhanced by physiologic concentrations of norepinephrine, indicating a direct impact of host stress responses on infection [61].

2.3 The immune system

The gut microbiota is involved in the regulation of the gut associated lymphoid tissue, which comprises a significant portion of the body's immune system. Structural components of gut microbes stimulate a tonic low-grade activation of the innate immune system that affects beyond the intestinal mucosal surface to the entire body [62]. For instance, bacterial cell wall lipopolysaccharides (LPS) induce synthesis of IL-18 [63]; bacterial peptides induce intestinal macrophages and T cells to produce interleukin-1beta (IL-1b) and tumor necrosis factor alpha (TNFa) [64]. Excessive stimulation due to dysbiosis, small intestinal bacterial overgrowth or increased intestinal permeability may induce systemic and/or CNS inflammation, and has been associated with several CNS disorders [29, 59].

2.4 Microbial metabolites

Short-chain fatty acids (SCFAs) such as butyric acid, propionic acid and acetic acid are the main products of bacterial metabolism. Through inhibition of histone deacetylases (HDAC) and activation of G-protein coupled receptors (GPCR), these metabolites have widespread regulatory effects throughout the body [65]. These bacterial metabolites can stimulate the sympathetic nervous system, cause mucosal serotonin release, and influence memory and learning process [68-71]. As such, the deregulation of HDAC and GPCR has been implicated in the pathophysiology of several neurodegenerative diseases [59, 66, 67]. Manipulation of the micro-

biota through diet has been found to influence behavior in mice. Mice fed with a diet promoting gut microbiota diversity exhibited an increase in physical activity, memory and a decrease in anxiety-like behavior [72].

Bacterial enzymes may also produce neurotoxic metabolites such as D-lactate and ammonia. D-lactate is a product of microbial fermentation of carbohydrates. High plasma levels of D-lactate resulted from intestinal hyperpermeability or abdominal surgeries were associated with symptoms of chronic fatigue syndrome [73-76], which could be improved by administration of specific dietary supplements [77-79]. Ammonia, produced in the intestinal tract by bacterial ureases, is normally taken up by the liver and consumed in the urea cycle; however, cirrhosis may allow absorbed ammonia to escape hepatic metabolism and lead to direct neurotoxic injury [80]. In addition, ammonia may alter the function of the blood-brain barrier, impairing intracerebral synthesis of neurotransmitters serotonin and dopamine [81].

3. Relevance in neurodegenerative diseases

Given the various interactions between the gut microbiota and the brain, a hypothesis has emerged to link gut microbes to multiple neurodegenerative disorders, ranging from AD and PD to multiple sclerosis, and amyotrophic lateral sclerosis [52]. A common mechanism is thought to be an impaired gut barrier associated with aging, bacterial overgrowth or abdominal surgeries [51]. Increased intestinal permeability allows gut microbiota-induced immune activation to lead to a systemic inflammatory response, which impairs the blood-brain barrier and promotes neuroinflammation, ultimately leading to neural injury and degeneration [21, 82-88]. This aberrant microbiota-to-CNS pathway results in the characteristic neuropathological features of AD and PD, namely the deposition of beta-amyloid in AD [82, 87, 88] and misfolding and aggregation of alpha-synuclein in PD [89].

3.1 Alzheimer's disease

IAD is characterized by an extracellular aggregation of amyloid plaques and intracellular deposition of tau in neurofibrillary tangles. Such aberrant protein accumulation is involved in neurodegeneration and cognitive impairment. Increasing lines of evidence in the form of animal models and correlational studies support the involvement of gut microbes in the pathogenesis of AD [52, 82, 85-88].

Recent preclinical studies suggest a correlation between amyloid plaque deposition and changes in the gut microbial composition. In a mouse model carrying mutated human genes associated with AD, the gut microbiota diversity was shown to regulate host innate immunity and affect beta-amyloid deposition [90]. In addition, preparation of germ-free AD mice reduced amyloid plaque deposition in the brain [91]. The recolonization with gut microbes from conventional AD mice, but not wild-type mice, restored amyloid plaques [91], suggesting the necessity of the gut microbiota in the development of AD pathology. Other AD mouse models have also been shown to possess an altered gut microbiota, which became more pronounced with advancing age [92, 93]. Furthermore, AD mice treated with probiotics from early ages showed changes in gut microbial composition, which led to a reduction in AD progression as demonstrated by brain structural markers and cognitive performance [94].

Although few studies in human patients have been published, these correlational studies also indicate a link between AD and alterations in the gut microbiota. In particular, a lower abundance of anti-inflammatory taxa Eubacterium and high abundances of pro-inflammatory taxa Escherichia and Shigella in the stool were associated with pro-inflammatory cytokines and amyloid deposition in the brain [95].

The link between AD and gut microbes is related to increased gut permeability, which allows microbes and microbial products to enter the circulation and reach the brain, contributing to the development of AD pathology. In particular, infections with microbes such as Herpes simplex virus type 1, Chlamydia pneumonia and spirochaete are thought to be involved in the pathogenesis AD or increase the disease risk [96-100]. Notably, LPS was found in amyloid plaques in the AD brain [101] and present in elevated levels in the plasma of AD patients [102]. Rats injected with LPS in the ventricles also showed inflammatory and pathological features seen in AD patients [103]. In addition, bacteria and fungi secrete a large amount of amyloid proteins that may accumulate in the CNS, leading to increased AD risk [104]. Amyloid proteins derived from gut microbes may prime the innate immune system to enhance the inflammatory response to cerebral amyloid proteins [105].

3.2 Parkinson's disease

PD is characterized by an accumulation of alpha-synuclein in the brain, which affects nerve cells that produce dopamine, leading to motor and non-motor symptoms. Gastrointestinal disturbances commonly precede motor symptoms by several years [106]. Similar to AD, the importance of gut microbes in the pathophysiology of PD has been demonstrated by mouse models and correlational studies in human patients.

In a PD mouse model overexpressing alpha-synuclein, antibiotic treatments improved its motor functions whereas oral administration of specific microbial metabolites promoted motor deficits and alpha synuclein aggregation [107]. In addition, colonization of these mice with feces from PD patients, but not healthy donors, aggravated existing neuroinflammation and motor deficits [108].

A number of published human cross-sectional studies also show an altered gut microbial composition in PD patients compared to appropriate controls, as shown in fecal or mucosal samples [52]. Collectively, these studies show lower abundances of anti-inflammatory Blautia, Coprococcus, Roseburia and Fecalibacterium [109], and higher abundances of pro-inflammatory Proteobacteria [109] and Enterococcaceae [110]. The pattern of changes is consistent with the features of peripheral and central inflammation seen in PD patients.

In addition, PD patients also showed increased abundances of Akkermansia, Lactobacillus and Bifidobacterium and a decreased abundance of Lachnospiracaea [111]. Functional analysis related these changes to pathways involved in the metabolism of plant-derived compounds and xenobiotic degradation, suggesting that diet may influence the progression of PD [111]. Interestingly, a decrease in Prevotellaceaeand an increase in Enterobacteriacaea were shown in German and Finland cohorts [112, 113], but not in U.S. cohorts [109, 110, 114, 115]. Such differences may be related to, among other factors, different dietary habits in European and North American populations.

The link between PD pathology and alterations in the gut mi-

crobiota may be related to a pro-inflammatory intestinal state triggered by gut microbial products, leading to alpha synuclein deposition in the ENS that reaches the brain via the vagus nerve. This has been supported by the fact that the earliest PD brain lesions appear in the motor nucleus of the vagus nerve [116]. Local inflammation likely induces microbial products, such as LPS, to leak out from the gut causing systemic pro-inflammatory status, or reach the brain via the bloodstream or the vagus nerve to worsen neuroinflammation and alpha-synuclein deposition [51]. Indeed, increased levels of serum LPS have been found in PD patients [117].

4. Limitations and clinical implications

4.1 Challenges and limitations

It is important to emphasize that the majority of evidence supporting the role of the gut microbiota in the pathophysiology of neurodegenerative diseases has come from observations in animal models [52]. While directly demonstrating the causation of altered gut microbial compositions in the progression of neurodegenerative diseases, these animal models may harbor differences in neurophysiology, immune responses, enteric microbiology, and therefore do not fully recapitulate the complete human phenotype.

Although several human studies demonstrate a link between gut microbes and AD or PD, these studies also have certain limitations. In particular, most were small in size and rely on fecal sampling to determine the gut microbiota population. Therefore, the study population may or may not have represented the general disease population, and could have been confounded by several factors, such as diet, medication and comorbidity that could influence the composition of gut microbiota [118-120]. Despite efforts to correct confounders in a few studies [121-122], it may prove difficult to assign the relative contributions of different factors to the microbial pattern in AD or PD patients. Given the bidirectionality of the gut-brain axis, the direction of causation between alterations in gut microbiota and neurodegenerative diseases could not be determined in these correlation studies [118, 123-125].

Nonetheless, establishing a direct link between the gut microbiota and key features of neurodegenerative diseases will open up new diagnostic and therapeutic opportunities. The last sections will briefly discuss clinical implications for the involvement of gut microbiota in neurodegenerative diseases.

4.2 Biomarkers

Gut microbes produce various metabolites, many of which are involved in host metabolism or maintenance of a healthy gut environment. These metabolites are detectable in the blood, urine, feces, or breath of the host. Disease-related microbes and metabolites, if identified, could serve as novel biomarkers, allowing for a useful and non-invasive method of identifying persons at risk for or in early stages of neurodegenerative diseases. In particular, high-throughput sequencing, metagenomics, metabolomics and other techniques allow for the study and profiling of microbes and metabolites in individuals [29].

4.3 Potential Therapeutic Interventions

Several animal studies use novel strategies to modify microbial compositions and rescue disease phenotypes [91, 94, 107, 108], revealing the therapeutic potential of altering the gut microbiota by the means of diet, probiotics, prebiotics and fecal transplantation. Probiotics are microbes administered to the host to confer health benefits [126], while probiotics are molecules metabolized by gut bacteria to favor specific changes in the activity and composition of the gut microbiota that benefit host health [127]. Diet, prebiotics and probiotics have been shown in clinical trials to lead to improvement of a variety of diseases and changes in emotional reactivity and brain activity [128-136]. As such, management of the gut microbiota via diet, prebiotics, probiotics and fecal transplantation may hold potential in the realm of preventive medicine and treatment of neurodegenerative diseases [29].

Conclusion

The gut microbiota changes throughout life in response to various factors and plays a critical role in human health, including brain function. Preclinical studies have established the significance of the gut-brain axis in the progression of neurodegenerative diseases. While animal studies have linked neurodegenerative diseases to altered microbial compositions, research in humans relies largely on correlative analysis of patient populations and has several limitations. Hence, high-quality human clinical studies are needed to elucidate the actual impact of the human microbiota on neurodegenerative diseases. In particular, the directionality of causation and relative contributions of gut microbes to disease pathology remain to be determined. Such clinical investigations may pave the way for novel diagnostic tools as well as more feasible and readily accessible means of interventions, in the forms of diet, prebiotics, probiotics and fecal transplantation.

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JULS RESEARCH

A Pilot Study of Microbubble-Delivered Gene Therapy Using High Intensity Focused Ultrasound (HIFU)

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Abstract

Oncolytic adenovirus is a frequently used viral vector for gene therapy in cancer treatments. However, they are highly susceptible to liver-drainage or rejection by the immune system upon injection. This leads to an inadequate gene-delivery to the target tumor or unnecessary delivery to healthy tissues, causing necrosis in different organs. This not only decreases the effectiveness of the therapy but also creates additional damages that may lead to severe side effects, outweighing the benefit of the treatment. In order to efficiently deliver the viral vector to the target tumor and tumor tissue only, the adenovirus is inserted into microbubbles. Microbubbles have been used to locally, temporally and reversibly open the blood-brain-barrier (BBB) under the influence of high intensity-focused ultrasound (HIFU). This increases the amount of gene delivered, decreasing the dose needed to successfully induce cell apoptosis in tumor cells. Understanding that HIFU induces the opening of BBB and degradation of microbubbles at proximate sites of the target tissue, it is still unknown whether the adenovirus can efficiently and effectively be delivered to cause successful apoptosis in local tumor cells. Our study investigates the possibility of minimally-invasive gene delivery using microbubbles with MRI-guided HIFU and confirms that the adenovirus can successfully be delivered into target tumor tissues.

Introduction

One of the popular mechanisms for brain tumor treatment is gene therapy using an oncolytic viral vector targeted to tumor tissue. However, due to the blood-brain barrier (BBB) that is selective against large and therapeutic molecules, gene therapies are limited to intracranial injections [1]. This highly invasive technique can be replaced by a minimally-invasive technique in which viral vectors, like adenovirus, are injected intravenously and delivered to the brain through the bloodstream. The BBB can be opened temporally with microbubble contrast and focused ultrasound (FUS), allowing oncolytic viruses to overcome the barrier and reach target tumor tissue [2].

Ultrasound has traditionally been used as a diagnostic tool in the medical field. However, recent developments of the FUS introduced a therapeutic aspect – high frequency, high amplitude waves induce an ablative effect while low frequency, low amplitude waves induce oscillations that can be targeted to the to temporally and reversibly open the BBB [3]. These ultrasound waves are focused through a mechanism of actions similar to that of a magnifying glass [4]. Because the ultrasound waves are absorbed by the skull and are refracted, FUS incorporate thousands of transducers, leading to a more localized and penetrative delivery of sonification without the opening of the skull [3]. According to previous research, BBB opening was successfully carried out in both rabbits and humans with FUS and ultrasound contrast agents, guided by real-time MRI [5, 6].

Previous studies have shown that adenovirus itself has a limited ability in cell-to-cell spread and induction of apoptosis in tumor tissues [7]. However, the expression of relaxin gene (RLX) in adenovirus has led to an increase in even penetration and distribution of virus in tumor tissues [4]. By using relaxin-expressing adenovirus, tumor tissues can undergo apoptosis more effectively, eliminating the need to deliver higher doses of the adenovirus. However, intravenous injections are highly prone to dosage loss as most drugs are drained to the liver or unnecessarily delivered to healthy tissues [8]. This may create additional problems like liver damage or side effects that can outweigh the advantages of the treatment. Therefore, microbubbles can be used to localize adenoviruses to the target tumor area to allow lower and safer dosages while preventing unnecessary damage to healthy cells. Starting from injection of these microbubbles, real-time MRI can be used as a guiding-tool to observe adenovirus-enclosed microbubbles in the bloodstream. When these microbubbles reach the BBB, low frequency FUS can be used to open the BBB and high frequency FUS for the disintegration of microbubbles, releasing the adenovirus at the site of target tumor cells. In this study, we evaluated whether therapeutic ultrasound improves the successful delivery of GFP-tagged adenovirus into tumor cells in the brain.

Materials and methods

Cell and Animal Preparation

The human breast cancer cell line, MDA-MB-231, was grown in medium with high-glucose DMEM with 10% fetal bovine serum. A mixture of 5x104 cells in 2 µL matrigel was prepared and sterotactically injected 0.5 mm anterior and 2 mm lateral to the bregma, 3 mm deep from brain surface. Immunocompromised athymic nude mice were used in this study. After three weeks of tumor growth, tumor size was confirmed through MRI spatial coordinates of FUS positioning system co-registered to that of a 7-Tesla MRI scanner (BioSpin 7030).

Blood-Brain-Barrier Opening

After sufficient tumor growth, gadolinium (GAD) contrast agents were intravenously injected and BBB opening was verified using MR-guided focused ultrasound (MRgFUS). T1- and T2-weighted images were taken to confirm BBB opening and to detect any hemorrhage.

Oncolytic Adenovirus

GFP-tagged relaxin-expressing oncolytic adenovirus (Ad-ΔE1B-RLX) with significant viral distribution was intratumorally injected with a dosage of 8x1011-VP/kg and intravenously injected with a dosage of 1.6 x 1012 VP/kg in separate mouse models. Four mouse models were used in this study. One sample was given an intravenous injection of GFP-tagged adenovirus, delivered to normal tissue and another sample was given an intravenous injection of GFP-tagged adenovirus, delivered to tumor tissue. Another mouse model was given an intratumoral injection of GFP-tagged adenovirus and the last sample was given an intravenous injection of GFP-tagged adenovirus, delivered to tumor tissues with MRgFUS. Intravenously injected adenovirus with GFP was injected prior to MRgFUS-induced BBB opening. MRgFUS RK100 system with 1.136 MHz spherically focused transducer was used. Microbubbles were intravenously injected (20 µL/kg) before the mice was treated with FUS to create oscillations in the BBB to cause the barrier to open.

Frozen Section for GFP Confirmation

Mice were sacrificed one week after the treatment to harvest brain samples. Frozen section and fluorescence microscopy were used to confirm GFP expression of the adenovirus. Tumor cell nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI).

Results

MRgFUS induces localized and reversible BBB opening

A T1- and T2-weighted imaging of mouse model brains before adenovirus injection confirmed the opening of BBB and the absence of hemorrhage. T1-weighted MRI imaging showed a significantly greater presence of GAD in tumor tissue only after FUS treatment (Fig.1b). Tumor tissue did not display high levels expression of GAD before FUS treatment (Fig. 1a). T2-weighted MRI imaging showed a clear absence of hemorrhage after FUS treatment (Fig. 1c,d).

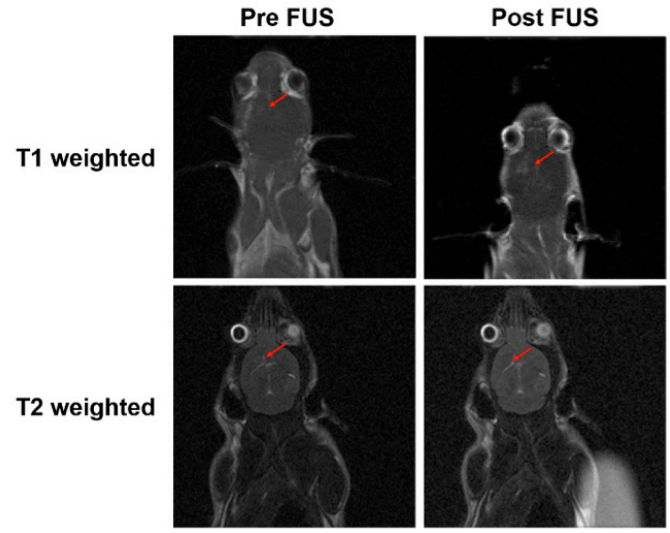

Figure 1. T1- and T2-weighted MRI of mouse model brains confirming BBB opening and absence of hemorrhage. a, T1-weighted MRI before FUS with no GAD expression and b, T1-weighted MRI after FUS with GAD expression indicating BBB opening. c, T2-weighted MRI before FUS and d, T2-weighted MRI after FUS showing no changes around tumor tissue area, indicating the absence of hemorrhage.

Intravenous or intratumoral delivery of GFP-tagged adenovirus yields poor distribution

An intravenous injection of GFP-tagged adenovirus to target normal brain tissue showed only the presence of DAPI nuclei staining and a lack of GFP expression, indicating the absence of adenovirus (Fig. 2a). Frozen section of an intravenously injected GFP-tagged adenovirus targeted to tumor tissue in the brain showed a slight increase in GFP expression, relative to normal tissue (Fig. 2b). However, adenovirus distribution was significantly uneven and the amount of adenovirus delivered was insufficient for successful gene therapy.

Intravenous delivery of GFP-tagged adenovirus using MRgFUS yields enhanced distribution and tumor penetration

Frozen section of the intratumorally injected GFP-tagged adenovirus targeted to tumor tissue confirmed the successful delivery of the virus across the tumor (Fig. 2c). Similarly, an intravenous injection of GFP-tagged adenovirus targeted to tumor tissue showed a comparably significant delivery and even distribution of the adenovirus across tumor tissue (Fig. 2d).

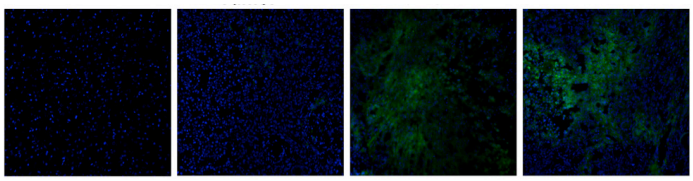

Figure 2. Frozen section of injection- and MRgFUS-dependent distribution of GFP-tagged adenovirus. a, GFP-tagged adenovirus injected intravenously to target normal tissue only displays expression of DAPI staining of nuclei. b, Intravenous injection of GFP-tagged adenovirus targeting tumor tissue in the brain has slight expression of GFP and c, Intratumoral injection of GFP-tagged adenovirus alone shows an average distribution of adenovirus across tumor tissue. d, MRgFUS-induced BBB opening prior to intravenous injection of GFP-tagged adenovirus shows similar adenovirus expression and distribution across brain tumor tissue.

Discussion

Overall, using MRgFUS significantly increased the amount of GFP expression, considering that the adenovirus was intravenously injected. This was possible due to the ability of the adenovirus to overcome the BBB, without creating novel side effects such as hemorrhage or permanent damage to the BBB. Although the intratumoral injection seems ideal and has been often the method

of gene therapy delivery to brain tumor tissue, it is considered a highly invasive treatment. In order to minimize pain and complications, treatment for tumors located deep inside the brain must be reached without opening the skull. Therefore, changing the method of delivery leads to a minimally-invasive treatment with other barriers. Because the BBB is known to keep molecules and therapeutic agents from entering the brain from the bloodstream, intravenous injection of the adenovirus, although tumor-specific, was insufficient for even distribution across tumor tissue [9, 10]. With MRgFUS that reversibly, temporally and locally opens up the BBB through oscillations in tight junctions, intravenously injected adenovirus was able to successfully reach tumor target tissue and with higher efficiency at the same dose. The dosage used for intravenous injections were much larger than the dosage used for intratumoral injections. This was to compensate for the dosage loss as the adenovirus travelled through the bloodstream. However, future experiments could further increase tumor penetration efficiency through genetic engineering and continue to lower the dosage requirement for much lower side effects and safer treatment.

Even with potent oncolytic adenovirus, there is still an uneven distribution of the virus across tumor tissue. This may be due to the different densities across the tumor or the ratio of adenovirus binding to the tumor cells. Further experiments should examine the possibility introducing microbubbles to enact oscillations in the BBB and additionally, function as another transportation vehicle for the adenovirus to reach targeted tumors. This will lead to an accurate and local release of the adenovirus and ultimately, an increase in their distribution and tumor penetration.

For this study, our goal was to overcome the BBB using MRgFUS, examining the possibility that intravenously injected relaxin-expressing adenovirus has in increased distribution in brain tumor tissues and, therefore, transform what was once a highly invasive treatment into a minimally-invasive cancer treatment. Further investigation will allow us to utilize this technique to monitor actual tumor size reduction and confirm its therapeutic efficacy by further enhancing localization and dosage compensation.

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JULS RESEARCH

Statistics and Research Methodology Training Needs in Medical Imaging

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Abstract

Purpose:

The purpose of this study is to recommend improvements for research methodology and statistics training resources specifically designed for medical imaging residents, fellows, and faculty.

Methods:

In 2017, survey and interview questions related to existing standards and ideal characteristics for a statistics and research methodology training program in medical imaging were created and administered to a large academic department prospectively. To explore common usages of statistics and research methodology skills in medical imaging, articles published in 2013 from all departmental faculty members were retrieved from Scopus and a review was conducted retrospectively. A mixed methods approach was used, including standard protocols for online surveys, phone interviews, and a cross-sectional review.

Results:

All interviewees stated that a statistics and research methodology training program would be beneficial to the department. 51% of survey respondents either only had informal or no training experiences in statistics and research methodology. 322 articles were published in 2013 by 177 faculty members. The median journal impact factor was 2.7 (IQR = 1.7 – 3.7) and median h-index of the corresponding authors was 10 (IQR = 4 – 22). 65% of the articles considered in the review used statistical methods with 79% of these employing a "basic" level of complexity (basic I, basic II, intermediate, and advanced).

Conclusion:

Results from this study support the need to offer broad training in research methodology. A better emphasis on basic level statistics that enables a more efficient use of departmental resources may be connected to a more cost effective and enabling education program.

Introduction

Medical trainees and clinicians are responsible for making informed choices about patient management based on accurate interpretations of research evidence. This requires an understanding of statistics and research methodology [1]. This has been demonstrated in trainee and clinician populations, including emergency medicine and undergraduate medical school programs [2,3]. Clinicians also undertake impactful research, requiring statistical knowledge for analyses, and interpretation of results [4].

Postgraduate medical students find statistics difficult, are frequently uncertain about the meaning of statistical terms, and struggle with selecting research methodologies in their research [5]. Graduate medical students who have taken quantitative university courses, including mathematics, also perceived statistics with feelings of anxiety [6]. The needs and preferences of medical imaging trainees and clinicians are important when considering ways to support this group of learners in conducting high quality research and providing high standards of patient care. The purpose of this study is to recommend improvements for research methodology and statistics training resources, specifically designed for medical imaging residents, fellows, and faculty. For this study, research methodology and statistics training was analyzed in the context of skills to analyze results. The objectives were: (1) survey and interview medical imaging residents, fellows, and faculty to explore the optimal depth, learning environment, and set of content priorities for a statistics and research methodology program; (2) review articles published in one year from faculty members in the Department of Medical Imaging at the University of Toronto, in order to determine commonly utilized statistical methods and; and (3) use the results to assist in the design and implementation of a data science support unit within the department.

Methods

Study Design

A prospective mixed methods approach was used, including standard protocols for online surveys, phone interviews, and a cross-sectional review [7,8]. Verbal informed consent was obtained from interviewees, and The University of Toronto's Health Sciences Research Ethics Board approved this study (#34191).

Online Survey and Phone Interview

Participants. Residents, fellows, and faculty from the Department of Medical Imaging were invited to participate in an online survey and a phone interview. The list of participants, including their contact information, was provided by Department of Medical Imaging. There were no exclusion criteria.

Outcomes. The standards for existing statistics and research methodology training opportunities were explored. Additionally, insights about the optimal depth, learning environment, and content for an ideal statistics and research methodology training program in a medical imaging department were to be identified through the survey and interviews.

Intervention. In 2017, quantitative data was collected online using Survey Monkey surveys (see Figures S1-S3). After surveys were completed, qualitative insights were collected through 10-minute semi-structured phone interviews. Questions 1-3 collected background participant information, including roles previously or currently held, formal research methodology or statistics course experiences, and preferred software. Questions 4-5 focused on gathering insights on content priorities, gained through comparing competent content areas in question 4 and the top three content areas for careers in question 5. Question 6 focused on ideal pedagogical formats, asking participants for the formats of learning they prefer. The phone interview elaborated on participants' responses to the survey, following the same topics. Participants were given the option to participate in one or both parts of the study.

Data collection. Recruitment for the online survey was based on emails sent directly to residents, fellows, and faculty in the Department of Medical Imaging. Instructions, a link to the survey, and an invitation for a phone interview were included. Survey Monkey software was used, because it is reliable, well-known, and allows personalized question formats to be created. Participants were given the option to complete the surveys on any electronic device with internet access. Consent was obtained at the beginning of online surveys via a mandatory question that required answering before proceeding. To advance questions, user pressed a 'Next' button.

Phone interviews were conducted after the surveys and at a time that was convenient for the participant, scheduled via email. Responses were recorded using an audio recorder, if consent was provided, and recordings were later transcribed for qualitative analysis. No personal information was collected during the interview, and any personal information mentioned accidently was removed while transcribing. The phone interview was semi-structured, based on the order of survey questions. At the end, participants were asked to discuss any additional information they felt was valuable.

Data analysis. Quantitative data from the surveys were used to calculate univariate statistics (frequencies/percentages). Qualitative results were analyzed using inductive thematic analysis to identify any themes emerging from phone interviews with participants [9]. A question-focused content analysis approach was used to determine patterns of responses. The interview data was transcribed verbatim and anonymized from recordings, and was then coded and analyzed for key themes.

Review of Published Articles

Search Strategy. The articles published by the University of Toronto's Department of Medical Imaging in 2013 were retrospectively searched on Scopus. Publications were retrieved by searching for faculty member names from the department within the list of authors.

Study Selection. Out of the 322 abstracts retrieved, a randomized sample of 170 articles (53%) were reviewed. A randomized sample was selected because this was an unfunded undergraduate project. A full review was not possible due to restricted resources and time. Simple randomization was used. No exclusion criteria were applied to full text articles, as all articles in the sample would be examined to find distribution of type of study, impact factor, h-index, and if applicable, level of statistical analyses. A flow diagram of the literature search is represented in Figure S4.

Outcomes. The review identified the difficulty and impact of the statistical methods that were most commonly used in articles published by the University of Toronto's Medical Imaging Department.

Intervention. A review of abstracts from articles published from faculty in the University of Toronto's Medical Imaging Department in 2013 was conducted.

Data collection. The full-text versions of the articles were independently evaluated by authors HL and AM. Data collected consisted of article citation, bibliometric indicators, study methodology and presence of statistical analysis. If statistical analysis was performed, the statistical software and methods were recorded. Disagreements in the data extracted by the two authors HL and AM were resolved by PNT.

Data analysis. For each abstract for studies that included statistical analysis, the statistical methods performed were categorized into four levels, based on standards in biostatistics consulting by our department: Basic I, Basic II, Intermediate, and Advanced (see Figure S5). Journal impact factors were retrieved from InCites Journal Citation Reports (Thomson Reuters). Descriptive univariate statistics were calculated (frequencies/percentages) and displayed in graphs, as well as tables.

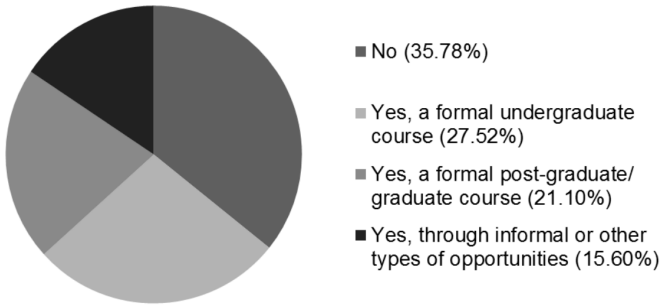


Figure 1. Previous statistics and research methodology experiences for residents, fellows, and faculty in the University of Toronto's Medical Imaging Department in 2016. This figure illustrates the proportion of participants, in percent, for different types of statistics and research methodology experience.

Results

Online Survey

Sixty residents, ninety fellows, and 184 faculty from the Department of Medical Imaging at the University of Toronto were invited to participate in the online survey and a 10-minute phone interview. A total of 117 residents, fellows, and faculty from a single Medical Imaging Department responded to the survey. Participants were given a list of professional experiences and they selected all the options with which they self-identified. The participants had varied professional experiences; 65.14%, 47.71%, 45.87%, 35.78%, 14.68%, and 8.26% of participants were a trainee (resident or fellow), co-principal investigator, staff or faculty, principal investigator, research assistant, and teaching assistant, respectively.

Previous experiences and software preferences. Based on responses to question #2 in the survey and as shown in Figure 1, participants had varying experiences including no experience

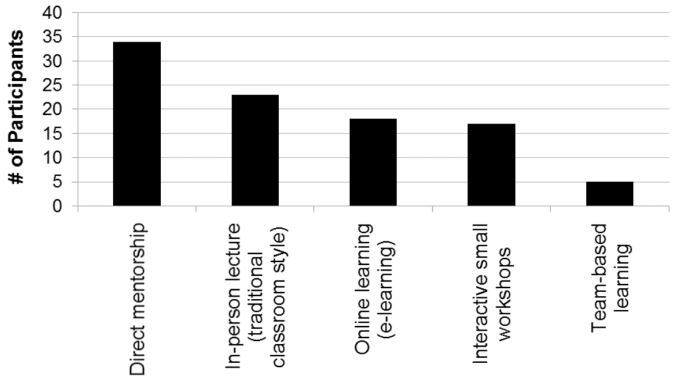


Figure 2. Preferred format to learn statistics and research methodology, selected by residents, fellows, and faculty in the University of Toronto's Medical Imaging Department in 2016. This figure illustrates the frequency, in number of participants, for different learning formats. Participants ranked all the learning formats and this graph illustrates the distribution of choices for rank #1, or the first choice.

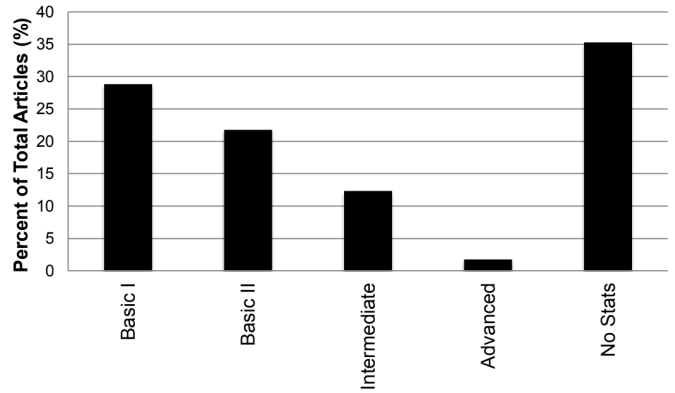


Figure 3. Distribution of difficulty levels for statistical methods used in articles published by the University of Toronto's Medical Imaging Faculty in 2013. This figure illustrates the portion of total articles published, in percent, for each difficulty level category.

(35.78%), a formal undergraduate course (27.52%), a formal post-graduate or graduate course (21.10%), or only an informal learning experience (15.60%). Informal learning experiences may include online learning. The most preferred software for participants was also diverse with a preference for SPSS (42.65%), Excel (27.94%), MATLAB (10.29%), SAS (7.35%), R (2.94%), STATA (2.94%), or other software (5.88%).

Content priorities for statistics and research methodology training. In response to question #4 in the survey, participants selected the top three content areas they felt were valuable to their career. 'Research manuscript writing', 'use of statistics for estimation and hypothesis testing' and 'research proposal writing' were selected as the first, second, and third most common choice.

In response to question #5 in the survey, participants also selected all the content areas about which they felt competent. 'Research manuscript writing', 'use of statistics for estimation and hypothesis testing' and 'research proposal writing' were selected by 66.0%, 26.0%, and 45.0% of participants, respectively.

Preferred learning formats. In response to question #6 in the survey, participants ranked different formats of learning according to their preference for how to receive training in statistics and research methodology. A graph depicting how many participants selected learning formats as a first choice is depicted in Figure 2. Direct mentorship was the most popular first choice option. In-person lectures were the second most popular first choice, closely followed by workshops.

Interviews

To augment the online surveys, five semi-structured ten-minute interviews were conducted to gain insights on the reasons behind their preferred learning format, as well as feelings towards their current level of statistics and research methodology knowledge. Since these are qualitative results, the quantitative significance of the interviews is not relevant. Participants were also asked how they thought a statistics and research methodology training program would impact the department.

Current level of statistics and research methodology knowledge. All the interviewees had different previous experiences with statistics and research methodology, but they all felt their knowledge could be improved. Key concerns expressed were: (1) uncertainty about when to perform different procedures, (2) the importance of more related practice, and (3) lack of confidence. It was frequently mentioned that an expert in statistics is usually hired for research projects, rendering it irrelevant to know procedures in-depth. Instead, knowing how to choose different statistical procedures was identified as important. It was noted that the lack of a preference for the SAS and R programs was related to limited knowledge and experience with the software.

Preferred learning format. Four interviewees selected 'direct mentorship' as their preferred learning format. Key constructs expressed were: (1) interactive experience, (2) individualized learning experience, (4) comfortable environment, and (5) convenience. Analysis indicated that data saturation had been reached, as the final two interviews did not result in new information. One interviewee selected 'in-person lecture' as their preferred learning format. Key constructs were: (1) familiarity with learning format and (2) comfort.

Impact of a training program. All interviewees agreed that a statistics and research methodology training program would be beneficial to the Medical Imaging Department at the University of Toronto. Key constructs expressed were: (1) improved ability to interpret research and (2) improved ability to work with experts on research projects.

Review of Departmental Publications

The review identified 322 articles published in 2013 by 177 faculty members of the Department of Medical Imaging at U of T, including 26 full professors, 43 associate professors, 92 assistant professors, and 16 lecturers. The 322 articles were randomized, and a representative sample of 170 articles were reviewed. The most common study types were retrospective cohort (22%), prospective cohort (17%), review (15%), and case-report (14%) (see Figure S6).

Depth of statistics knowledge utilized. Figure 3 shows that 110 (65%) of the articles analyzed used statistical methods. After recording the type of statistical analysis for each of the reviewed articles, the level of statistics that an article contained was deter-

mined. The level categories are described in Figure S5. The most common level of statistics used was Basic I, which was the case for 49 (44%) of the articles analyzed. The second most common level of statistics used was Basic II, which was the case for 37 (34%) of the articles analyzed. 21 (19%) of the studies analyzed required a level of statistics that would be classified as Intermediate. Only 3 (3%) of the studies analyzed required a level of statistics that would be classified as Advanced.

Impact of research. In terms of the journal articles published, 82.0% of the articles had an impact factor of 0-5. The median journal impact factor was 2.7 (IQR = 1.7 – 3.7). The three most common frequent journals to publish in were the American Journal of Neuroradiology, Pediatric Radiology Journal, and Canadian Association of Radiologists Journal, in which six, four, and three percent of articles were published, respectively. Results from a previous study conducted by Tyrrell et al. found that the median h-index of faculty in the department for the year 2013 was 10 (IQR = 4 – 22). Only 20% of corresponding authors were faculty from within the department [10].

Discussion

Medical imaging trainees and clinicians have unique statistics and research methodology training needs, as well as pedagogical preferences. These data support the continued need to offer broad training in research methodology, but suggest that a more cost effective and enabling education program would result from emphasis on basic level statistics. It was found that: almost half of the participants in the survey had no experience or only informal experience with statistics and research methodology; knowledge gaps existed, especially in the 'use of statistics for estimation and hypothesis testing'. Gaps in statistics and research methodology preparation for medical imaging trainees and clinicians exist, illustrating not only the importance of an evidence-based training program, but also specific content recommendations.

A baseline level of knowledge with regards to statistics and research methodology is important. The ability to 'work with experts who have a background in statistics on research projects' was highlighted as particularly important in interviews. The ability to communicate with experts was found to be valuable, rather than the capacity to complete analyses. To meet these needs, a training program that focuses on a basic level of statistics is valuable, rather than in-depth knowledge of advanced statistical procedures.

Medical trainees and clinicians have learning style preferences. Expectedly, direct mentorship and workshops were preferred, noting that they are pedagogical formats that provide an 'interactive experience', an 'individualized learning experience', a 'comfortable environment' and 'convenience'. Tailoring the learning format of statistics and research methodology training programs may be beneficial; aligning teaching methods to participants' learning styles and preferences improves engagement, as well as success [11]. Surprisingly, in-person lectures were also preferred, a counterintuitive finding given the current trend towards interactive teaching methods. Reasons in interview responses for preferring in-person lectures included 'familiarity with learning format' and 'comfort'. This may be linked to didactic learning being a dominant form of instruction in the field of medical imaging already [12]. The interview and survey findings both supported the importance of research methodology and statistics training for medical imaging trainees and clinicians.

Review of Department Publications

The review of published articles elucidated needs of the department. Most published articles were retrospective or prospective cohorts, and the level of statistics employed in most of them was at a basic level. This implies that instruction on basic statistics would be highly beneficial. Only a small percentage of the research from the department was published in high impact factor journals, and that the median h-index of the corresponding authors was relatively low. These results emphasized the value of fewer but impactful publications, rather than numerous low-impact publications [10]. Since high impact journals typically have a wider readership, publishing in these journals may increase the dissemination of research findings. Therefore, with the aim of maximizing the impact of articles, researchers need to consider ways to improve the quality of their publications, such as incorporating more advanced statistical analysis. However, it is important to keep in mind that journals with relatively higher impact factors do not necessarily have higher statistical standards, which has been shown in a study reporting the wide variability in statistical practices across journals with high or low impact factors [13]. Regardless, implementing a research methodology training program may increase researchers' knowledge of the different statistical methods available for analyzing and interpreting their data, thereby enhancing their studies' methodological quality and potentially increasing the chances of the studies being published in a journal with a high impact factor. Indeed, a dedicated resident research program has previously increased quantity and quality of publications by orthopaedic residents [14].

Limitations

Due to the nature of self-reported data, the findings of the study cannot be generalized. There are limitations in self-reported accounts of personal experiences and skills, as well as the determination of what learning environment would be best for the partcipants [15]. The study only included participants from the Department of Medical Imaging at the University of Toronto, and the review was conducted over a randomized sample of the department's publications over one year. Furthermore, characterization of the use of statistics in the department may have been more accurate if a review of all published articles was conducted instead. Findings from this study may not reflect other institutions.

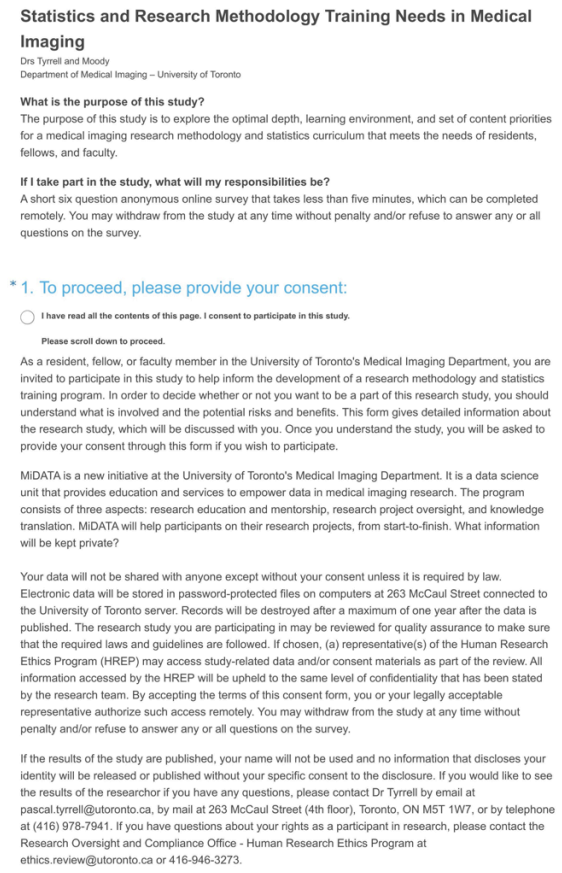
Future work

The findings of this study will be used for the research team to support the design of statistics and research methodology training programs at our department. In response to a preference for learning through direct mentorship, efficient and creative teaching through a mentored learning environment will be emphasized.

Opportunities that are tailored to participants' professional responsibilities, concentrate on statistics for medical research and medical image feature analysis, and include research project oversight, will be better integrated.

Conclusion

Academic departments need to address their own training needs in order to better support the development of research methodology curricula. Results from this study support the need to offer broad training in research methodology and suggest that a more cost effective and enabling education program would result from better emphasis on basic level statistics.



Statistics and Research Methodology Training Needs in Medical Imaging

Drs Tyrrell and Moody
Department of Medical Imaging – University of Toronto

What is the purpose of this study?
The purpose of this study is to explore the optimal depth, learning environment, and set of content priorities for a medical imaging research methodology and statistics curriculum that meets the needs of residents, fellows, and faculty.

If I take part in the study, what will my responsibilities be?
A short six question anonymous online survey that takes less than five minutes, which can be completed remotely. You may withdraw from the study at any time without penalty and/or refuse to answer any or all questions on the survey.

* 1. To proceed, please provide your consent:

◯ **I have read all the contents of this page. I consent to participate in this study.**

Please scroll down to proceed.

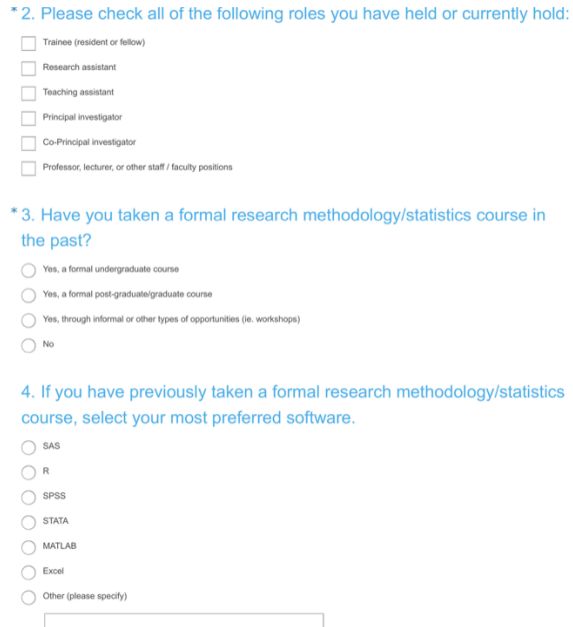
As a resident, fellow, or faculty member in the University of Toronto's Medical Imaging Department, you are invited to participate in this study to help inform the development of a research methodology and statistics training program. In order to decide whether or not you want to be a part of this research study, you should understand what is involved and the potential risks and benefits. This form gives detailed information about the research study, which will be discussed with you. Once you understand the study, you will be asked to provide your consent through this form if you wish to participate.

MiDATA is a new initiative at the University of Toronto's Medical Imaging Department. It is a data science unit that provides education and services to empower data in medical imaging research. The program consists of three aspects: research education and mentorship, research project oversight, and knowledge translation. MiDATA will help participants on their research projects, from start-to-finish. What information will be kept private?

Your data will not be shared with anyone except without your consent unless it is required by law. Electronic data will be stored in password-protected files on computers at 263 McCaul Street connected to the University of Toronto server. Records will be destroyed after a maximum of one year after the data is published. The research study you are participating in may be reviewed for quality assurance to make sure that the required laws and guidelines are followed. If chosen, (a) representative(s) of the Human Research Ethics Program (HREP) may access study-related data and/or consent materials as part of the review. All information accessed by the HREP will be upheld to the same level of confidentiality that has been stated by the research team. By accepting the terms of this consent form, you or your legally acceptable representative authorize such access remotely. You may withdraw from the study at any time without penalty and/or refuse to answer any or all questions on the survey.

If the results of the study are published, your name will not be used and no information that discloses your identity will be released or published without your specific consent to the disclosure. If you would like to see the results of the researchor if you have any questions, please contact Dr Tyrrell by email at pascal.tyrrell@utoronto.ca, by mail at 263 McCaul Street (4th floor), Toronto, ON M5T 1W7, or by telephone at (416) 978-7941. If you have questions about your rights as a participant in research, please contact the Research Oversight and Compliance Office - Human Research Ethics Program at ethics.review@utoronto.ca or 416-946-3273.

Figure S1. Survey question one.



* 2. Please check all of the following roles you have held or currently hold:

☐ Trainee (resident or fellow)
☐ Research assistant
☐ Teaching assistant
☐ Principal investigator
☐ Co-Principal investigator
☐ Professor, lecturer, or other staff / faculty positions

* 3. Have you taken a formal research methodology/statistics course in the past?

◯ Yes, a formal undergraduate course
◯ Yes, a formal post-graduate/graduate course
◯ Yes, through informal or other types of opportunities (ie. workshops)
◯ No

4. If you have previously taken a formal research methodology/statistics course, select your most preferred software.

◯ SAS
◯ R
◯ SPSS
◯ STATA
◯ MATLAB
◯ Excel
◯ Other (please specify)

Figure S2. Survey questions two to four.

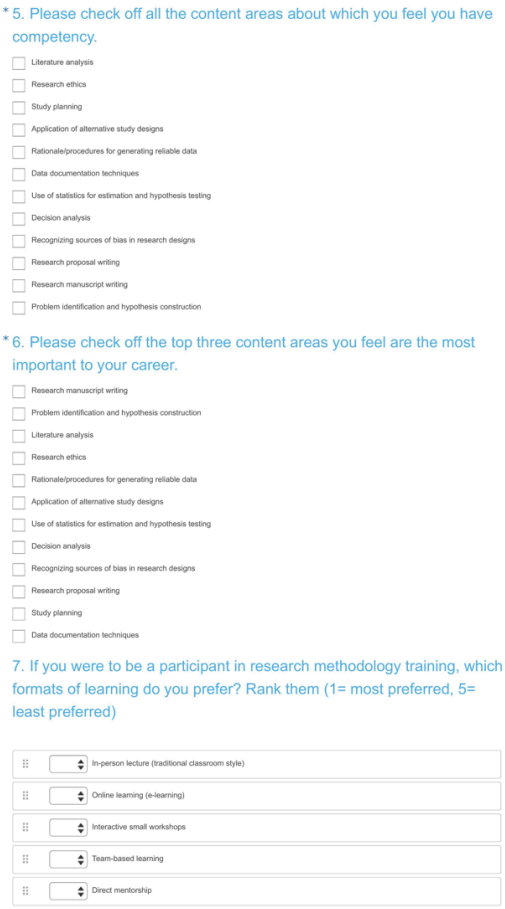


Figure S3. Survey questions five to seven.

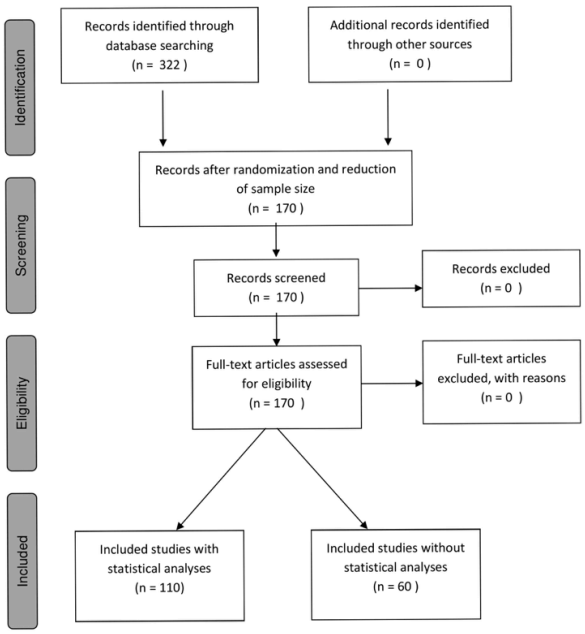


Figure S4. Study selection flowchart.

Basic I	Basic II	Intermediate I	Advanced
t-tests	Includes Basic I	Includes Basic II	Includes Intermediate I
Chi squared-tests	Up to 20 variables	Up to 30 variables	Up to 50 variables
Descriptive Stats	ANOVA	Complicated repeated measures ANOVA	Complicated repeated measures ANOVA
< 5 variables to test against 2 groups	Some categorical data	Standard complex designs for categorical data	Nonstandard designs
Clean data by our standards	Classical designs	Extensive modeling	Nonstandard complex designs for categorical data – research required
Estimated Time: 5hrs	Regressions – model building w/ variables given by investigator	A few multivariate techniques may be necessary	Extensive modeling – variables to be defined by statistical methods
	Data modification	Some data modification	Multivariate techniques should be performed
	Estimated Time: 20hrs	**Estimated Time: 40hrs**	Data Modification
			Write stats section – research required
			Estimated Time: 70-100hrs

Figure S5. Four levels of biostatistics with estimated time for analysis.

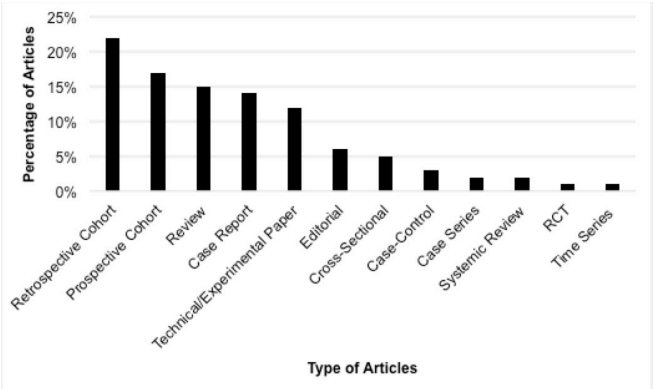


Figure S6. Distribution of the articles randomly sampled from the articles published by the University of Toronto's Medical Imaging Department in 2013 by study type.

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Dr. Sunit Das

Dr. Sunit Das is a clinician-scientist and a neurosurgeon at the St. Michael's Hospital.

Shveta Bhasker

Q: Can you please describe your basic and clinical research in oncology and what your main research focus is?

SD: I am a clinician-scientist at the St. Michael's Hospital at the University of Toronto. I am an adult neurosurgeon and I find myself taking care of patients that have had a lot of trauma. However, my elective practice is in neuro-oncology and is focused on taking care of tumors in the brain and spine. Most of my practice focuses on taking care of patients with primary and secondary brain tumors, such as gliomas, which can arise in the brain or in patients who have systemic cancers which have metastasized. In this scope, I do a lot of minimally invasive surgery and a lot of surgeries where patients are usually awake. I also run a basic science laboratory at the Hospital for Sick Children and I do adult brain tumour research there within the Brain Tumour Research Centre at SickKids. My group at the lab focuses on understanding the way primary brain tumors like gliomas develop and evolve with different therapies. I also do clinical research which stems from the work that I do as a clinician which falls into two different categories. One, I do a lot of work understanding brain neuroanatomy and function and that involves working with people from medical imaging who do functional imaging before surgery and then doing intraoperative brain mapping during surgery. I also do knowledge translational research where I use clinical trials or translational science to guide practice.

Q: Why did you choose to research oncology being a neurosurgeon?

SD: There are multiple different sub-specialties in neurosurgery like oncology, spine surgery, vascular neurosurgery, functional neurosurgery, pediatric neurosurgery, and skull-based neurosurgery. My research interest developed as a result of my clinical experiences. As a medical student, who had never done basic science research before, I found fundamental science really exciting and that the environment of academic medicine was something I wanted for myself. In medical school, I started to think of a career that involved some type of academic imprint, and the dual-identity seemed like the right choice for me. I was surprised to find neurosurgery to be what I ended up in. I went to medical school to become a psychiatrist because of my growing interest in neuroscience. I was very disinclined about thinking of neurosurgery as a career. During the third year of medical school, you rotate through multiple different fields to build a general knowledge base of different specialties. I spent my first rotation in psychiatry and had a spectacular experience with amazing residents and staff and came out of that month thinking that I am going to become a psychiatrist. I took a month off afterwards to write a paper on philosophy and then came back and spent a month on neurology and had a spectacular month again. I was in awe of staff and residents, and thought I should do neurology instead of psychiatry. I then spent three months doing internal medicine and also had an amazing experience and really enjoyed it, but I wanted to do something clinical which involves working with questions about the brain, which I did not find in internal medicine. I then started my three-month rotation in surgery and I had an amazing experience spending time with someone who became a mentor to me. He was a surgical oncologist, and I revered him as a person, a doctor, and a caregiver. I had a similar experience when I was on neurology working with a neuro-oncologist whom I found greater than life. I think my experience is not much different that you hear from many other doctors – all of us try different fields but at the end, we all find someone that inspires us, and we think to ourselves, "this is what it means to be a doctor" or, "this is what I should aim for". For me, the two people that I met like that were both cancer doctors, and I think that if there is

"We all find someone that inspires us"

anything that prompted me towards thinking about oncology, then it was my initial meeting with them. I also spent two months in general surgery and then one month doing neurosurgery, which I selected as an elective because I initially wanted to do psychiatry or neurology. However, going into the neurosurgery rotation, I didn't think that I would want to be a neurosurgeon.

Q: Prior to medical school, you studied English Literature and Philosophy. How has studying these subjects helped you as a physician and a scientist?

SD: I would argue that that the processes required to be a good literary critique is not much different from the requirements to become a good physician. As a physician, you are essentially performing a close reading interpretation. The bigger answer is that we live in such a rich world. There is so much beauty and so much that is remarkable, and I feel lucky to have been given training that lets me spend time in these worlds that are remote from my own. I got to spend six years living in a life that is not typical and reading the literature and the philosophy came with it. I also got to spend time with people who exposed me to jazz, classical music, foreign films, and just a world that is richer than I think I would be exposed to if I were just on track for the life that I am lucky enough to have today.

It's hard to say that without it sounding like a value judgment and I don't mean it to be that – I feel lucky that I somehow stumbled into a world that is really amazing. What I studied during undergraduate studies makes me a better doctor because they give me personal and emotional strength. I stumbled onto the Centre for Ethics here at U of T and luckily sat down with the director, becoming more involved at the centre because I wanted to get back into the world of ethics and philosophy. I just recently published a paper in the ethics of social innovation with other co-authors on the ethics of artificial intelligence in medicine.

"My focus is to understand the ethics of artificial intelligence in medicine. I am very interested in understanding what professional identity and professionalism mean specifically in medicine. "

Q: Can you please describe your research in Artificial Intelligence (AI)?

SD: My focus is to understand the ethics of artificial intelligence in medicine. I am very interested in understanding what professional identity and professionalism mean specifically in medicine. Medicine as a field holds a unique place within society, and physicians are ambassadors of that professional identity. If you think about what doctors have a responsibility towards, we obviously have a relationship with our patients and a responsibility which we are bound to. We also have a responsibility towards each other as physicians, which is to treat each other appropriately and be of assistance to one another.

If you look at popular definitions of AI, they all seem apocalyptic which will make us the lesser of the intelligent entities on Earth. There are ways in which the rules of the game will fundamentally change because of AI. If you think about ways in which AI will integrate into medicine and how it may change what we can do and how we can do it, it's remarkable. It's hard because what we're saying right now is based on imaginative ideas of what AI will be and we haven't really manifested what that means in the real world. All of those things I would argue are going to fundamentally require us to reimagine how we think of ourselves as physicians and what we see our relationship with patients. When I say these are fundamental questions about professionalism and professional identity, they are as if not more important than the ethical realm as they are practical. The practical realm will be figured out by its own, but how we evolve iteratively is the bigger question in my mind.

Q: The benefits of AI in medicine for a healthcare practitioner is that it helps improve efficiency and it creates more free time for clinicians to focus on other tasks. Where is the patient in this paradigm? How much does that patient benefit from all of the AI automation?

SD: So, we don't know that answer to that yet. So far, the automation has not made life better for physicians and the processes have not beneficially affected patient and doctor relationships. For example, family doctors spend an average of 8 minutes with patients and it is thought that for every hour of patient care, there is one hour required for documentation through the electronic medical record. In saying this, there is hope that AI will make what we do, more efficient. So how could that happen? We could imagine things that are really mundane and really spectacular. Imagine a 64-year-old woman with a history of asthma that presents to the ER with a cough and shortness of breath. Basically, this is a patient who has complicated pneumonia and will be admitted to the hospital. All of us are interested in trying to identify biomarkers, clinical or blood, that could help us discriminate between patients that will be fine without intervention, and those that will crash if we don't intervene in more aggressive ways. We don't have the ability to do that yet, and we hope that machine learning would be able to discern large data sets allowing us to identify patients that need help in ways that we can't. There may be an algorithm that goes beyond our own scope of ability to discern. A mundane situation would be if I am talking to my patient and I write down what they're saying and when I'm done, I come and dictate the information, which I have to make corrections to. In the US, many medical schools have hired scribes who write notes as the doctor interviews the patient. Why can't a robot record the interview and if it had the ability to do natural language processing? It could then generate a note based on the template of a medical record. Technology could radically change how doctors and patients spend their time.

Q: There is a debate regarding the socio-legal implications of AI. In the case of malpractice, who do you think should be responsible for the result? The clinicians using AI or the developers of

AI systems?

SD: If someone is in a driverless car and it strikes someone across the road, who is liable? Right now, the contract that medicine depends on, or the idea of why physicians have a particular relationship with patients is based on three fundamental principles: expectation that patients can believe that the physician will always attempt to do what is in their best interest, secondly physicians are bound to protect the patient's privacy, and lastly patients understand that physicians have taken the responsibility for the patient's health. As a surgeon, that means that if something goes wrong in surgery, I will take the weight of the implications of that error. These are all fundamental to the physician-patient relationship.

My inclination to this question's answer is this: physicians have access to many different adjuncts that add to what we do, ranging from the otoscope to an MRI. Using adjuncts does not take away your responsibility as a physician. I expect that AI will simply be another adjunct and mechanism by which we take care of a patient. This does raise the difficult question of wondering whose decision should be privileged. In other words, if the clinician's decision is to be antagonistic to the output of an algorithm, then what do you do there? But I will say that as a clinician, we are always in this position. I am often telling my patients that we don't have certainty. I would hope that the element of counselling and decision making between the patient and physician remains an identity of the physician regardless of what tool informs it.

Q: If AI were to take over, do you think the trust towards the physician may be transferred over to the AI?

SD: I think there are elements of what people are looking for in their caregivers that are particularly human. At the moment, it is difficult to think what AI will mean. When we speak of AI right now, we think about algorithms that may move the the way we process databases and large amounts of data. This is very different than thinking about a robot that has a warm voice and puts its hand on your shoulder when giving bad news. Maybe this is a possibility of what AI may mean. Then we get into questions that are beyond medicine. If you throw medicine away, what happens if or when we create these entities that have intelligence and sentients, but aren't human?

Q: What is your favourite part about being a clinician scientist?

SD: I enjoy the technical part of being a surgeon. I find the friendships I develop with my patients incredibly meaningful. Being a mentor to the students in my lab is remarkably rich. I like thinking about science. I like the fact that my 6-year-old kid jokes around and cuts brain tumors out of his Play-Doh. I like the fact that my wife is an Infectious Disease doctor and I am proud to be married to her. I like the fact that my life allows me to meet new students all the time and that I get to share my excitement of what I do. I love that I get to see my residents grow and take on abilities that they did not have before. It is a real privilege to be a doctor. It is such a long road, but it's amazing and so rewarding.

Nishila Mehta

Nishila Mehta is a first-year medical student at the University of Toronto and is part of the AI in Medicine Student Society at U of T Medicine.

Shveta Bhasker

Q: Can you please describe your research and how it is related to Artificial Intelligence (AI)?

NM: I'm a research fellow in Ethics of Artificial Intelligence at the University of Toronto's Center for Ethics. I'm particularly interested in how emerging AI technologies will impact healthcare, and have two ongoing research projects in the area. The first aims to understand medical students' knowledge and perceptions of AI in medicine through an electronic survey. We want to gage whether students are aware of the technologies behind AI and their limitations, and also learn more about their beliefs towards AI's impact on their future medical practice and professional scope. This will give us insight into key areas that medical curricula need to address in order to ensure that medical professionals are prepared to ethically and safety work in the AI-integrated healthcare environments of the future. For example, understanding that algorithms are prone to bias may prevent doctors from blindly trusting the predictions of AI tools and potentially harming patients.

My next project is a scoping review and commentary on how AI will impact health equity locally and globally. With any new emerging technology, we must ask ourselves which populations will be able to access it, who it benefits, and who it may harm. In this study, I hope to analyze what has been written on this topic in the literature, and add concerns from my background in global health. Some concerns include bias of algorithms through exclusion of data from vulnerable populations, access to technology needed to implement AI in the developing world, and reinforcement of discrimination and disadvantage through predictions of AI tools. Through this study, I hope to be able to alert policymakers as to the importance of social considerations in AI dissemination, and foster discussion on how to prevent AI from deepening existing inequities.

Q: There have been proposals of using fully automated clinical systems where clinicians are not needed in clinical settings and algorithms can incorporate all of the clinical presentations to make a decision for the diagnosis by its own. What is your opinion on fully automated clinical systems?

NM: While there may be benefits to fully automated clinical systems, I don't see these adequately replacing care provided by clinicians. Some benefits for patients may be improved diagnostic accuracy, lower wait times, and increased rural access to clinical diagnoses. Clinicians may have to spend less time in clinic, and can dedicate time towards other endeavors such as community health education initiatives or research. Health systems may benefit through reduced human resource costs. That being said, clinical care goes far beyond just making a diagnosis. In medical school, we're taught to listen to patients' stories, empathize with them, and incorporate compassion into every aspect of clinical care. Diagnoses has to be followed by counselling and treatment discussions, which can be complex and sensitive topics. So ultimately, I see the role of AI tools to support and augment clinical decision making, rather than replacing clinicians entirely.

"Diagnoses has to be followed by counselling and treatment discussions, which can be complex and sensitive topics."

Q: The benefits of AI in medicine for a healthcare practitioner is that it helps improve efficiency and it creates more free time for clinicians to focus on other tasks. Where is the patient in this

paradigm? How much does that patient benefit from all of the AI automation?

NM: I think patients will benefit directly from physicians having more free time. When physicians spend less time documenting in patient charts, and researching diagnoses and treatments, it leaves more time to actually listen to patients and discuss treatment options. Patients will benefit from this increased attention from clinicians. As I mentioned previously, patients also benefit from increased diagnostic and treatment accuracy with AI tools, increased access in rural and remote areas, and in many more ways.

Q: How do we optimize workflow with AI such that when AI is not perfect, we can still allow clinicians to catch any misdiagnosis by AI?

NM: This is an important topic, and many people are working to answer this question. I think it starts with making sure clinicians are aware of the different ways in which AI can be imperfect (e.g. inaccurate algorithms, biased training data, etc). Through my research, I hope to promote the idea that this should be instilled at the medical school level, and build on this throughout the rest of medical training. Further, I think this is where collaboration and communication between computer scientists, AI tool developers and clinicians is extremely important. By developing a shared understanding of the appropriate use and limitations of specific tools, errors can be prevented. Lastly, I think there needs to be a process in place to double check predictions. Right now, when a clinician makes a diagnosis, patients can choose to seek out a second opinion from another clinician or a specialist. I think that the practice of double checking predictions with clinicians should remain an option for patients.

Q: There is debate regarding the socio-legal implications of AI. In the case of malpractice, who do you think should be responsible for the result? The clinicians using AI or the developers of AI systems?

NM: Another important question! There currently is little consensus on this. I personally believe that it depends on where exactly things went wrong. If there was an error in the actual AI tool or algorithms that the developers did not anticipate, I'd argue responsibility could fall on the developers, or perhaps the regulating body that permitted the tool to enter medical practice. If there were known risks and issues with the medical tools, I think clinicians have a responsibility to understand these prior to using these tools in patient care. I think we need to keep these discussions going, and make sure to have appropriate legal and policy standards equipped to manage errors before AI tools infiltrate healthcare environments.

Q: How can we address racial biases in machine learning algorithms? How can we make sure that data in AI models are generalizable to the general population?

NM: It all comes down to the quality of the data used to train the AI, which in computing is expressed by the phrase "garbage in, garage out". If data is based on years of biased human decision making (e.g. court judges with racial biases who are more likely to convict black people), the AI will inherit these biases and learn that being black is a risk factor for criminal activity. I think it becomes extremely important then to try and diversity data sources as much as possible prior to the development of AI tools. It's also important to be able to identify when an algorithm is not performing well on certain demographic groups, and prevent harm from occurring. This is what inspired me to pursue my research at the Center for Ethics- there is potential for racially biased AI tools to inflict harm on population health and we need to get ahead of the curve.

Q: How can we achieve optimal adaptation and a good uptake of AI models globally?

NM: I think it's all about who you have at the table. Working with the Center of Ethics has allowed me to interact with people from the worlds of ethics, medicine, humanities, computer science, and education. At the weekly seminars, I observed how having these diverse perspectives allowed for a more comprehensive analysis of ethics issues around AI. As AI tools become more ubiquitous, and there is increased uptake by institutions and patients globally, I think it's important to have interdisciplinary expertise to guide their implementation. Through this, we can optimize outcomes for patients, clinicians, and health systems.

Vinyas Harish

Vinyas Harish is a second-year MD/PhD student at the University of Toronto and co-founded the AI in Medicine Student Society at U of T Medicine.

Shveta Bhasker

Q: Can you please describe your research and how it is related to Artificial Intelligence (AI)?

VH: There are a couple things I'm currently working on right now. A good friend and fellow MD/PhD classmate (aka mudphud as we like to call it), Felipe Morgado, and I founded the 'Artificial Intelligence in Medicine Student Society' at U of T Med. Over the last year, we have been working closely with our faculty mentor and neurosurgeon-scientist, Dr. Sunit Das, on various philosophical, commentary-style pieces. One of them deals with the inherent nature of uncertainty in medicine and how artificial intelligence will not drastically change that. We have also been working closely with the director of the Foundations curriculum in the MD Program, Dr. Marcus Law, to advocate for and pilot teaching on artificial intelligence in the preclerkship medical curriculum both at U of T and across Canada.

In addition to the projects above, I'll be starting my PhD in Clinical Epidemiology this July at the Institute for Health Policy, Management and Evaluation. I'm excited to be working at Dr. Laura Rosella's Population Health Analytics Laboratory, a group that seeks to leverage cutting-edge computer science in the creation of learning tools for population health decision-making. For my doctoral research, I am interested in the application of machine learning methodologies to the creation of a global early warning system that predicts the emergence and spread of infectious diseases. Given today's social commentary and widespread 'techlash', I also plan to examine broadly the ethical implications of artificial intelligence as it's used in public health decision-making systems.

Q: There have been proposals of using fully automated clinical systems where clinicians are not needed in clinical settings and algorithms can incorporate all of the clinical presentations to make a decision for the diagnosis by its own. What is your opinion on fully automated clinical systems?

VH: I don't think that's on the horizon anytime soon. The sorts of AI applications that are appearing in the peer-reviewed literature still deal with relatively narrow tasks (e.g. diagnosing a skin lesion, or predicting the likelihood of sepsis). Any systems that will be approved for use on patients will have to remain 'human-in-the-loop' for the foreseeable future – meaning an AI system will be like any other diagnostic test that a clinician will have to interpret in the context of the entire patient's presentation to come with a diagnostic impression and management plan. Moreover, it's important to remember that diagnosis is only one aspect to a clinician's role. Management of a medical condition, whether medical or surgical, remains even harder to automate away completely.

"I think, if implemented carefully, AI can help clinicians by helping put patients at the center of the healthcare paradigm."

Q: The benefits of AI in medicine for a healthcare practitioner is that it helps improve efficiency and it creates more free time for clinicians to focus on other tasks. Where is the patient in this paradigm? How much does that patient benefit from all of the AI automation?

VH: I think, if implemented carefully, AI can help clinicians by helping put patients at the center of the healthcare paradigm. Atul Gawande has recently published a fantastic piece in the New Yorker about the advent and adoption of electronic medical records -- and the surge in physician burnout that has followed them in what appears to be lockstep. Various AI-powered tools (e.g. a digital scribe) can facilitate a therapeutic relationship between doctors and patients where a doctor isn't typing frantically on their computer

during the encounter and can bear witness to the patient's story in a more empathetic and humanistic way. By spending less time 'charting', or creating documentation about the patients that are being seen, and more time directly caring for patients, optimists expect to see happier and healthier patients and physicians alike. I want to emphasize the 'careful' bit about AI implementation. I'm sure similar promises were made about electronic medical records twenty years ago.

Q: How do we optimize workflow with AI such that when AI is not perfect, we can still allow clinicians to catch any misdiagnosis by AI?

VH: Like I mentioned earlier, I don't think we can get around having human-in-the-loop systems. Whether it's reviewing the literature, referring a patient to specialists (perhaps even more than one specialist of the same type), or building in metrics that quantify an AI decision-support system's 'confidence' around a diagnosis – I think there should be multiple points in a workflow that allow for an opportunity to consider the possibility of errors including misdiagnosis. In keeping with my interests in medical education, I believe that it's essential to train clinicians on how to interpret the output of AI decision-support systems and critique them. These critical appraisal skills should be cultivated much like how clinicians learn to interpret the medical literature in terms of bias, internal and external validity, and generalizability. Finally, there should be a mechanism of improvement built into the system such that it can learn from what clinicians identify to be errors (both prospectively and retrospectively.

Q: There is debate regarding the socio-legal implications of AI. In the case of malpractice, who do you think should be responsible for the result? The clinicians using AI or the developers of AI systems?

VH: That's an interesting question, and I'm not sure if anyone really has an answer to that yet. I think it's reasonable to assume that precedent will be drawn from similar cases in medical technology, but I definitely am not a lawyer. A lot of responsibility is placed on clinicians to create the safest environment possible, and that's another reason why I am hesitant about 'fully autonomous' systems delivering care. When it comes to medical error, we are taught about the importance of having a 'just culture' in order to balance accountability without being excessively punitive. An 'incident decision tree' can be followed along the following dimensions in this order: determining if there was deliberate harm, determining if there was any evidence of impairment, determining if there was a deviation away from established protocol, and finally if another colleague in the same manner would have acted similarly. If it can be established that there was a system failure using an incident decision tree, as opposed to an individual at fault, I can imagine the courts and regulators would have their work cut out for them in determining culpability.

Q: How can we address racial biases in machine learning algorithms? How can we make sure that data in AI models are generalizable to the general population?

VH: The issue of racial bias, and bias more broadly speaking, remains a huge issue. There are two main ways that come to mind on how to address it. With machine learning and other areas of computer sciences, trainees quickly learn firsthand the meaning of the adage 'garbage in, garbage out.' In other words, the insights one can draw from models are only as good as the data that's used to generate them. Readers may be familiar with the 'WEIRD' phenomena in psychology – that much of the research is done on Western, educated (e.g. college and university students participating in studies for extra credit) students in industrialized, rich, and democratic countries. Understandably, the results of these studies can have issues depending on how you want to interpret them. Similar issues exist with machine learning algorithms, within (e.g. diagnosing a skin lesion) and outside of (e.g. using facial recognition to unlock your computer) applications in healthcare. Algorithms for both the aforementioned use cases performed worse on individuals of Fitzpatrick type skin.

The second method of addressing the issue of bias comes from who is actually researching, developing, and scaling AI models. The technology industry has received considerable criticism for being overly white, male, cis-gendered, heterosexual, and wealthy. These individuals, like any other group, bring with them a certain lens they use to view the world into their work. That in itself is not problematic, but it can be if we disregard or exclude the views of everyone else that doesn't fit that mould. Medical schools are moving towards having students whose backgrounds reflect the demographics of the populations they serve; I think we should think of AI developers the same way.

Q: How can we achieve optimal adaptation and a good uptake of AI models globally?

VH: There is a growing worry about the asymmetric concentration of AI developers in countries that are industrial superpowers (e.g. the USA or China) or in powerful multinational corporations (e.g. The Tech 'Big Four'). There are many ways to ease those concerns including but not limited to:

1. International agreements that set standards on what sort of AI research and development is considered acceptable
2. The promotion of diverse workforces that create, implement, and scale AI systems in the public and private sectors
3. Supporting open-source communities that empower all individuals to work on cutting-edge AI solutions to interesting problems, irrespective of resources
4. Promoting thriving start-up ecosystems that prevent a brain-drain into multinational corporations
5. Incorporating public opinion around AI at all levels from international policy to the development of products for individuals

Ruwandi Kariyawasam

Ruwandi Kariyawasam is a PhD student at the University of Toronto.

Shveta Bhasker

Q: Why did you choose Leishmania as your major area of study for your thesis topic for your PhD?

RK: Prior to my PhD, I was working as a research technician on a project focusing on drug resistance in malaria. I always knew I wanted to go to graduate school to study infectious diseases. Fortunately, my current PhD supervisor, Dr. Andrea Boggild was willing to take me on as her graduate student. Her research portfolio focuses on American Tegumentary Leishmaniasis, which is a parasitic infection like malaria. And so I began my MSc.

Q: What makes Leishmania a neglected tropical disease? Why is there not enough funding in neglected tropical diseases?

RK: Neglected tropical diseases (NTDs), by definition, encompass multiple different communicable diseases which affect people in impoverished areas, primarily in the tropical and subtropical parts of the world. According to the WHO, NTDs affect more than one billion of the world's poorest people and cost the economy billions of dollars each year. Leishmaniasis is one disease that falls into this category. Given that these diseases are endemic in many developing countries, there is not enough funding to support research of NTDs.

Q: Is there an increase in Leishmaniasis in Canada?

RK: I do believe there is an increase of Leishmaniasis in Canada. This is supported by a number of studies that have shown an increase in travel-acquired infections in parts of the world that are endemic for Leishmaniasis. Here in Canada, we see quite a lot of cases in travelers as well as people who immigrate from these parts of the world.

Q: What prevention strategies do you think can be implemented in endemic areas for Leishmaniasis (e.g. countries like South America, Central Africa, South and Central Asia)?

RK: At the moment there are no licensed vaccines, however there have been significant developments regarding experimental animal models allowing for phase 1 and 2 clinical trials which is exciting news! For now, I would say active case detection where health workers reach out to the community to screen and document cases of leishmaniasis could aid in prevention strategies as seen by the successful campaigns in the Indian subcontinent.

"For me, I needed to see the applicability of my research."

Q: What got you into research?

RK: In my fourth year of undergraduate studies, I was working on a project which focused on evolutionary genetics in the context of fruit flies. By the time I completed my degree, I told myself I wanted nothing to do with research! Interestingly, that summer I was offered a summer research student position in a microbiology laboratory. It was there that I realized that it's not that I don't like research, it was the kind of research I did that changed my perspective on research. For me, I needed to see the applicability of my research. When I was working in a clinical microbiology laboratory, I found that the research I was doing could directly impact patient care and guidelines and how my work could be translated to the medical community.

Q: Why did you switch into the PhD program after starting in your masters?

RK: I always knew I wanted to become a scientist and oversee my own laboratory. The only way to accomplish this is to get a PhD. When I initially began my MSc, I had not known about transferring into my PhD until Dr. Boggild brought it up. Given my interest in my current project, I thought it would be a great idea to expand on my MSc thesis chapters and get a PhD.

Q: Research comes with its ups and downs – how much does success and rejection impact you?

RK: It's hard not to be excited when you are awarded a grant or when your paper gets accepted. These are all great but, at the same time, you want to think about why you got into research. There should be a greater motive that drives you to do research. It also helps keep you grounded so you don't get carried away with all the success and rejection that happens in research. Success is definitely a lot easier to swallow than rejection, but when you go into research, you need to have an open mind. There will be tough days where your experiments fail, you receive rejection letters for grant applications, but there are also days when your hard work pays off. This is the beauty of research – I do my best not to let success and rejection impact me.

Q: What are your interests outside of research? How do you make time for your passions?

RK: I love music, volleyball, cooking and travelling! I have played the piano since the age of 5, joined numerous volleyball teams throughout my life, and always enjoy cooking a meal at home. I find these interests outside of research help me balance graduate school. These hobbies may not be a part of my full-time job, but they all add up to who I am and how I carry myself. It makes the tough days much easier to handle, and allow me to refocus and go back to the lab ready to persevere.

Q: One piece of advice to aspiring researchers that are questioning if research is for them, since it is a very competitive environment.

RK: If you've ever thought about research, I would recommend trying it. You never know until you try! It's better to get some experience early on so you could make a decision as to whether or not research is for you. You don't need a formal position as a research student, you can volunteer in a lab as well. Talk to people, network, volunteer, be open to collaboration, and don't limit yourself to only one field. At the end of the day, research is highly interconnected. If you're worried about research being unavailable for a certain topic, think again. Speak to a supervisor, bounce your ideas around with them and who knows where it will take you!

Lina Elfaki

Lina Elfaki is a Master of Science student at U of T

Shveta Bhasker

Q: Can you please describe the cardiovascular research for your masters?

LE: An abdominal aortic aneurysms or AAA is in simple terms a swelling that occurs in the largest blood vessel that feeds into all our organs – the aorta. Blood is flowing at a high pressure through the aorta, so once the wall starts to distend, there is a very high risk of the wall rupturing. Aortic rupture is associated with an 80% mortality rate, making it the 10th most common cause of mortality in Western countries.

My research project aims to use a novel technique called Ultrasound-targeted Microbubble Destruction or UTMD to deliver small genetic sequences – microRNAs – that we believe are therapeutic to the aneurysms, speciically to the diseased aorta. To simplify it, these microRNAs, are being coupled to the microbubbles. We then inject the microbubbles into the blood and focus high power ultrasound beams only at the abdominal region where the diseased aorta lies. That detonates the microbubbles there and ensures targeted delivery. All the bubbles circulate in our blood again and again until they are all permeabilized with the ultrasound only at the site of vascular injury, which minimizes side effects of the treatment.

Q: I see that a main focus for your masters is abdominal aortic aneurysm (AAA). How many available therapies are there for AAA? Are there any limitations for the therapies?

LE: Pharmacological approaches, like ACE inhibitors or β-blockers, for AAA therapy have largely remained unproven. Therefore, currently, the only treatment option available for AAA is surgical repair. Even that is only available to larger aneurysms, so you could live for years only monitoring the aneurysm's diameter and waiting for it to become large enough to require surgery. Surgery, of course, carries it own complications, such as post-operative myocardial infarction and renal insufficiency. Therefore, there is substantial research effects to find effective therapy for AAA. Since it's a multifactorial disease, the use of microRNAs that target entire functional networks (rather than one pathway) has yielded some promising results. Hopefully, my research project will bring us one step closer to identifying a clinically-relevant, safe and effective therapeutic for AAA.

Q: Who is mainly affected by AAA? How many people does it affect?

LE: AAA affects 5% of patients who are 60 years of age or older. Smoking, old age and genetic predispositions all put individuals at a great risk for the disease. As well, it is reported that AAA affects males to females in a 4:1 ratio. However, that ratio is compromised by the threshold (30 mm) for AAA that may underestimate incidences in females as they have a smaller normal aortic diameter to begin with, compared to males. What's more, when women develop aneurysms, they grow faster and are 3-4 times more likely to rupture, leading to worsened outcomes in women.

Q: Why do you think there is a research gap when it comes to cardiovascular research for women?

LE: I believe science is very subjective! We try to pretend it's not, and that only impedes us from addressing how our individual prejudices influence the scientific discoveries we make. The Principle Investigator (PI) of a lab is the person who usually decides what kind of research projects to pursue. Currently, there is still a massive gender gap between the number of male and female PIs in Canada and that leads to gender biases when it comes to the diseases and populations we study. Often, studies will cite female hormones as the reason they choose to only consider male disease models. That communicates that male hormones do not add their own complications to the research findings or that we are only aim-

ing to use therapies on one half of our population – males. There are many layers to this issue but it's past time that we made science as inclusive as the populations affected by its findings.

"The fact that an organ the size of my hand can pump blood to the farthest cell of my toe is marvelous."

Q: Why did you decide to go into cardiovascular research?

LE: I have been fascinated with cardiovascular research since high school. The fact that an organ the size of my hand can pump blood to the farthest cell of my toe is marvelous. That led me to gravitate towards cardiovascular science courses and research in my undergraduate degree. Honestly, the more I learned about all the intricate molecular and thermodynamic processes that are being utilized during each cardiac cycle, the more interested I became in pursuing it at the graduate degree.

Q: Can you please describe your advocacy in STEM and what Step into STEM is?

LE: Step into STEM is a UofT-recognized club that aims to eliminate barriers for youths who are from under-represented minorities. I founded the club with a group of friends who are all very passionate about promoting inclusion and diversity in STEM fields. For example, we created an extensive guidebook that has jobs, scholarships, mentorship, and volunteer opportunities, all for students from under-represented minorities. Our goal is to provide youths – mostly high school students – with practical tools and advice to help them explore and navigate their interest in science so that they can pursue it at higher educational levels.

Q: Why did you decide to create Step into STEM?

LE: As an immigrant Muslim Black woman studying at U of T, I have had to creatively carve out new paths and find ways around challenges I faced daily. For example, the lack of representation of faculty and students with similar backgrounds as mine at my undergraduate program made me regularly question if I belonged there so I got involved in various outreach and inclusion advocacy initiatives. However, soon I realized that these programs tend to attract a specific demographic – downtown Toronto residents with family support and an above-average financial status; very rarely were there students who looked like me or shared my experiences. That's when I felt a responsibility to expand my advocacy work. I wanted to give youths like myself connections and opportunities I often missed out on. I wanted to make their journeys a little easier than mine so that they can go a little further and become empowered to advocate for their communities too.

As minority students, all Step into STEM executives and I are grateful for privilege of attending elite universities, so we want to share our advice and guidance to youths in our communities. In the past year, all of our events have sold out in record-breaking time and we have received overwhelming support from other organizations and funding agencies, which tells us how much these initiatives are needed in these various communities.

Q: What can people do on an everyday basis to break down barriers for underrepresented female youth and encourage them to go into STEM?

LE: I think it's important to first reflect on your own privileges and disadvantages you have overcome. Once you know where you stand and what guidance you can offer youth, go the extra mile to participate in programs that are truly inclusive. Its much harder to reach students who are not usually engaged in these programs, due to geographical, financial and accessibility barriers, but its what needs to be done to ensure outreach programs are truly effective. I should point out how much more meaningful your graduate school experience, and life in general, feels when you are playing an active role in advancing your community. It gives me so much joy and a sense of achievement when a student tells me that they are applying to a scholarship they heard about at our event or that they appreciate seeing themselves represented in the scientists we invite to our events.

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