Sciential

McMaster Undergraduate Science Journal

CONTENTS

DEAR READER,

It is our privilege to welcome you back to Issue 2 of *Sciential*! Science communication is the avenue for scientific discovery, advancement, and awareness. Since written communication has pioneered in the field of science, the network of experts has exponentially grown with contributors willingly sharing their findings and expertise leading to the intertwinement of scientific fields. Now, fields of chemistry, biology, physics, and mathematics collaborate to inaugurate new paradigms, contributing to and advancing human legacy. Join us to explore the interdisciplinarity of scientific fields by enjoying the featured Academic Literature Reviews, News and Views, as well as impactful Interviews, all shedding light to the diversity and accessibility of science.

Issue 2 opens with a featured academic literature review by Alisa Nykolayeva and Aiman Shahid, describing heavy metal exposure impact on the morphology of an immortal *Hydra*, widely used as a model organism for toxicity studies. Following, Gursharan Sohi et. al. authored a systematic literature review introducing clinical characteristics, pathophysiology, and treatment regiments of two Glomerular diseases, providing insight on current gaps and future directions on glomerulopathies.

Recognizing McMaster University's impact on scientific discovery, Issue 2 features personal insights of four interviewees on their fields of discipline. An interview conducted by Sophia Khani pitched the careers of two Physician Assistants (PAs) through the lens of Kristen Burrows, the Assistant Dean of the Physician Assistant Education Program, and Ohood Elzibak, a practicing PA in Orthopedic Surgery. In the realm of career options in science, Tyler Redublo manuscripted his interview with an Assistant Professor at the School of Interdisciplinary Sciences, Dr. Katie Moisse, describing careers in the field of science communication. Following, Dr. Maikel Rheinstadter, professor in the Department of Physics and Astronomy, shared his team's advancements in discerning the origins of life while utilizing the newly engineered planet simulator.

The last section of the second issue is dedicated to the novel submission type, News and Views, where authors present recent research milestones and critically gauge the information, providing personal insight on the chosen topic. Freeman Paczkowski interpreted the findings of a study that suggested the implications of a cell shape promoting protein complex as a potential therapeutic for *Helicobacter pylori* infections. Additionally, Bichoy Labib described a lethal bacterial mechanism that may likely be proposed as a novel antibiotic treatment as it also provides an insight into the mechanism of antibiotic resistance. Presented next is a neuroscience article authored by Netri Pajankar that introduces the use of neurophysiological scales in diagnosing the disorders of consciousness. The issue concludes with an insight into the implications of Artificial Intelligence in health care, where the author, Anchana Kuganesan, speculates that the technology will promote higher efficiency health services and lower health-related costs for patients and their families.

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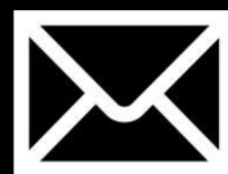
Immortal *Hydra* as a Model Organism for Metal Toxicity Studies

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ABSTRACT

Toxicology is an interdisciplinary scientific field that explores the impact, epidemiology, and treatment regimens for exposure to various toxic compounds and elements. Many toxicants such as metals have not yet been comprehensively examined, and a plethora of metal-related conditions are currently untreatable. *Hydra* is an immortal freshwater organism that serves as an excellent model for toxicity studies due to its natural availability, anatomical simplicity, yet comparatively complex physiology. This review will examine the significance of *Hydra* toxicity studies, outline current experimental designs, as well as summarize the most commonly tested metals. Altogether, comprehensive toxicity studies on *Hydra* might provide promising breakthroughs in the understanding of toxicity-related physiology, and can be applied to clinical research and practice to ultimately improve health and wellbeing of those affected by metal-related disorders.

Keywords: Model organism, *Hydra*, metal toxicity, morphology, experimental setup, regeneration

INTRODUCTION

Model Organisms

In research, model organisms are non-pathogenic living organisms that are carefully and extensively studied to contribute to the understanding of biological processes.[1] They are usually easily sustainable in laboratory conditions, bred in large quantities, have a short life-span, and are cost-effective to maintain.[1] Depending on the research incentive, these organisms are studied to understand genomic and genetic phenomena, disease origin and/or progression, anatomical and histological structures, developmental events, and pharmacologic outcomes, with the prospect that findings obtained from these studies will be applicable to more complex organisms such as humans.[1]

Hydra Anatomy and Regenerative Potential

Carnivorous cnidarian *Hydra* is a model organism used for genetic, developmental, pharmacological, and toxicity studies, and is simple enough to allow for effi-cient *in vivo* experimentation.[2] Biologically immortal, *Hydra* is a freshwater polyp that is said to be ageless due to the unlimited capacity to replace all cells in its body, and it is theorized to be able to survive for over 1400 years in ideal conditions.[3] The organism, thus, possesses the ability to regenerate a missing fragment or a wound upon injury anywhere along the body column, including the peduncle (foot), and the hydranth (head).[2] The hydranth consists of a hypostome, which is the oral tip made of epithelial cells that can temporarily open due to cell rearrangement to allow the entry of prey and excretion of regurgitated food.[4] The hypostome is surrounded by the tentacles that carry terminally differentiated cells called cnidocytes, that are involved in paralyzing and capturing prey.[5] The animal's body column is composed of the gastric section for food digestion, the budding section where a genetically identical polyp grows, and the peduncle.[4] At the very bottom of the peduncle, the basal disk, secretes various types of glycan-releasing granules that enable the *Hydra* to adhere to surfaces.[6] See Figure 1 for anatomical overview.

Hydra is a small diploblastic animal,[7] which makes it extremely sensitive to any environmental change.[4] It is
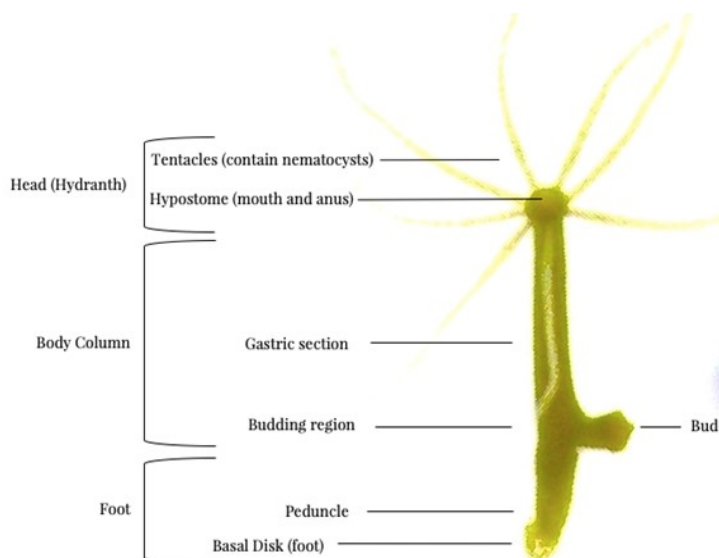
Figure 1. Overview of *Hydra* Anatomy. This figure identifies specific anatomical features of *Hydra* including the head, the body column, and the foot, as well as the developing bud.

composed of only two cell lineages: the epithelial cell lineage which builds up the endodermal and epidermal tissue layers, as well as the interstitial cell lineage dispersed throughout the interstitial space.[8] Both cell lineages possess multipotent stem-cell properties allowing the cells to undergo continuous mitotic divisions, and upon the influence of various intracellular and extracellular factors — permits cell differentiation.[4] The tissue layer between the epidermal and endodermal tissue is called the mesoglea, which is a modified, fibrous extracellular matrix that is evolutionary similar to a basement membrane that is known to partake in molecular signalling.[4] *Hydra's* regenerative capacity is not yet comprehensively understood since there is a multitude of cellular and molecular events which contribute to the phenomenon.[4] However, the mesoglea might have crucial implications in understanding stemness since it regulates intracellular signalling while also acting as a substratum for cell migration.[9]

Phylogenetically, *Hydra* is a very divergent organism from higher animal forms; however, studying this organism provides a crucial knowledge base for development, stemness, differentiation, regeneration, and evolutionary mechanisms such as symbiosis.[10] Overall, roughly 80 different species of cnidarian *Hydra* were identified, and they were clustered into four species groups due to morphological similarity.[10] The genus *Hydra* originated an estimated 60 million years ago with *Hydra viridissima,* green *Hydra*, being the first diverging group, followed by three clades of brown *Hydra* that share a common ancestor.[10] The three clades are called *Braurie, Oligactis,* and *Vulgaris,* in the order of divergence.[10] Green *Hydra* carries photosynthetic endosymbiotic algae, *Chlorella,* which are

known to modulate carbohydrate metabolism. However, other evolutionary advantages of the symbiont have been proposed and require further investigation.[11] Brown *Hydra* lack a symbiont, thus they are called aposymbiotic, and present with efficient lipid metabolism,[12] however, these species tend to exhibit higher protein loss during periods of starvation, which negatively affects their survival.[13]

Complete *Hydra* regeneration, either from a cut segment or a pellet containing aggregates of cells, occurs within a few days.[4] Numerous studies have proven that only the interstitial stem cells are required for complete regeneration of the animal since they are able to differentiate into any other cell type in *Hydra's* body.[14] Signalling to achieve differentiation and regeneration is accomplished by morphogens that are activated and/or upregulated during injury to ensure that the positional information is communicated to regenerate the missing segment.[9]

Since the animal is diploblastic, its epithelial layer is in continual contact with the external environment, and toxins only need to diffuse through two-cell layers to penetrate the organism.[10] This sensitivity and *Hydra's* regenerative capacity makes it suitable for acute and chronic toxicity studies, which are discussed further in this review.

Water Contamination by Heavy Metals

Although roughly 70% of Earth is covered in various types of water bodies, only 3% of it is freshwater, and only roughly 1% is usable, drinkable water. Canada is in a possession of 20% of the planet's freshwater supply, where only 7% is considered renewable due to industrial, medicinal, and nuclear wastes. The Canadian guidelines for drinking water quality have been established by the Federal-Provincial-Territorial Committee on Drinking Water (CDW), and are annually updated by Health Canada. The guide provides information on various types of water contaminants, accompanied by several parameters such as the maximal acceptable concentration (MAC), common sources of a parameter in water, health considerations, and a comments section.[15] Moreover, the guidelines in the document are systematically updated through extensive primary literature appraisal carrying relevant findings. Thus, the guide serves as a foundation to begin toxicity experimentation focusing on specific groups of compounds. Metal toxicity screening combined with clinical studies provides insight into chronic effects, acute toxicity, and birth defects associated with metal compounds in drinkable water. There are various metals, however, that are essential to human health in trace quantities. Such metals include copper, zinc, and chromium, and they are involved in mediating a multitude of biochemical processes. Non-essential metals include lead, mercury, arsenic, and cadmium, and exposure

often results in accumulation of the compound in organ tissues leading to metal overload. The main methods by which these metals are ingested by humans are through poorly filtered drinking water, as well as through the food chain.[16] Therefore, studying the implications of long-term, small-dose exposure is essential to uncover acute and chronic health effects of the aforementioned metals to human health.

Wet laboratories use model organisms to test the toxicity of metal compounds to obtain insight into the effects, mechanisms, and treatment implications of the research. The findings are also used by the government to develop regulations for drinking water quality and testing since optimal water filtration is limited in many areas of the world. This review discusses the common experimental designs used to perform toxicity screens on *Hydra,* specific metals tested in the screens, as well as the species of *Hydra* used for experimentation. Current limitations in any aspect are appraised by the review, and it includes a suggestion to explore a novel experimental design that will specifically target the regenerative potential of *Hydra's* stem cells. This design permits a more comprehensive approach to explore toxicity on a cellular level, and provides insight into the teratogenic and otherwise toxic potential of metal compounds.

DISCUSSION

Hydra Culturing and Experimental Design

Hydra exhibit a very dynamic body column, and some species extend up to 2 cm in length at the most relaxed state.[4] The tentacles of the animal, in a healthy state, are long and flowy, and are usually the first to provide a response to external stimuli.[5] At the state of morphology deterioration leading to the disintegration of the organism, the cnidarian exhibits notable physical features, allowing the observer to quantify the morphological, most commonly, by using the Morphology Scale, which was established by Wilby in 1988 (see Table 1).[17] The scale describes the organism's physical appearance from the stages of thriving to disintegration (death). Disintegrated *Hydra* presents as a cluster of cells attached to its substrates, such as rock or vegetation in the natural environment, or a glass dish, in the lab. The apparent morphological changes in *Hydra*, especially in response to toxicity, provide yet another reason the organism serves as an excellent model for toxicity experimentation.[18]

Naturally, *Hydra* is found in shallow, slow-moving freshwaters such as lakes and rivers, living in cultures of millions of *Hydra*.[8] As environmental conditions change, *Hydra* locomote using various methods that require muscle fibre contraction, tentacle movement, or floating.[8] To mimic natural habitat conditions of

Table 1. Adaptation of Wilby's (1998) Morphology Scale. The scale presents visual characteristics of the *Hydra* quantified with scores 10 (healthy state) to 0 (disintegrated state). The use of this morphology scale is useful in toxicity studies, as it contributes to consistency in data collection and analysis permitting accurate species response quantification and metal impact comparison.

Score	Morphology
10	Extended tentacles and reactive body column
9	Partially contracted tentacles; slow reactions to stimuli
8	Clubbed tentacles and slightly contracted body column
7	Shortened tentacles and a slightly contracted body column
6	Shortened tentacles and body column
5	Totally contracted body column, short and visible tentacles
4	Totally contracted body column, with no visible tentacles
3	Expanded body column, yet visible, short tentacles
2	Expanded body column, with no visible tentacles
1	Dead but intact
0	Disintegrated; appears as a cluster of cells at the bottom of the substrate

the animal in the lab and to prevent mass disintegration of *Hydra* culture, individual *Hydra* must be placed in glass dishes or bowls and filled with *Hydra* Medium (HM).[4] HM, similar in composition to freshwater, is a mixture of highly diluted salts in double distilled water. Namely, it consists of calcium chloride salt ($CaCl_2$ ● $2H_2O$), TES buffer (N-tris [hydroxymethyl]methyl 1-2-aminoethanesulfonic acid buffer), and EDTA (Ethylenediaminetetraacetic acid) that are diluted in double distilled water.[18] The composition of the HM, however, might vary amongst the laboratories. The culture plates are usually kept in a temperature-controlled incubator, at around 20°C; however, the temperature can vary amongst the laboratories and species used.[18]

The 1997 study by Trottier S. et al., is used here as a general example for the experimental setup of a typical *Hydra* toxicity study. The organisms are starved for 24 hours prior to experimentation, ensuring that the regurgitated waste does not contaminate the solution.[18] Feeding does not occur in the duration of the experiment,[19] likely to control for contamination as well as potential budding, since feeding stimulates an increase in the rate of asexual reproduction.[19] This was a cross-sectional study tracking three *Hydra* per treatment group, once daily.[18] A 12-well microplate was used, where a third of the wells were dedicated for the control variable which is the HM in this case.[18] The screen was carried on for 96 hours, where at every 24-hour time-points the *Hydra* were qualitatively assessed and imaged using a stereoscope.[18] Budding *Hydra* in the

experimental set-up were avoided, ensuring each individual well was occupied by three *Hydra* only.[18] Test solutions were diluted in HM using logarithmic dilution for pure substances and serial dilution for aqueous environmental samples.[18] Prior to placing the animal into the experimental microplate with its respective solutions, the animal was washed in a petri dish filled with the respective test compounds.[18] Different concentrations of the compound are utilized to observe the gradient of its effect on *Hydra* morphology.[18] Usually, replicate microplates are set up to increase the sample size for improved statistical power at the time of analysis. Two measures of drug potency are generally applied to determine the effect of a drug on an organism:[18] EC50 - half maximal effective concentration and LC50 - half maximal lethal concentration. EC50 is used to measure the induced response of the compound halfway between the baseline and maximal effect at some duration of exposure, as opposed to the LC50, which estimates the concentration of the compound that kills half of the population at some duration of exposure. In the case of *Hydra* toxicity screen in Trottier's study, EC50 is applied as the *Hydra* exhibit clubbed tentacles, whereas LC50 is applied as the *Hydra* disintegrate.[18] Thus, the study measured sub-lethal toxicity of the compound, as well as lethal effects, utilizing the same experimental setup.[18]

A 1998 study by Beach M. J. et al. utilized a 6 mL-well of a repli dish with a single *Hydra* populating the space. This was a longitudinal study, which tracked an individual *Hydra* over time, after having been exposed to a particular compound.[7] The overall sample size of the study was 20 individuals per each tested metal concentration, and the study randomly selected both, budding and non-budding subjects during the setup.[7] Beach's study measured toxicity similarly, but with the addition of a feeding protocol for the sub-lethality screen.[7] Similarly to Trottier's study, 24-hour time intervals were used to collect data on the animal's morphology and any associated conditions, and the experiment lasted for a total of 96 hours for the acute toxicity screen. For sub-lethal toxicity, at the 48-hour time point, the subjects were presented with five *Daphnia* neonates as their prey.[7] Feeding behaviour was recorded after each 20-minute interval for two hours and the response was assessed by the amount of shrimp ingested by each organism.[7] Differently from Trottier, however, *Hydra* cultures were kept at 18 hours light and six hours dark lighting regiment, at 20 +/- 1°C.[7]

A 2000 study by Karntanut W. and Pascoe D. conducted an experiment similar to Beach; however, testing was performed in 3 mL glass vials carrying 1 polyp per space, with a total of ten subject replicates.[17] As opposed to recording the LC50 and EC50, Wilby's scale was used to assess the trend of *Hydra* response, allowing for consistent numerical categorization of *Hydra*

Normal morphology:	Clubbed/shortened tentacle morphology:	Tulip morphology:	Loss of regulation morphology:	Disintegrated morphology:
Extended/partially contracted tentacles; Reactive, extended body column	Clubbed/shortened tentacles; Contracted body column	Slightly visible, but completely contracted tentacles; Variable body column	No visible tentacles; Dead but intact	Appears as a cluster of cells at the bottom of a substrate

Figure 2. **Condensed Overview of *Hydra* Morphology in Response to Toxicity.** Stages of morphological deterioration were adapted from Wilby's (1988) Morphology Scale (Scores 10-0) are portrayed in a condensed manner. The overview features five stages: normal, clubbed/shortened tentacle, tulip, loss of regulation, and disintegration morphologies.

morphology per time-point. LT50 or median lethal time response was applied in addition to LC50 to further compute the response of the animal to the toxin.[17] See Figure 2 for the condensed overview of *Hydra* morphology in response to toxicity.

Numerous other studies have been performed using similar, but not identical experimental setup. This, although inconsistent, allows for further understanding of *Hydra's* physiology in relation to metal toxicity, as every study provides for a unique set of variables. Some designs are cross-sectional, but longitudinal studies are commonly performed to avoid various co-variables such as the state of deterioration, which may affect neighbouring *Hydra* to disintegrate as well. If the subject occupies the space alone, this variable would be eliminated. Irrespective of the experimental design, physiological toxicity can be concluded based on the experimental data. The main compelling advancement to the studies would be to perform toxicity screens where the *Hydra* is dissected and allowed to heal and regenerate over a period of time. This will provide a more comprehensive insight into metal toxicity since it will target regeneration mechanisms and its susceptibility to metal toxins.

Compounds Tested and Species Response

Hydra have been shown to accumulate metals through waterborne and foodborne routes,[20] evident through sampled nematocysts contents that led to reduced prey capture.[4] Traces of metals were also found in the algae of the symbiotic *Hydra*.[4] Thus, *Hydra* is an excellent bioindicator for metal toxicity studies, as it lacks metallothionein, a binding protein, that aids with sequestration of metals.[4]

The 2002 study by Karntanut W. and Pascoe D. used *Hydra vulgaris* (pink *Hydra*) and copper sulfate ($CuSO_4 \bullet 5H_2O$), cadmium chloride ($CdCl_2 \bullet 21/2 H_2O$), and zinc chloride ($ZnSO_4 \bullet 7H_2O$) as test compounds to conduct their study. Ranges of concentrations for the study were 0.018–0.32 mg l^{-1} for copper, 0.10–3.20 mg l^{-1} for cadmium, and 18.0–56.0 mg l^{-1} for zinc. LC50 values at the 96hr time point were 0.025–0.084 mg l^{-1}, 0.16–0.52 mg l^{-1} and 11–14 mg, respectively, and this was the study's terminal point.[21] Data analysis identified that copper exhibits more toxicity than cadmium or zinc, however, cadmium was comparatively more toxic than zinc. Qualitative observations identified that *Hydra* morphology changed in response to the toxic compound, beginning from progressive tentacle clubbing, shortening, and detachment.[21] The body of the organism seemed to be progressively contracted.[21] These descriptions have been quantified by morphology scores, adapted from Wilby (1998). The regular HM mimics true living conditions of the organism and promotes proper conditions for

osmoregulation.[4] However, during exposure to the compound, the body of the organism was swollen,[21] indicating an osmoregulatory impairment.[4]

Hydra viridissima has its distinctive green colour due to symbiote photosynthetic algae, which might have been affected by the targeting metal, since copper is a potent algaecide.[22] The pink *Hydra*, which does not carry the symbiont, however, was not affected until subjected to higher copper concentration. Pollino C. and Holdway D. A. (1999), have also reported that green *Hydra* have been more sensitive,[22] perhaps, due to the inhibition of photosynthesis performed by the algae.[23] This finding was also confirmed by Karntanut W. and Pascoe D. (2005) study, where amongst four species of *Hydra: Hydra oligactis,* two strains of *Hydra vulgaris,* and *Hydra viridissima,* the later exhibited higher sensitivity to both copper and cadmium.[22] Karntanut W. and Pascoe D. study has also discovered that bleached *H. viridissima* exhibited higher sensitivity to low concentrations of copper without its symbiont, in opposition to *H. viridissima* with intact symbiont that was more affected.[24] Karntanut hypothesized that *Chlorella* sequesters the copper, which results in symbiotic green *Hydra* being more resilient to the metal at low concentrations.[24] This benefit however, is indifferent to higher copper concentrations, at which green *Hydra* appear to be more sensitive than other species.[24]

The 2001 study conducted by Holdway A. D. et al., screened cadmium and zinc on two *Hydra* species: *Hydra vulgaris* and *Hydra viridissima* (green *Hydra*). Mortality of green *Hydra* occurred at cadmium concentration of 1.6 μg/L, whereas the pink *Hydra* stayed resistant to the compound until the concentration reached 100 μg/L.[25] At t-96h, the terminal time-point of the study, the LC50 values for 3 μg/L of cadmium chloride were 27 times higher for green *Hydra* than pink *Hydra*.[25] Zinc toxicity testing has also exhibited higher resistance in *Hydra vulgaris,* with total mortality occurring at 8,000 μg/L, whereas the green *Hydra* appeared disintegrated at 2,000 μg/L.[25] Such drastic differences in impact of metals on species morphology and survival likely occur due to differences in species-specific physiology. Further research by Holdway concluded that elimination of light in experimental design had no significant impact on *Hydra* regeneration, concluding that photosynthesis is not being affected by cadmium.[25] The impact is speculated to have targeted the differences in metabolism as the green *Hydra* primarily utilizes carbohydrates for energy, rather than lipid-centered metabolism demonstrated by the pink *Hydra*.[25] Further studies are needed to determine how cadmium interferes with carbohydrate metabolism. Cadmium is a transition metal with a very lengthy half life, varying by the isotope.[26] This feature allows cadmium to accumulate over time, and there are no proven effective interventions to reverse cadmi-

um over load.[26] Cadmium and zinc have similar bioligands, thus, upon exposure, cadmium interferes with many zinc-mediated metabolic processes.[26] The differential zinc impact has occurred due to defective osmoregulation that occurs in *Chlorella* as zinc triggers altered cell membrane permeability.[4] Nitrogen fixation and photosynthesis are also known to be affected, thus, reduced rates of mitosis are observed leading to disintegration of the organism.[4] Comparing the metals' impact, cadmium is noticeably more potent at lower concentrations than zinc. This phenomenon occurs on the basis of the fact that cadmium is not an essential metal, as opposed to zinc. Zinc is used for numerous cellular functions, such as signalling, cell membrane structure, and is a common biochemical cofactor. Thus, its effect might be sequestered by the organism, until the concentration crosses a certain threshold.

CONCLUSION

The freshwater polyp *Hydra* serves as excellent model organism for metal toxicity studies due to its ease of culture, sensitivity to toxic compounds, and timely response to exposures. Experimental setup of toxicity screens is very efficient allowing for both, cross-sectional and longitudinal studies to be performed. The review has appraised multiple studies that tested the impact of different ranges of copper, zinc, and cadmium on *Hydra* morphology. Copper and zinc are essential metals and partake in a multitude of biochemical pathways. Specifically, copper is known to participate in biochemical cascades and reactions involving transcriptional regulators, chaperones and storage proteins, cell surface/secretory compartment transporters and receptors, oxidoreductases, oxidases, monooxygenases, and electron transfer-involving proteins.[27] This heavy metal has been found in both eukaryotes and prokaryotes, and specifically in *Hydra*, copper is known to impact reactive oxygen species (ROS)-mediated reactions that result in DNA damage, activation of programmed cell death called apoptosis, and modulation of antioxidant biomarker genes, all evident through morphological changes in response to exposure.[28] Limited information is available regarding the role of zinc in modulating toxic responses in *Hydra*. However, zinc is an essential trace metal and it is involved in a large multitude of biochemical processes including cell proliferation and differentiation, prevention of free radical formation, and binding of metallothioneins, that are absent in *Hydra*.[29] More research is suggested to determine the molecular mechanisms of zinc toxicity on *Hydra*. Cadmium, on the other hand, is not an essential metal, and tends to accumulate in cells due to its long half life, resulting in a toxic response at low concentrations. Thus, findings from *Hydra* toxicity screens are relevant and applicable for use during the compilation and revisions of the Guidelines for Canadian Drinking Water Quality. This will

be used to develop policies and regulations ultimately improving human health. Additionally, *Hydra* is a small animal found attached to rocks or vegetation, as well as it may be found floating in water. Naturally, *Hydra* may be ingested by larger freshwater organisms, thus contributing to the food chain and further bioamplification of metals. Further research needs to be conducted to investigate how metals impact *Hydra's* regenerative potential, therefore, regeneration assays are recommended to help investigate current understanding of metal-related pathologies.

ACKNOWLEDGEMENTS

REFERENCES

(1) Ankeny RA, Leonelli S. What's so special about model organisms? Studies in History and Philosophy of Science Part A. 2011;42(2):313–23.

(2) Bosch TCG, Anton-Erxleben F, Hemmrich G, Khalturin K. The Hydra polyp: Nothing but an active stem cell community. Development, Growth & Differentiation. 2009;52(1):15–25.

(3) Khokhlov AN. On the immortal hydra. Again. Moscow University Biological Sciences Bulletin. 2014;69(4):153–7.

(4) Quinn B, Gagné F, Blaise C. Hydra, a model system for environmental studies. The International Journal of Developmental Biology. 2012;56(6-7-8):613–25.

(5) Steele RE. Developmental Signaling in Hydra: What Does It Take to Build a "Simple" Animal? Developmental Biology. 2002;248(2):199–219.

(6) Rodrigues M, Leclère P, Flammang P, Hess MW, Salvenmoser W, Hobmayer B, et al. The cellular basis of bioadhesion of the freshwater polyp Hydra. BMC Zoology. 2016;1(1).

(7) Beach MJ, Pascoe D. The Role Of Hydra Vulgaris (Pallas) In Assessing The Toxicity Of Freshwater Pollutants. Water Research. 1998;32(1):101–6.

(8) Thorp JH, Covich AP. Ecology and classification of North American freshwater invertebrates. San Diego: Academic Press; 2001.

(9) Barzansky B, Lenhoff HM. On the Chemical Composition and Developmental Role of the Mesoglea of Hydra. American Zoologist. 1974;14(2):575–81.

(10) Hemmrich G, Anokhin B, Zacharias H, Bosch TC. Molecular phylogenetics in Hydra, a classical model in evolutionary developmental biology. Molecular Phylogenetics and Evolution. 2007;44(1):281–90.

(11) Cook CB. Benefit To Symbiotic Zoochlorellae From Feeding By Green Hydra. The Biological Bulletin. 1972;142(2):236–42.

(12) Pardy RL, White BN. Metabolic Relationships Between Green Hydra And Its Symbiotic Algae. The Biological Bulletin. 1977;153(1):228–36.

(13) Cook CB, Kelty MO. Glycogen, protein, and lipid content of green, aposymbiotic, and nonsymbiotic hydra during starvation. Journal of Experimental Zoology. 1982;222(1):1–9.

(14) Bosch TCG. Hydra and the evolution of stem cells. BioEssays. 2009;31(4):478–86.

(15) Guidelines for Canadian Drinking Water Quality Summary Table [Internet]. 2010 [cited 2019Mar28].Availablefrom: https://www.canada.ca/content/dam/hc-sc/migration/hc-sc/ewh-semt/alt_formats/pdf/pubs/water-eau/sum_guide-res_recom/sum_guide-res_recom-eng.pdf

(16) Akpor OB, Muchie M. Remediation of heavy metals in drinking water and wastewater treatment systems: Processes and applications. International Journal of the Physical Sciences. 201;5(12):1807-17.

(17) Karntanut W, Pascoe D. A comparison of methods for measuring acute toxicity to Hydra vulgaris. Chemosphere. 2000;41(10):1543–8.

(18) Trottier S, Blaise C, Kusui T, Johnson EM. Acute toxicity assessment of aqueous samples using a microplate-based Hydra attenuata assay. Environmental Toxicology and Water Quality. 1997;12(3):265–71.

(19) Rodrigues M, Leclère P, Flammang P, Hess MW, Salvenmoser W, Hobmayer B, et al. The cellular basis of bioadhesion of the freshwater polyp Hydra. BMC Zoology. 2016;1(1).

(20) Karntanut W, Pascoe D. A comparison of metal accumulation by the cnidarian Hydra vulgaris directly from water or through contaminated prey and effects upon reproduction and regeneration. Songklanakarin Journal of Science and Technology. 2007;29(3):869-80.

(21) Karntanut W, Pascoe D. The toxicity of copper, cadmium and zinc to four different Hydra (Cnidaria: Hydrozoa). Chemosphere. 2002;47(10):1059–64.

(22) Pollino CA, Holdway DA. Potential of Two Hydra Species as Standard Toxicity Test Animals. Ecotoxicology and Environmental Safety. 1999;43(3):309–16.

(23) Jouany J, Ferard J, Vasseur P, Gea J, Truhaut R, Rast C. Interest of dynamic tests in acute ecotoxicity assessment in algae. Ecotoxicology and Environmental Safety. 1983;7(2):216–28.

(24) Karntanut W, Pascoe D. Effects of removing symbiotic green algae on the response of Hydra viridissima (Pallas 1776) to metals. Ecotoxicology and Environmental Safety. 2005;60(3):301–5.

(25) Holdway DA. Hydra Population Reproduction Toxicity Test Method. Small-scale Freshwater Toxicity Investigations. 2005;:395–411.

(26) Waisberg M, Joseph P, Hale B, Beyersmann D. Molecular and cellular mechanisms of cadmium carcinogenesis. Toxicology. 2003;192(2-3):95–117.

(27) Festa RA, Thiele DJ. Copper: An essential metal in biology. Current Biology. 2011;21(21).

(28) Zeeshan M, Murugadas A, Ghaskadbi S, Rajendran RB, Akbarsha MA. ROS dependent copper toxicity in Hydra -biochemical and molecular study. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 2016;185-186:1–

(29) Stefanidou M, Maravelias C, Dona A, Spiliopoulou C. Zinc: a multipurpose trace element. Archives of Toxicology. 2005;80(1):1–9.

A Review of Glomerular Diseases: Focal Segmental Glomerulosclerosis (FSG) and Minimal Change Disease (MCD)

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ABSTRACT

Purpose: Idiopathic focal segmental glomerulosclerosis (FSGS) and minimal change disease (MCD) are chronic glomerulopathies which may compromise patients' quality of life, and for which there is no cure. This literature review aimed to summarize our current understanding of the pathophysiology, clinical characteristics, and best available treatment for the two conditions in order to outline a consolidated treatment protocol and identify future research considerations.

Methods: PubMed was systematically searched by a single reviewer in order to identify primary studies pertaining to the diagnosis, treatment and classification of FSGS and MCD. Additionally, a hand search of UpToDate was conducted to glean further information about the best available evidence as summarized for clinician use. Relevant information was extracted and synthesized.

Results: Primary FSGS and MCD result from distinct pathogenic mechanisms, hypothesized to involve kidney injury via immune dysregulation. Patients require a kidney biopsy for diagnostic purposes. First-line treatment involves glucocorticoids (i.e. prednisone), although patients' responsiveness may be inconsistent; second-line treatment is immunotherapy.

Conclusion: This review summarized clinically-important information about FSGS and MCD, and emphasized the need for further research in the field of clinical nephrology. Large scale trials such as the Cure Glomerulonephropathy should be conducted to gather information about the affected population.

Keywords: Focal segmental glomerulosclerosis, minimal change disease, Glomerulonephropathy, histology, review paper, clinical management

INTRODUCTION

Glomerulonephropathy (GN) refers to a broad category of inflammatory glomerular diseases, which often manifest with proteinuria, hypoalbuminemia, and edema.[1] Focal segmental glomerulosclerosis (FSGS) and minimal change disease (MCD) are two specific GN diseases which are the subjects of this review. Each may present idiopathically (*primary GN)* or consequent to kidney injury by systematic disease *(secondary GN)*. Diagnosis is exclusively based on histological characteristics seen on renal biopsy.[2] However, little is known about the unique pathophysiology of each condition and consequently, treatment options are rather limited. This review seeks to better understand the clinical symptoms and histologic features of FSGS and MCD, as well as to summarize the current treatment protocol for these diseases in the general adult population.

Millions of individual nephrons within each kidney process blood to produce urine through filtration, secretion, and reabsorption. The glomerular capillary

tuft mechanically filters the components of blood by size on a pressure-based system, barring individual cells and large proteins from entering the tubular nephron. Several cell types make up the glomerulus: parietal cells, capillary endothelial cells, podocytes, and mesangial cells.[1] Glomerular injury can disrupt the fine homeostatic balance maintained by this system, frequently resulting in proteinuria and hematuria as the filtration membrane is widened. Proteinuria ≥3.5 g/day, accompanied by edema, hyperlipidemia and hypoalbuminemia constitutes nephrotic syndrome (NS), an umbrella term for symptoms which are often the first indicators of FSGS or MCD.[2] These two diseases present with similar clinical features, yet each has a distinct pathogenic mechanism, necessitating separate treatment plans following pathological investigation and diagnosis.[3] Further clarifying what is known about these processes may aid clinicians in advising patients, as well as guiding further research to address unanswered questions.

METHODS

Search

Six search terms were entered into the PubMed database (September-October 2018): "(idiopathic minimal change disease) AND (biopsy)", "(idiopathic focal segmental glomerulosclerosis) AND (biopsy)", "(idiopathic minimal change glomerulonephritis) AND (treatment)", "(idiopathic focal segmental glomerulosclerosis) AND (treatment)", "(primary focal segmental glomerulosclerosis)" and "(primary minimal change disease)". The search was exhausted once duplicate results appeared frequently. Results were filtered to include studies from the last 10 years (2009-2018) and pertaining to humans only. The rationale for selecting relatively recent studies was to gather information about developing treatments on the forefront of research efforts, as well as to ensure the feasibility of the search given that a single reviewer would be responsible for screening titles. The search yield using these terms was 1380 citations. Additionally, a manual search was conducted of the UpToDate database, a resource which provides summative resources for clinicians use. This proved more relevant to information-gathering within the scope of this research paper. The following search terms were used: "minimal change disease", "idiopathic focal segmental glomerulosclerosis", "Canadian society of nephrology clinical guidelines", and "focal segmental glomerulosclerosis, minimal change disease". In order to find the seminal studies which determined clinical protocol, the citations of top search results were manually searched. These search terms were crafted based on prior knowledge of the subject and consultation with an expert clinician. Consequently, the manual search was instrumental for amassing clinically-relevant informa-

tion to bolster the findings of this review.

Eligibility and Critical Appraisal

Primary studies investigating MCD and/or FSGS which were published in a peer-reviewed publication in the English language within the last 10 years (2009-2018) were included during the systematic search. Studies were excluded for irrelevance if they focused on: nephrotic syndrome generally (without making specific reference to MCD and/or FSGS in the title or abstract), secondary disease rather than idiopathic MCD/FSGS, or genetic markers of disease. Genetic factors were not assessed because the scope of this paper addresses clinically available markers of disease and treatment. Opinion pieces, abstracts, book chapters, editorials, nonhuman studies, and case reports were excluded.

Study Identification and Selection

Screening of 1380 non-duplicate titles and abstracts yielded a cohort of 234 citations to review in full. Of the 218 excluded papers: 102 were considered out of scope (i.e.: not pertinent to the questions posed in this review), 14 were specific to genetic markers of disease, 74 had an ineligible study design, and 28 addressed GN broadly rather than focusing on FSGS, MCD or both. These citations were assessed in their entirety, and 16 manuscripts were included. The methodological quality, risk of bias and precision of each study was qualitatively assessed at this point based upon the reviewer's prior experience with medical literature, and poorly-conducted studies were excluded. A formal critical appraisal using a risk of bias tool was not performed primarily due to the mix of study types being evaluated. A manual search of UpToDate was conducted to gather more specific information about treating glomerular diseases, which resulted in additional studies being included (Figure 1). All searches, screening and data extraction were completed by a single assessor. The findings of this review apply specifically to
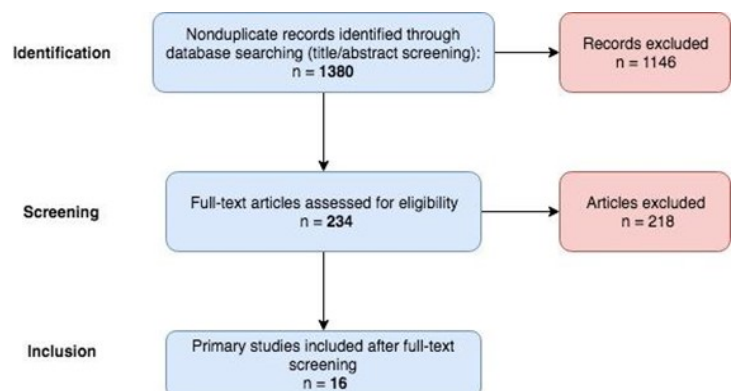
Figure 1. PRISMA flow diagram detailing the study inclusion process for this review.
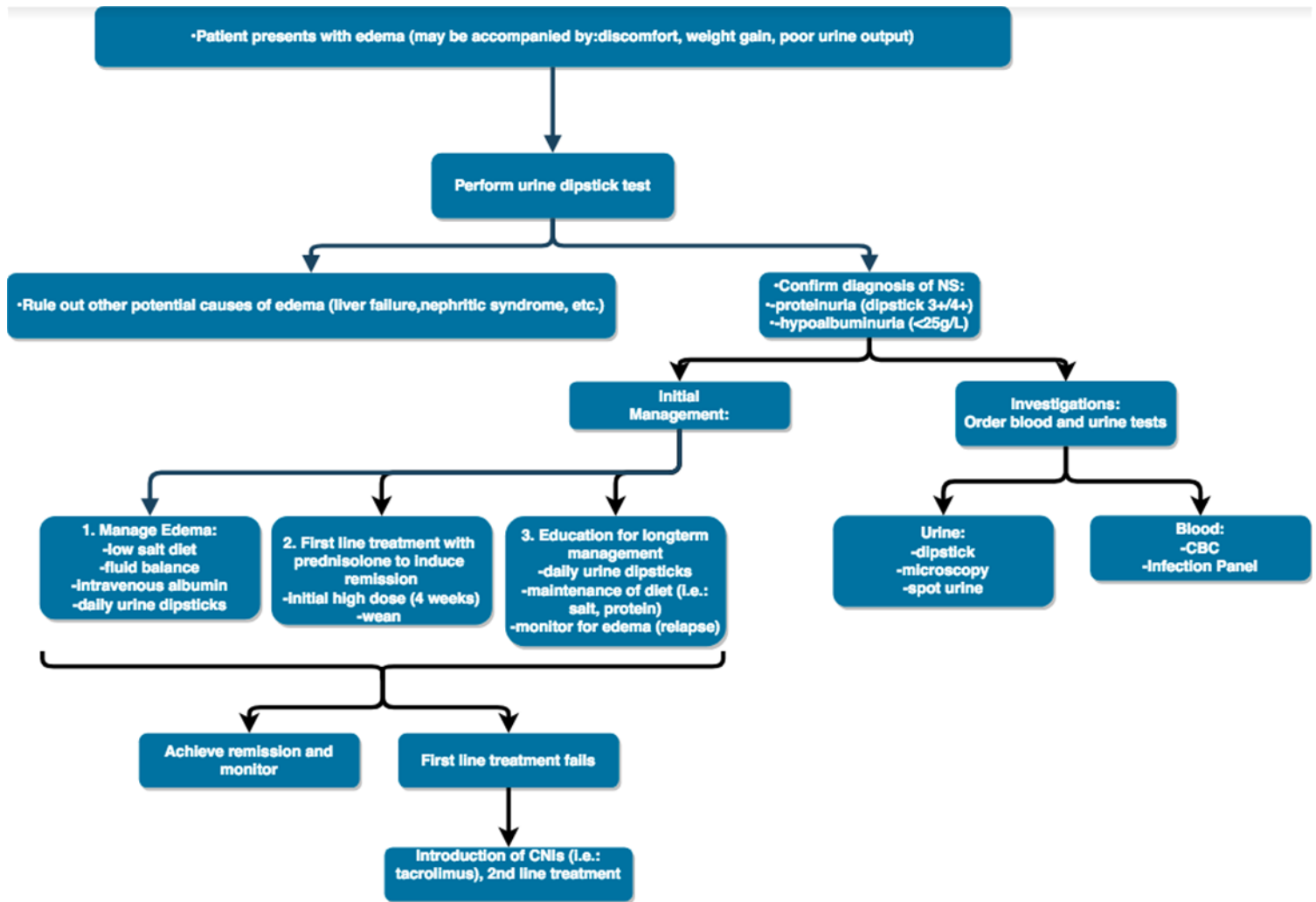
Figure 2. Standard clinical plan for diagnosis and management of patients presenting with NS.

idiopathic FSGS and MCD in adults; secondary disease warrants separate evaluation as its pathogenesis, treatment, and prognosis differ significantly from primary disease.

RESULTS

### Clinical Presentation and Symptoms

Patients suspected to have glomerular disease may present with unexplained weight gain, foamy urine and peripheral edema upon physical examination (Figure 2).[4] Frequently, they may also have a history of hypertension and in cases of MCD particularly, may have experienced an explosive onset of symptoms.[4] Bloodwork and a random urine test should be ordered at this point. Typically, lab results indicate significant proteinuria, often, in the NS range, along with hypoalbuminemia and hyperlipidemia.[2, 5] This evidence may suffice to diagnose GN, however, a more specific diagnosis of FSGS, MCD, or another condition necessitates renal biopsy.

A biopsy is nearly always warranted, exempting two rare cases: i) performing the biopsy itself would result in significant harm to the patient, or the patient is unwilling to proceed, ii) glomerular injury secondary to systemic disease is strongly suspected (e.g. patients with Type II Diabetes who are experiencing diabetic nephropathy).[6, 7] In general, FSGS is more common in adults and is frequently secondary, although causative factors may be difficult to determine.[8, 9, 10] The pathologist's determination of MCD, FSGS, or related glomerular disease is made at this point, after which treatment can be pursued. Standard treatment for both conditions includes a steroid regimen which frequently gives rise to toxic side effects and significantly compromises patients' quality of life.[2] For this reason, the renal biopsy is imperative to confirming diagnosis prior to pursuing an intensive treatment course.

Diagnostic Criteria and Biomarkers

Pathological determination of FSGS or MCD using electron microscopy is the current gold standard in GN diagnostics. Light microscopy cannot detect variation

amongst and between individual glomeruli to sufficient detail to detect podocyte effacement.[11] As its name implies, MCD is particularly difficult to diagnose given that it appears nearly identical to an undamaged specimen when examined with light microscopy – only electron microscopy suffices to view the podocyte foot process effacement (Figure 3), which causes disease symptoms.[11] Biopsy results must be interpreted in the context of clinical and laboratory findings, especially given that FSGS and MCD share a number of histologic features. The key differentiating factor between the two is the hardening of intraglomerular mesangial cells (mesangial sclerosis) which compromises capillary structure in FSGS (Figure 4).[11] However, extracted samples may be unclear (due to poor technique or damage, for example), or suffer from sampling error by failing to include an adequate number of glomeruli (at minimum, n = 23) to make a definitive diagnosis.[9, 11] These technical challenges of detecting sclerotic lesions and the upstream implications for disease treatment prompt the need for alternative diagnostic techniques at the biopsy level.


A   B
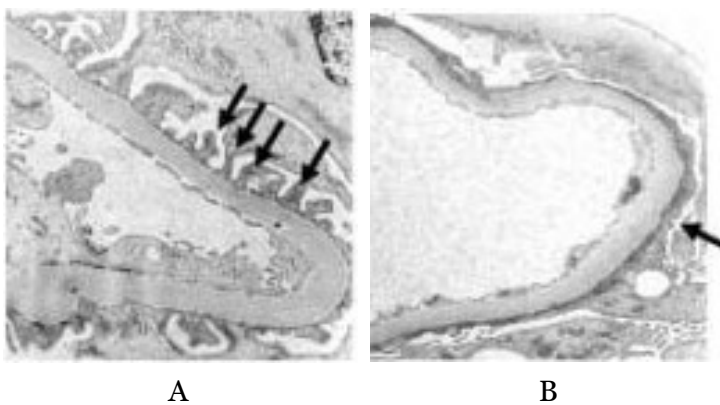
Figure 3. Glomerular foot processes in a normal (a) and MCD (b) renal biopsy viewed with electron microscopy; the latter shows significant foot process effacement.[15]

The diagnosis of FSGS presents an additional challenge because the disease may be expressed as one of five pathologic subtypes which are distinct in their prognostic implications (Figure 3).[12] The variants of FSGS according to the Columbia classification system are as follows: collapsing (≥1 glomerulus showing segmental or global collapse), tip (segmental lesion on the glomerular cells nearest the proximal tubule), not otherwise specified (NOS) (segmental damage to the glomerular capillary loop), perihilar (lesions at the glomerular pole) and cellular (damage to the glomerular capillary loop with hypercellularity).[12] Pathologists should attempt to describe the subtype of FSGS when analyzing a biopsy sample because each subtype may respond differently to treatment, despite their similar clinical presentation.[12] Collapsing FSGS frequently presents with heavy proteinuria and progresses rapidly, often leading to end-stage renal disease (ESRD).[12-14]

Cellular FSGS has been similarly described; this may be due either to a common causal mechanism underlying the two variants, or because of diagnostic challenges which limit our ability to detect their differences on biopsy.[12-14] Patients with collapsing and cellular variants are also more likely to be steroid-resistant upon usual treatment, further supporting the hypothesis that the two subtypes are at least closely related if not truly identical.[12, 14] The tip variant of FSGS may also demonstrate rapid disease progression, however these patients are less likely to experience CKD and/or ESRD.[13, 14] Some studies have described this variant as having a relatively less-severe prognosis, however findings did not reach statistical significance. Goals for further research should more clearly distinguish cellular versus collapsing FSGS variants, as well as plan for an adequately-powered and timed study to bolster findings related to disease prognosis.

Several studies have investigated the utility of certain biomarkers in discriminating between FSGS and MCD. Although not yet validated through extensive

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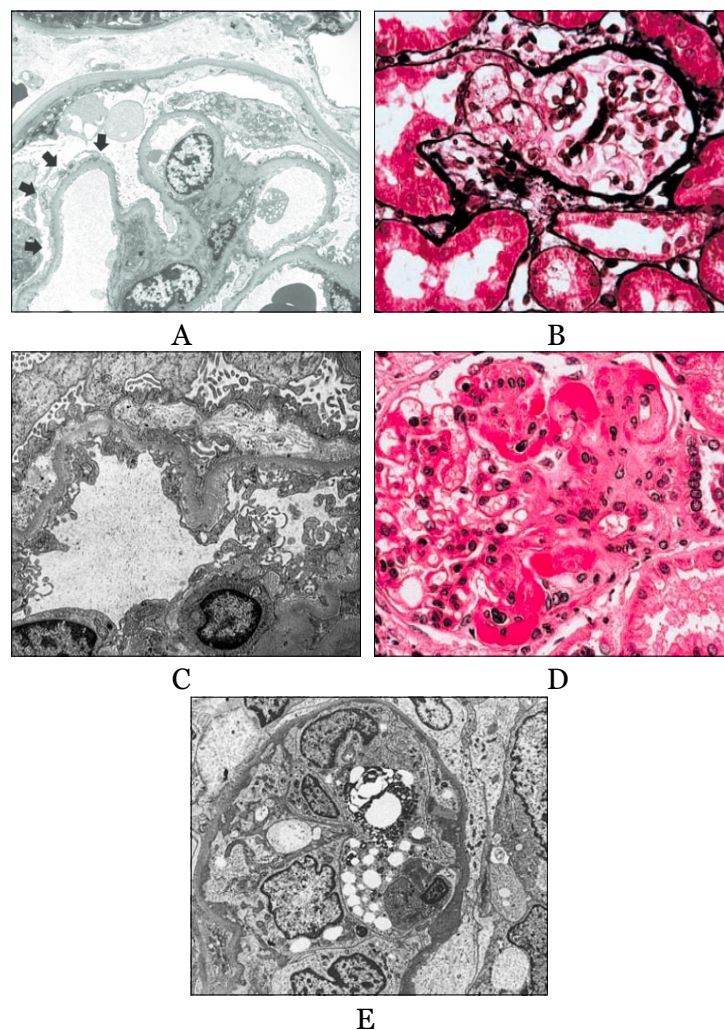
Figure 4. Microscopy images representing FSGS variants (A) collapsing, (B) tip, (C) NOS, (D) perihilar and (E) cellular.[16]

study, cell-surface adhesion receptor CD44 shows some potential as a diagnostic marker of FSGS. CD44 is expressed on activated parietal epithelial cells (PECs), which are involved in the formation of glomerular sclerotic lesions in FSGS.[11] Pathological studies conducted in 2012 and 2014, respectively, detected sclerotic lesions in the same glomerular regions as CD44 immunostaining.[3, 11] Furthermore, CD44 expression was more robust and widely-distributed in patients with advanced disease, which may inform hypotheses regarding the mechanism and temporality of FSGS incidence.[3] Other biomarkers which have shown some success in distinguishing GN diseases include malondialdehyde, an indicator of oxidative stress, and fibrinogen, a soluble glycoprotein.[8-10] Both are elevated in FSGS biopsies and reflect glomerular irritation which may result in sclerotic lesioning.[8-10] Further research involving more patients at varying stages of disease is necessary to confirm the utility of these indicators as robust markers of FSGS vs. MCD.

Disease Mechanism

GN diseases such as FSGS and MCD may develop either idiopathically or secondarily to a systemic disease or genetic condition. FSGS is more likely attributable to secondary causes in adults, which may include: HIV, obesity, other renal diseases, or sickle cell anemia.[8] The sclerotic lesions evident upon biopsy are simply a symptom of kidney injury due to an underlying pathology. MCD is more likely to result idiopathically, often presenting with a sudden onset of symptoms.[6] Infrequently, some secondary causes such as neoplasms, atopy and certain infections may create renal damage characteristic of MCD.[6] The proteinuria which distinguishes these disorders is caused by effacement of podocyte foot processes (see Figure 5), as seen on biopsy.[8] However, there is no consensus regarding the causal factors which may originally cause glomerular injury in idiopathic cases.[6]

It has become clear that primary FSGS and MCD develop from separate disease processes. There are two primary theories addressing the etiology of MCD. Firstly, increasing evidence implicating T-cells and the cell-mediated immune response has recently come to light as the injurious factor in primary MCD.[17] For example, Garin and colleagues (2015) demonstrated that MCD patients respond, albeit temporarily, to immunotherapy targeting CTLA-4, a T-cell surface receptor and CD80 inhibitor, while FSGS patients were unresponsive.[18] These findings are reinforced by independent reports of elevated urinary CD80 excretion in MCD proteinuria.[3,18] This suggests that dysregulation of the CD80 pathway within podocytes may be responsible for initiating glomerular injury in MCD. Additionally, other medications targeting the cell-mediated immune response such as cyclophosphamide have shown some efficacy in treating MCD which further

lends weight to this theory.[17] The second hypothesis regarding MCD pathogenesis describes an unknown circulating glomerular permeability factor as initiating glomerular injury. Some studies have described T-cell mediated interleukins, particularly IL-13 as potential causative factors. However, the evidence for this theory is not robust and warrants further investigation.[17]
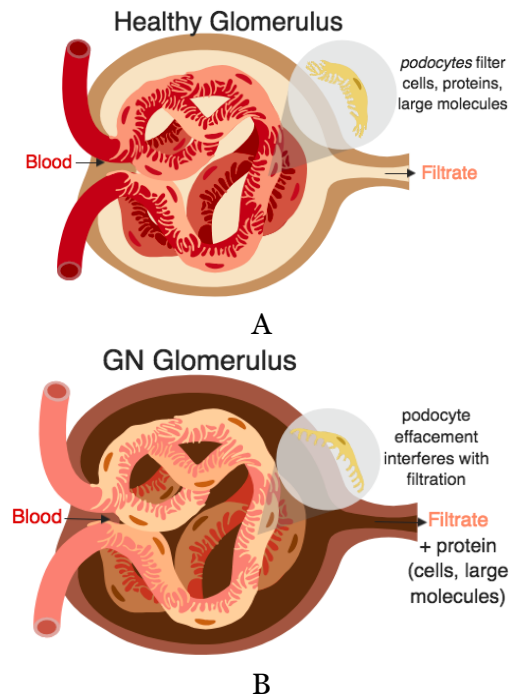
Figure 5. Illustrated glomeruli highlighting podocyte effacement in GN diseases; glomerular damage is theorized to be caused by local immune dysregulation (MCD) or by circulating permeability factors (FSGS).

Theorized mechanisms of idiopathic FSGS pathogenesis tell a similar story. Although unconfirmed, it is likely that some circulating factor is responsible for causing initial damage to PECs within the glomerulus.[18] Several pieces of evidence support the veracity of this mechanism. Firstly, animal studies have shown that injected serum from FSGS patients initiates proteinuria in otherwise healthy rats.[19] Additionally, FSGS has a relatively high rate of recurrence following remission or the receipt of a kidney transplant, which indicates the pathogenic factor is likely distal to the kidney. Soluble urokinase receptor (suPAR) has been investigated as a potential instigator of FSGS symptoms, given its functional role in adhering podocyte foot processes to the glomerular basement membrane.[18, 20] Serum suPAR levels are markedly elevated in FSGS patients, however the association is only correlational.[20] Other studies have considered microRNA (miRNA) as the circulating factor potentially responsible for FSGS. Some miRNA subtypes have been found inhibit expression of crucial podocyte-associated proteins in wildtype mice, and in human studies miRNA levels have been shown to be elevated in primary FSGS

patient glomeruli compared to controls.[21] Although the cause of injury has not yet been elucidated, the development of sclerotic lesions in FSGS is better understood:

1. Glomerular injury results in PEC activation, marked by notable cell proliferation and the production of fibrous proteins (i.e.: elevated levels of CD44 as seen on biopsy).[3, 11]
2. Local irritation results in the accumulation of matrix proteins and aggravates podocytes.[3]
3. Podocytes are terminally-differentiated cells which show low regenerative capabilities; hence they react to initial injury by hypertrophy which disrupts the filtration barrier.[3]
4. The structural changes to the glomerulus appear to produce the non-inflammatory sclerotic lesions that characterize FSGS, further underscoring the distinctness of this pathogenic mechanism from that of MCD.[3, 11, 18]

Regardless of the event responsible for initial kidney injury, both FSGS and MCD patients experience heavy proteinuria as a result of podocyte damage which compromises the glomerular filtration barrier. Consequently, large proteins from the blood are able to pass into the glomerular duct system. The excretion of protein in the urine contributes to: hypoalbuminemia (loss of albumin from the blood), edema, foamy urine (high protein content lowers liquid surface tension) and abnormal lipid metabolism (hyperlipidemia triggered by a decrease in blood oncotic pressure).[2, 4, 22] There is evidence that symptoms are reversible upon efficient diagnosis and treatment of disease.[22]

Treatment

Gold standard treatment for FSGS outlined by the International Society of Nephrology consists of high-dose glucocorticoid medication (e.g. prednisone, prednisolone) tapered after initial response.[7, 23] Patients with secondary FSGS or those with very low-grade proteinuria are not treated with steroids; conservative options including blood-pressure management through pharmaceuticals and lifestyle modifications are pursued as first-line treatment in this population. To date, this is the only routine proven efficacious by randomized-controlled trial data for the treatment of primary FSGS patients.[7] However, not all patients are responsive to prednisone and relapse rates - especially for FSGS - are high.[7, 24] What follows is a discussion of current clinical management followed by an introduction to recent findings in exploratory treatments for FSGS, then MCD.

Idiopathic FSGS is typically treated initially with glucocorticoids if patients demonstrate NS symptoms, which may be able to achieve remission with prolonged use.[7, 19, 23] Other immunosuppressants may also

be used rather than prednisone/prednisolone. While glucocorticoid dose tapering is often effective, certain patients may demonstrate steroid-resistance or steroid-dependence during this phase of treatment.[19] Steroid-dependent idiopathic FSGS patients are those who relapse either while receiving, or soon after stopping glucocorticoid treatment. Conversely, steroid-resistant FSGS constitutes patients who fail to respond at all to initial treatment. Both groups who are ineffectively treated with glucocorticoids are subsequently treated with second-line therapy, which includes calcineurin-inhibitor drugs (CNIs).[19]

CNIs such as tacrolimus and cyclosporine A (CsA) have been thoroughly investigated for their efficacy in addressing the autoimmune dysregulation underlying FSGS, either in combination with low-dose prednisone or alone. Gorsane and colleagues (2016) retrospectively analyzed 23 patients with idiopathic FSGS, concluding that CsA was effective at achieving complete or partial remission in 57% of patients after approximately one year of treatment, although some nephrotoxic side effects were noted.[7] Similarly, tacrolimus has proved efficacious in the treatment of primary FSGS with estimated remission rates of approximately 60% reported.[19, 25] Studies investigating tacrolimus also report a relatively low incidence of adverse effects, the most severe of which seemed to be diarrhea and/or worsened hypertension in some patients (approximately 12%).[25, 26]

In some cases, second-line treatment may also prove ineffective, prompting clinicians to explore other treatment options which may only be supported by low or mid-level evidence. Immunosuppressants such as adrenocorticotropic hormone (ACTH) gel and similar analogues have been used with moderate success in treating some GN cases, particularly in patients with membranous nephropathy.[27, 28] Investigators seeking to evaluate its utility in treating FSGS have reported complete or partial remission in approximately 30% of patients treated with biweekly subcutaneous ACTH injections.[27] Furthermore, multiple studies of ACTH for steroid-unresponsive idiopathic FSGS patients have been plagued by high attrition and a significant incidence of adverse effects as a result of treatment.[27, 28] Despite trends towards remission of proteinuria demonstrated in these studies, the lack of robust benefit and frequency of adverse effects associated with ACTH therapy bars it from consideration as a plausible second-line treatment currently. Furthermore, ACTH therapy is extremely expensive, which creates a financial barrier to using it in exploratory or clinical settings. Other treatments options are also being explored. For example, the success of CNIs led Cho et. al to investigate the efficacy of sirolimus, a molecule with a similar structure but different immune target than tacrolimus (mammalian target of rapamycin, rather than calcineurin phosphatase) in treating FSGS.[29]

Their case-series of 6 patients was stopped early for safety reasons after 5 patients experienced severe adverse effects including worsened proteinuria.[29] It is clear that better treatment options are needed beyond first-line steroid treatment for idiopathic FSGS.

Initial management for MCD is often targeted towards management of hypertension and edema in addition resolving glomerular injury using immunosuppressant medication.[30] Patients are typically advised to follow a low-salt diet and potentially prescribed an antihypertensive medication along with glucocorticoid therapy.[30] Prednisone or prednisolone are generally very effective in achieving remission of idiopathic MCD within a few months of treatment, although approximately 10% of patients are steroid-resistant.[30] In these patients, CNIs may be used as second-line therapy. Furthermore, patients who exhibit frequent relapses of MCD when treated with tapering doses of glucocorticoids are often prescribed a continuous low dose of the medication to manage symptoms, which may be combined with a CNI in some cases.[30] Currently, there are few alternative treatments available for idiopathic MCD and consequently, steroid-responsiveness is an asset. Nakayama and colleagues (2002) retrospectively studied 62 adults with biopsy-proven MCD to determine which prognostic factors, if any, influence a patient's degree of steroid-responsiveness.[31] The majority of patients (n=53) were treated with prednisolone only, while the remainder received combination treatment with a second-line immunosuppressant.[31] Late responders (remission of proteinuria <3 g/day after 8 weeks of treatment) had more severe hematuria and renal impairment at presentation, and also displayed a larger interstitial volume on biopsy.[31] Notably, there was a significant negative correlation between age of onset and frequency of relapse, meaning that younger patients tended to benefit less from the treatment and seemed at higher risk for steroid-dependence.[31] Other studies have presented similar findings; moreover, the side-effects of first-line treatment make it a less-than-ideal long-term option even if patients respond well initially.

DISCUSSION

Key Conclusions

This paper sought to summarize our current understanding of the pathogenesis, diagnosis and treatment of idiopathic FSGS and MCD respectively, and to make recommendations for future research. These two GN diseases are difficult to distinguish between and consequently, closely associated in clinical settings. Patients with either disease often present with nephrotic-range proteinuria and warrant a renal biopsy to specify

a diagnosis. Biopsy must be performed using electron microscopy and evaluate an adequate number of glomeruli to detect any focal lesions characteristic of FSGS; otherwise the two pathologies may be easily confused to even the well-trained eye. Thus, it may be advantageous to identify biomarkers of each disease which can be tested for upon biopsy. This review found that CD44 may be a feasible and effective marker of activated PECs in FSGS but not MCD, albeit these findings are limited and not yet clinically useful. Clarification of diagnosis, including FSGS subtype, is also important for prognostic reasons and treatment planning. It is clear that FSGS and MCD each develop from unrelated initial events and thus should differ in management per patient. Disease management is standardized, but not ideal. Although the Kidney Disease Improving Global Outcomes guidelines suggest steroid therapy (frequently prednisolone) as a first-line treatment, rates of non-responsiveness, disease relapse or steroid-dependence, respectively, are high, rendering this treatment option far from ideal. Recent studies have explored other methods of treatment to varying degrees of success. For instance, CNIs such as tacrolimus have proved efficacious in achieving remission, thus recommending it as a second-line treatment option; however the long-term consequences of CNI use are yet unknown. Similarly, ACTH therapy has been recently evaluated in treating FSGS however the results are not promising, suggesting that this treatment should be refined or abandoned.

Limitations

This narrative review of the literature was limited by several factors. Firstly, the literature search, development of eligibility criteria and inclusion of papers was conducted by a single reviewer who had no particular expertise in the field of nephrology. For the sake of feasibility only one database (PubMED) was systematically searched; this was supplemented by a hand-search of UpToDate to glean more specific information. Unpublished data, gray literature and conference abstracts were not included, however the many small and inconclusive studies included makes it unlikely that the review suffered from publication bias. The majority of the included studies were relatively small, primary experiments and there was considerable heterogeneity between studies with regards to geographic location, patient population, methodology and even results. Thus, readers should proceed with caution when assessing the conclusions made in this paper and use it as general information rather than as a decision-making tool. This paper's research question was rather unfocused and consequently this paper addressed FSGS and MCD as a broad overview rather than answering clinically-relevant questions. Finally, much of the included research was purely qualitative and/or would have contributed to significant heterogeneity, which discouraged the pooling of data to esti-

mate overall findings. For these reasons the results of this study should be used as background information to learn about FSGS and MCD, and perhaps to inform the development of future research questions.

This review sought to provide a fundamental overview of the rare glomerular diseases FSGS and MCD. It touched upon clinical signs and symptoms, underlying pathogenic mechanisms, as well as ongoing research regarding biomarkers of the diseases and treatment options. Key findings include the affirmation that FSGS and MCD stem from separate disease processes despite their similar presentation and that certain biomarkers may help to distinguish between the two pathologies on renal biopsy. The evidence and productivity of research in this subfield of nephrology is relatively weak. Consequently, it would be irresponsible to draw robust conclusions from the findings of this paper. It has become sufficiently clear that bigger and better studies are needed to learn about GN diseases. Cure Glomerulonephropathy is an example of one such study which hopes to gather enough data to learn about nephropathies in the general population and ultimately lead to improved patient outcomes (see *Next Steps, below*).

Next Steps: Cure Glomerulonephropathy (CureGN)

The findings of this paper serve to provide a basic overview of recent literature published on the topic of FSGS and MCD. More importantly, it is able to point out glaring gaps in our knowledge of these diseases such that effective research can be done towards improving patient outcomes. CureGN is an ongoing multi-centre, cohort study which recognized the need to gather more information about GN.[32] It began enrolling patients in December, 2014 and aims to include n=2400 adult and pediatric patients diagnosed with idiopathic GN diseases including FSGS and MCD. As of August 3, 2018, 2202 patients were enrolled, an impressive feat considering the relative rareness of primary GN diseases in the general population (estimated period prevalence 2007-2011 in the US population= 306/100 000 persons).[32, 33] The study aims to establish a longitudinal cohort of GN patients, collecting biospecimens and patient-reported outcome information over a 4 year period. Concurrent to a literature search, the authors of this paper gathered anecdotal information about current research in GN diseases by assisting with data collection for the CureGN study under the supervision of Dr. P. Boll and Martin Romano at Credit Valley Hospital in Mississauga, Ontario. The study requires participants to undergo an initial diagnostic biopsy within 5 years of enrollment. The sample is reviewed by a pathologist, undergoes both electron microscopy and immunofluorescent assessment, and is stored at a biorepository site in Michigan.

Following enrollment, patients are assessed 4 times per year; blood samples and urine specimens are procured at each visit if possible.[32] Outcomes of the study are broad and varied within the following categories: epidemiology (including demographics, medical history, etc.), biomarkers (renal biopsy, blood and urine samples), genetics (analysis of blood and urine samples) and patient-reported outcomes (quality of life).[32] The miscellaneous nature of CureGN's target outcomes reflects the severe lack of concise information regarding these diseases in the literature currently. The study aims to standardize data-collection methodology amongst a large sample which is representative of the general population, hoping to spur more specific ancillary studies derived from this initial database. The cacophony of fragile and/or conflicting results from small, observational studies renders research progress slow and unproductive in the field of GN disease. Hence, this multicentre trial is a necessary first step towards providing a more robust characterization of the patient population and leading the way to more personalized treatment options.

ACKNOWLEDGEMENTS

REFERENCES

(1) Vaughan, M.R., Quaggin, S.E. How do mesangial and endothelial cells form the glomerular tuft? JASN. 2008: 19;1: 24-33. Available from: doi https://di.org/10.1681/ASN.2007040471 [Accessed November 20, 2018].

(2) David S. Liebeskind. Chapter 26 - Nephrotic syndrome. Editor(s): José Biller, José M. Ferro. Handbook of Clinical Neurology, Volume 119. 2014. Pages 405-415. Available from: https://doi.org/10.1016/B978-0-7020-4086-3.00026-6 [Accessed November 20, 2018].

(3) Fatima H, Moeller MJ, Smeets B, et al. Parietal epithelial cell activation marker in early recurrence of FSGS in the transplant. Clin J Am Soc Nephrol. 2012;7 (11):1852-8. Available from: doi 10.2215/CJN.10571011 [Accessed November 20, 2018].

(4) Dantas, M. et al. Foamy urine in nephrotic syndrome. Clinical Kidney Journal. 2013. 6 (341). Available from: doi 10.1093/ckj/sft018.

(5) Kodner, C. Nephrotic syndrome in adults: diagnosis and management.

(6) Glassock, R. Secondary minimal change disease. Nephrology Dialysis Transplantation. 2003. 18(6):52-58. Available from: doi 10.1093/ndt/gfg1060

(20-7) Gorsane, I. et al. Cyclosporine therapy in steroid-dependent or steroid-resistant idiopathic focal and segmental glomerulosclerosis. Saudi Journal of Kidney Diseases and Transplantation. 2016. 27(5):958-965. Available from: doi 10.4103/1319-2442.190864

(8) Agrawal, V. et al. Reduced podocin expression in minimal change disease and focal segmental glomerulosclerosis is related to the level of proteinuria. Clinical and Experimental Nephrology. 2013. 17: 811-818. Available from: doi 10.1007/s10157-013-0775-y

(9) Nezhad, S. et al. Glomerular malondialdehyde levels in patients with focal and segmental glomerulosclerosis and minimal change disease. Saudi Journal of Kidney Diseases and Transplantation. 2010. 21:5: 886-891. Available from: PMID 20814126

(10) Wang. Urinary fibrinogen and renal tubulointerstitial fibrinogen deposition: Discriminating between primary FSGS and minimal change disease. Biochemical and Biophysical Research Communications. 2016. 478: 1147-1152. Available from: doi https://doi.org/10.1016/j.bbrc.2016.08.083 .

(11) Smeets, B. et al. Detection of activated parietal epithelial cells on the glomerular tuft distinguishes early focal segmental glomerulosclerosis from minimal change disease. The American Journal of Pathology. 2014. 184(12): 3239-3248. Available from: doi 10.1016/j.ajpath.2014.08.007

(12) Nickavar, A., Rahbar, M. A clinicopathologic study of children with idiopathic focal segmental glomerulosclerosis. Fetal and Pediatric Pathology. 2016. 355(3):159-166. Available from: doi 10.3109/15513815.2016.1153177

(13) Swarnalatha, G. et al. Focal and segmental glomerulosclerosis: does prognosis vary with the variants? Saudi Journal of Kidney Diseases and Transplantation. 2015. 26(1):173-181. Available from: PMID 25579744

(14) Arias, L.F. et al. Histologic variants of primary focal segmental glomerulosclerosis: presentation and outcome. Jornal Brasileiro de Nefrologia. 2013. 35(2):112-119. Available from: doi 10.5935/0101-2800.20130019

(15) UNC Kidney Centre. Minimal Change Disease. UNC School of Medicine. Available from: https://unckidneycenter.org/kidneyhealthlibrary/glomerular-disease/minimal-change-disease/ [Accessed February 5, 2019].

(16) D'Agati, V. Pathologic classification of focal segmental glomerulosclerosis. American Journal of Kidney Diseases. 2004. 43(2):368-382. Available from: doi https://doi.org/10.1053/j.ajkd.2003.10.024.

(31-17) Myrier, A., Radhakrishnan, J. Etiology, clinical features and diagnosis of minimal change disease in adults. UpToDate. April 17, 2017. Available from: https://www.uptodate.com/contents/etiology-clinical-features-and-diagnosis-of-minimal-change-disease-in-adults?search=MCD%20mechanism&source=search_result&selectedTitle=1~30&usage_type=default&display_rank=1#H2 [Accessed February 2, 2019].

(18) Garin, E. Case series: CTLA4-IgG1 therapy in minimal change disease and focal segmental glomerulosclerosis. Pediatric Nephrology. 2015. 30(3):469-477. Available from: doi 10.1007/s00467-014-2957-6

(19) Cattran, D.C., Appel, G.B. Focal segmental glomerulosclerosis: treatment of primary focal segmental glomerulosclerosis. UpToDate. November 14, 2018. Available from: https://www.uptodate.com/contents/focal-segmental-glomerulosclerosis-treatment-of-primary-focal-segmental-glomerulosclerosis?search=FSGS%20management&source=search_result&selectedTitle=1~146&usage_type=default&display_rank=1#H17 [Accessed February 2, 2019].

(20) Wei, C. Circulating suPAR in two cohorts of primary FSGS. Journal of the American Society of Nephrology. 2012. 23(12):2051-2059. Available form: doi 10.1681/ASN.2012030302

(21) Fervenza, F.C., Sethi, S. Focal segmental glomerulosclerosis: pathogenesis. UpToDate. November 5, 2018. Available from: https://www.uptodate.com/contents/focal-segmental-glomerulosclerosis-pathogenesis?search=FSGS%20mechanism&source=search_result&selectedTitle=1~146&usage_type=default&display_rank=1#H1859932283 [Accessed February 2, 2019].

(22) Radhakrishnan, J. Lipid abnormalities in nephrotic syndrome. UpToDate. 2018. Available from: https://www.uptodate.com/contents/lipid-abnormalities-in-nephrotic-syndrome [Accessed 5 December, 2018].

(23) Official Journal of the International Society of Nephrology. KDIGO Clinical Practice Guidelines for Glomerulonephritis. [2, 2]. June 2012. Available from: http://www.kdigo.org/clinical_practice_guidelines/pdf/KDIGO-GN-Guideline.pdf .

(24) Cara-Fuentes, G. et al. Angiopoietin-like-4 and minimal change disease. PLoS One. 2017. 12(4). Available from: doi 10.1371/journal.pone.0176198

(25) Kallash, M., Aviles, D. Efficacy of tacrolimus in the treatment of children with focal segmental glomerulosclerosis. World Journal of Pediatrics. 2015. 10(2):151-154. Available from: doi 10.1007/s12519-014-0484-y.

(26) Xu, D. et al. Tacrolimus improves proteinuria remission in adults with cyclosporine A-resistant or -dependent minimal change disease. Nephrology. 2017. 22:21-256. Available from: doi 10.1111/nep.12991

(27) Hogan, J. et al. Treatment of idiopathic FSGS with adrenocorticotropic gel. Clinical Journal of American Society of Nephrology. 2013. 8(12):2072-2081. Available from: doi 10.2215/CJN.02840313.

(28) Bomback, A.S. et al. Treatment of resistant glomerular diseases with adrenocorticotropic hormone gel: a prospective trial. American Journal of Nephrology. 2012. 36:58-67. Available from: doi 10.1159/000339287

(29) Cho, M. et al. Sirolimus therapy of focal segmental glomerulosclerosis is associated with nephrotoxicity. American Journal of Kidney Diseases. 2007. 49(2):310-317. Available from: doi 10.1053/j.ajkd.2006.10.020

(30) Meyrier, A., Radhakrishnan, J. Treatment of minimal change disease in adults. UpToDate. October 15, 2018. Availble from: https://www.uptodate.com/contents/treatment-of-minimal-change-disease-in-adults?search=MCD%20management&source=search_result&selectedTitle=1~30&usage_type=default&display_rank=1 [Accessed February 2, 2019].

(31) Nakayama, M. et al. Steroid responsiveness and frequency of relapse in adult-onset minimal change nephrotic syndrome. American Journal of Kidney Diseases. 2002. 39(3):503-512. Available from: doi 10.1053/ajkd.2002.31400

(32) Kretzler, M. et al. Cure GN: cure Glomerulonephropathy network core study protocol. Version 1.2. NIH-NIDDK, NephCure Kidney International. September 8, 2014.

(33) Wetmore, J. et al. Prevalence of glomerulonephritis in the U.S. Medicare population. Chronic Disease Research Group. 2012. Available from: http://www.cdrg.org/media/1369/2014-asn_wetmore_gn-prevalence.pdf [Accessed December 21, 2018].

(34) Nephrotic Syndrome. The Royal Children's Hospital Melbourne. 2018. Available from: https://www.rch.org.au/clinicalguide/guideline_index/Nephrotic_syndrome/ [Accessed December 21, 2018].

(35) Hull, R.P., Goldsmith, D.J.A. Nephrotic syndrome in adults. BMJ. 2006. 336:1185-1189. Available from: doi 10.1136/bmj.39576.709711.80.

(36) Kodner, C. Diagnosis and management of nephrotic syndrome in adults. American Family Physician. 2016. 93(6):479-485. Available from: t www.aafp.org/afp/2016/0315/p479-s1.Html.

(37) Moranne, O. et al. Primary glomerulonephritis: an update on renal survival and determinants of progression. QJM. 2008. 101: 215-224. Available from: doi 10.1093/qjmed/hcm142.

APPENDIX

Table 1. Diagnostic criteria for various disease states amongst GN patients.[34-36]

	24-hour urine protein	Albumin	Clinical Signs	Other common findings
NS Diagnosis	3.5g	<25g/L	• Peripheral edema • Abdominal cramps • Dizziness • Thrombosis • Peripheral hypoperfusion	• Hyperlipidemia (>350mg/dL) • Microscopic hematuria
Complete Remission	<0.3g		• Diminished edema • Large quantities of urine produced	
Partial Remission	0.3-3.5g		• Diminished edema • Large quantities of urine produced	
Relapse	3.5g/day for 3 consecutive days		• Peripheral edema • Abdominal cramps • Dizziness • Thrombosis • Peripheral hypoperfusion	• Hyperlipidemia • Microscopic hematuria

INTERVIEW

WITH KRISTEN BURROWS AND OHOOD ELZIBAK

HOW MCMASTER IS FULFILLING THE NEED FOR PHYSICIAN ASSISTANTS IN CANADA

Interviewer: Sophia Khani

McMaster University, Honours Life Sciences, Class of 2021

ARTICLE INFORMATION

ABSTRACT

The Physician Assistant (PA) profession has been growing over the past decade in Canada, and McMaster University was the first institution in Ontario to establish a PA program in 2008. The PA profession occupies a niche in the health care field and is needed to extend physician services contributing to the improvement of patient care. McMaster's PA Education Program (PAEP) fosters collaboration and problem-based learning (PBL) in order to provide students with skills that are transferable to the workplace. McMaster's PBL approach arms graduates with life-long learning skills that are practical in a clinical setting. The following interviews with PAs provide a more comprehensive understanding of the development of the PA niche and proffer a glimpse into how McMaster's PAEP equips its graduates with practical skills, which will allow them to excel in the workforce.

Kristen Burrows, BSc, BHSc (PA), MSc, CCPA

Kristen Burrows is the Assistant Dean of McMaster's PAEP, and was interviewed on the pedagogical principles behind the program and the importance of the PA profession. She was a member of McMaster's PAEP class of 2010 and is currently completing her Ph.D. on the benefits of and barriers to PA integration into the field of health care.

► What is a Physician Assistant?

Physician Assistants, or "PAs", are trained health care providers that help extend various health care services by working under the supervision of a physician. PAs are trained in the medical model to take a medical history, conduct a physical examination, order diagnostic tests, interpret results, diagnose and/or treat medical conditions, counsel, and manage patients.

► Describe the need for PAs in the health care field.

PAs are needed to help increase patient access to health care and to provide cost-effective services to patients. Although Physicians, Nurse Practitioners, and other advanced clinicians currently provide exceptional care, hospital and clinic wait times are still long and the current system might not always be the most cost-effective. PAs are trained to extend physician du-

ties and can fill a number of gaps in our current delivery of health services.

► **Is there any research, especially at McMaster University, on the impact of the integration of PAs in Canada's health care field?**

There is very little literature regarding the impact of PAs in Canada compared to other jurisdictions, such as the USA, where PAs have been fully integrated for decades. Graduates from McMaster's PAEP have published a few studies, such as on the impact of PAs on resident workloads, improving health care efficiency, and the role of PAs in health advocacy. I am currently conducting research on the benefits of and barriers to PA integration as part of my Ph.D. dissertation.

► **Describe the pedagogical principles of McMaster's PA program.**

McMaster's PA Education Program (PAEP) is part of the Michael DeGroote School of Medicine, in the Faculty of Health Sciences. It is a bachelor's program (BHSc Physician Assistant) that consists of 24 months of intensive clinical training. The program is modelled after the undergraduate medical school curriculum, with the first year comprising of problem-based tutorial cases, simulations, large group sessions, professional competencies, an emphasis on clinical skills, and communication courses. The second year of the program is comprised of various core and elective clinical rotations ("clerkship") where PAs observe certain procedures and learn to assist with them under the supervision of a clinical practitioner. PA students are integrated into clinical teaching units across the province in Emergency Medicine, Internal Medicine, Family Medicine, General Surgery, Psychiatry, Geriatrics, Pediatrics, and various other specialties.

► **How are these principles integrated into the education system for PAs?**

The PAEP's pedagogical approach has allowed for a flexible program that can be adapted based on where PAs are working and feedback from stakeholders. For example, a core rotation in Geriatrics was established in 2018. This was integrated into the program following community and stakeholder feedback regarding the importance of ensuring that our PA graduates have a strong skill set to apply holistic care to an aging population. The PAEP's educational strategy helps PAs appreciate lifelong learning and is an excellent way to learn about collaborative patient management.

"PAs help increase patient access to health care"

Ohood Elzibak, BHSc, MPAS, CCPA

Ohood Elzibak is a PA who practices in Orthopedic Surgery and was interviewed to describe how her training at McMaster successfully translated into her PA profession. Ohood currently holds an appointment at McMaster University as an Assistant Clinical Professor (adjunct) and is involved in musculoskeletal clinical teaching as well as a preceptorship in Orthopedic Surgery.

► **What is Orthopedic Surgery?**

Orthopedic Surgery is a specialty in medicine that involves the diagnosis and treatment of conditions of the musculoskeletal system, including bones and articulations of bones (joints), muscles, tendons, ligaments, cartilage, bursae, and nerves.

Orthopedic Surgeons are specialized physicians who complete medical school followed by a five-year residency and one or two years of fellowship training in a specific area of practice (e.g. arthroplasty, trauma, or sports). Orthopedic Surgery PAs are clinicians who work alongside Orthopedic Surgeons to evaluate and treat patients with musculoskeletal complaints. PAs complete undergraduate studies and then enrol in a professional PAEP, completing 12 months of a focused didactic program. This is followed by 12 months of hands-on rotations in a variety of practice areas (e.g.

Family Medicine, Emergency Room (ER), Internal Medicine, Surgery and Surgical Subspecialties, Psychiatry, and Pediatrics). As collaborative providers, PAs work with increasing autonomy to improve clinical efficiency and participate in all aspects of patient care (e.g. history taking, physical examination, diagnosis, nonoperative treatment, surgical treatment/first assist, and preventive care).

Orthopedic Surgeons and PAs are becoming increasingly sub-specialized to provide care in focused areas of practice, such as sports medicine, joint replacement (arthroplasty), or trauma. Additionally, many clinicians focus their practice on a subset of anatomical regions or populations (e.g. foot and ankle, upper extremity, spine, hip and knee, or pediatric versus adult populations) to provide up-to-date evidence-based care using advanced treatments.

"McMaster's PBL model allowed me to identify gaps in my knowledge and skill set"

▶ **What does your role as a PA look like on a day-to-day basis?**

I work in the field of sports orthopedics and arthroscopy; most of our patients present with shoulder pain (e.g. rotator cuff or labral tears), knee pain or instability (e.g. ACL tears or meniscal tears), elbow or wrist conditions (e.g. tendonitis), and occasionally, early stages of osteoarthritis.

My role is divided between the outpatient clinic, fracture clinic, and operating room. At the outpatient clinic, I complete consultations, follow-up assessments, and pre- and post-operative evaluations of patients with pain, instability, or stiffness. I take a history to better understand the nature of the patient's condition, perform a physical examination, review diagnostic reports and images (e.g. X-ray, CT scan, and MRI), discuss treatment options, and administer treatments (e.g. provide a physiotherapy referral, perform a joint injection, apply a cast, prescribe NSAIDs, or discuss surgery). Patients who are undergoing complex procedures or require a more specialized care have the opportunity to spend more time with my supervising physician or surgeon. This is to ensure that they understand the technical aspects of the surgery and have a clear understanding of surgical outcomes.

▶ **How do you think the PBL model of McMaster's teaching transfers to and benefits your work life in Orthopedic Surgery?**

I was first exposed to the PBL model in my undergraduate years in the Bachelor of Health Sciences program and had the opportunity to further utilize this style of learning in PA school. The first year of the PA Education Program at McMaster is heavily reliant on PBL; students participate in tutorial sessions to explore topics and generate questions. They then independently search for answers to fill any gaps in their knowledge. Afterwards, students re-group to discuss their newly acquired information and clarify concepts.

When I first started working in Orthopedic Surgery, I had to actively participate in learning both, on the job and on my own time after work. McMaster's PBL model allowed me to identify gaps in my knowledge and skill set, retrieve appropriate evidence-based resources to address those gaps, and apply theoretical concepts to my day-to-day practice. My past experience and comfort level with the PBL model simplified this process of knowledge acquisition. This enabled me to maintain my clinical efficiency while also participating in continuing medical education and ongoing learning.

CONCLUSION

PAs are fulfilling a unique role in health care by extending physician services and improving quality of care. McMaster University is making a significant contribution to the future of health care in Canada with its PAEP, which is designed after the undergraduate medical school curriculum. Kristen Burrows is a pivotal leader in this movement as a McMaster alumnus, current dean of the McMaster PA program, and a Ph.D. candidate for PA integration. The PBL model of the PAEP equips future PAs with the tools they need to be successful in the workforce, as illustrated by Ohood Elzibak's journey as a PA in Orthopedic Surgery. Since the role of a PA is relatively new to Canada, more pedagogical research and advocacy is needed in order for PAs to become fully integrated into the health care system and imbue it with benefits.

INTERVIEW

WITH DR. MAIKEL RHEINSTADTER

THE ORIGINS OF LIFE LAB AT MCMASTER UNIVERSITY

Interviewers: Ishita Paliwal[1] and Caitlin Reintjes[2]

1. McMaster University, Honours Integrated Sciences, Class of 2021
2. McMaster University, Honours Integrated Sciences, Class of 2021

ARTICLE INFORMATION

ABSTRACT

One of the many novel scientific studies being conducted at McMaster University focuses on uncovering the origins of life, specifically, how the first cells were formed. Dr. Maikel Rheinstadter, from the Department of Physics and Astronomy, has developed a planetary simulator that effectively replicates environmental conditions both on Earth and other potentially habitable planets in the solar system. His team works to discern how the first cells may have developed on these planets by investigating the idea that water-based life was formed in warm, volcanic ponds. The following interview provides further insight into Dr. Rheinstadter's research project.

Dr. Maikel Rheinstadter, Ph.D.

Dr. Maikel Rheinstadter is a professor and researcher in the Department of Physics and Astronomy at McMaster University, where he carries out ground-breaking research in the field of membrane biophysics.

► **Please briefly describe your research. How is your planetary simulator able to effectively replicate Earth's conditions?**

We are trying to understand how the first basic cells may have formed on Earth and other potentially habitable planets. To do so, we must understand how life came to be on Earth. Although there are different theories for the origins of life, we follow the idea that life was formed in warm little volcanic ponds, in rough and extreme conditions of various temperatures, acidity, and salinity. In these volcanic fields, there are biological molecules. Seasons, changes of temperature, and humidity played a significant role in the polymerization of RNA, DNA, proteins, and peptides. That is why we built this lab which has a planet simulator (simulation chamber) that can mimic early Earth conditions at different ages of the planet. For instance, the machine simulates how conditions on the Earth's surface changed from a lack of atmosphere to the development of oxygen.

▶ **Is the planet simulator specific to Earth?**

No, the simulator was built to investigate water-based life: it controls temperature (-20 to 120˚C), humidity (0 to 100% RH), radiation (145 to 1000 nm), pressure and atmosphere. The simulator can, for instance, create a desert which is hot and dry, a rainforest that is hot and humid, make it freeze to create arctic conditions, or create various forms of precipitation, such as rain, snow, and ice.

▶ **Why focus on water-based life?**

Life on Earth has developed in water, so water-based life simulations are essential to understanding where more complex organisms, such as humans, come from. There are also other proposals of life-based on different atomic elements. However, hydrogen, carbon, and oxygen are the most abundant elements, both on Earth and everywhere in the universe.

▶ **What do you attribute to your laboratory's success in researching the origins of life?**

For a long time, scientists favoured the proposal that life was formed inside the oceans, and that the earliest forms of life came out of the ocean, upon formation. Laboratory experiments tried to recreate life in water, however, never succeeded. When we tried this, it was hard to make chemical reactions occur underwater, especially, in salt water. For biological systems, salt molecules are disruptive to biological structures such as membranes. A few years ago, we started to work with Dr. David Deamer from the University of Santa Cruz, who brought forward the idea that life was formed in warm little ponds. He could show that when cycles in temperature and humidity are added to warm ponds, RNA was polymerized from single nucleotides. We started to collaborate and developed techniques to see them and visualize how they organize and react. These first results and publications formed the basis of the origins of Life Lab. Essentially, we did these preliminary experiments without a planet simulator and could present evidence that these conditions can polymerize the basic building blocks of life.

▶ **In previous interviews, you stated that basic cells were found to form in the simulator after just a few days, which is much faster than you had expected. How long did you originally think it would take for these cells to form? Why do you think they appeared faster than hypothesized, and what does this tell us about the origins of life?**

The simulator can mimic a day in a few minutes. When I designed the simulator, I felt this was important because as we know, it took a long time for life to form on Earth. The first occurrence of biological molecules to the creation of first cellular life took half

a billion years, so we expected the process to be very slow. However, when we performed the first experiments on the planet simulator, we observed evidence of basic cells and traces of RNA surprisingly quickly. The overall process of forming these elements turned out to be much faster than expected, which was surprising and totally unexpected for everybody. The sequence from using nucleotides to form RNA and making simple cells happened at the same time.

▶ **Do you think such rapid cell development occurred because you had provided the necessary conditions in the simulator? Whereas on early Earth it would have taken time for the conditions to come together?**

One potential explanation is that we probably found a 'sweet spot' in the conditions, which makes cell development more likely to occur. The problem is that while basic cells form relatively easily, not all cellular materials may be biologically relevant and functional. A lot of cells may not be biological cells; they may look like cells but may, for instance, not be able to reproduce. In each pond, one may find a particular genetic code which may or may not be functional. In order to produce functional cells, I think that we would have to combine the content of different ponds. As a result, the genetic material would also be combined because each pond may have produced different codes. This process likely took a lot longer.

"We observed evidence of basic cells and traces of RNA surprisingly quickly"

▶ **Recently your lab has succeeded in creating protocells, which are cells that are not yet considered to be alive as they are not fully biochemically functional. What are the specific differences between protocells and real cells that permit the latter to retain the capacities of metabolism and replication?**

Three basic components are required for metabolism and replication; a cell membrane and cell wall, enzymes, and genetic material to allow functioning as a biological system. Some molecules we produce in the simulator must function as enzymes and must promote the appropriate biological reactions in order to support life. Then, the RNA we synthesize must carry information such as a protein sequence, otherwise, the RNA cannot function. The cell membrane must retain some functionality to maintain the circulation of nutrients and waste into and out of the cell. Each component of a cell needs functionality. The prediction of functionality depends on the chemical and physical composition.

► What proof would you be looking for in order to change the classification of protocells into fully fledged cells?

That is something we are currently working on. Our first big accomplishment was to show that these cells form relatively easily and we must assume that this process is happening in the universe every second, millions of times. The number of potentially habitable planets that have conditions similar to Earth is in the millions, if not billions. So, every second these protocells form somewhere in the universe, which I find totally mind-blowing. However, not every planet may progress as Earth did and is capable of combining different protocells in a way that results in a living system. Now that we have these first protocells, the next step is to prove or disprove that these cells are capable of replication, demonstrating selectivity, and initiating some sort of metabolism.

► How could your lab's current findings be used to understand how life has formed in the rest of the solar system?

The fundamental question for us is, "Where did life come from on Earth?". However, many people are more interested in the question: "Are we alone in the universe?". The simulator is made to mimic different planets with varying compositions. We are able to simulate planets that orbit different stars with varying degrees of light intensity, frequency, and amounts of ultraviolet (UV)/infrared (IR) light in order to mimic any potentially habitable planets that astronomers find. The simulator is computer controlled, so we can add planetary rotation, the path of the planet around its star, temperatures, lengths of seasons and days, and the intensity of daylight to the simulation.

► More laboratories are now planning the construction of other planet simulators to further study the origins of life. Are there any improvements you would make to the current planet simulator in order to test future hypotheses?

Right now, we are still learning how to use the planet simulator. It is the first of its kind and not only a huge science project, but also a huge engineering accomplishment. It is very difficult to build such a machine and ensure that it is functional. Right now, the simulator does exactly what we want it to do. In the next generation of simulators, one might want to include analytical tools, for instance, to see how cells grow in situ. We could also attach a spectrometer within and search for biological signatures as a function of biogeochemical cycles.

► What is the biggest challenge that your team has faced so far in your research?

Every step of the way was challenging, otherwise,

someone else would have done this already. At every step, we had to overcome obstacles and limitations by improving techniques and tools.

► What tools do you use other than the planet simulator?

We developed and improved tools to detect cells and determine if RNA was formed. These tools and techniques had to be developed along the way, otherwise we could not prove or disprove anything that has happened within the simulator.

► Did you have to develop the tools from scratch or improve existing tools?

We had to develop these tools from scratch and are still improving these tools. For example, humans are composed of DNA, so there are many tools that exist that allow us to work with DNA. However, there are very few tools to work with RNA and particularly short strands of RNA that are only a few nucleotides (20-30) long.

► Finding the origins of life is a very complex process. How has forming an interdisciplinary research team aided in this process?

Having an interdisciplinary team did not help– it was essential. An interdisciplinary research question requires an interdisciplinary research team. For example, in the latest paper on antibiotic resistance, our team worked with colleagues and utilized techniques and materials from their disciplines, such as biochemistry and medicine.

► What tools do you use other than the planet simulator?

Next, we want to work towards fully functional protocells and prove they work as biological cells. We also want to identify a transcription mechanism for RNA through a non-enzyme-driven process, since they would not be available under prebiotic conditions. These are all fundamental and big questions ahead of us.

CONCLUSION

The research carried out by Dr. Rheinstadter and his team is interdisciplinary and revolutionary. They are seeking to answer what is potentially one of the largest questions in science to date, by working to uncover the origins of life. This research will not only satisfy scientific curiosity but could also have promising applications to the fields of medicine and neuroscience, making it a ground-breaking field with almost limitless potential.

INTERVIEW

WITH DR. KATIE MOISSE

AN INSIDE LOOK ON SCIENCE COMMUNICATION

Interviewer: Tyler Redublo

McMaster University, Honours Life Sciences, Class of 2019

ABSTRACT

Science communication is an emerging field that consists of a multitude of different career options, such as journalism, storytelling, and multimedia production. At its core, the field of science communication represents the bridge between scientists and the general public. Experts in this profession are concerned with how the complexities of scientific research can be presented to all audiences in ways that are engaging, comprehensible, and relevant. Today, innovative scientists continue to push the boundaries of knowledge and they are supported by science communicators who help to raise awareness and advocate for the research. Despite the fundamental role that these experts play, many people are unaware of the field of science communication and the vast array of career opportunities that it offers. The purpose of this interview is to shed light on science communication and to explore the associated skills, careers, and growth opportunities.

Dr. Katie Moisse, Ph.D., M.Sc., M.Sc.

Dr. Katie Moisse, an Assistant Professor in the School of Interdisciplinary Science at McMaster University, is an expert in the field of science communication. Since her appointment in January 2018, she has taught various undergraduate courses pertaining to science communication, including Global Health and Human Disease, as well as Science and Storytelling. Additionally, Dr. Moisse has four years of experience as a Digital Health Editor for ABC News, in which she covered various health stories, such as the Ebola Outbreak in 2014 and the aftermath of Hurricane Sandy in 2012. The following interview provides an insight to Dr. Moisse's experiences and an inside-look on science communication.

► **How were you introduced to the field of science communication and what motivated you to pursue a career in this field?**

My interest in science communication developed gradually, over time; I knew that I enjoyed learning about a broad range of scientific topics and communicating with researchers in a diverse array of fields. I was also intrigued by the unique roles that science communicators play, in that they are able to interpret the public's viewpoint on various scientific paradigms and discoveries.

Initially, I was completing my Ph.D. in Pathology, specifically focusing on Amyotrophic Lateral Sclerosis (ALS) at Western University. Following my experiences with collecting and analyzing data in research-based settings, I realized my affinity for the storytelling aspect of science by considering how data can be presented in order to create interesting narratives. In addition, I enjoyed presenting my findings to different audiences in unique settings, such as conferences or meetings. I learned more about science communication from various individuals in the field, including colleagues at Columbia University, and a friend who worked at the Toronto Star. They played a significant role in introducing me to the career opportunities in this field. Eventually, I made the decision to enroll in a Master's program in journalism at Columbia University. During this time, I also applied myself in various settings where I was able to expand my repertoire of skills that would help me become a stronger communicator. This included volunteering for the ALS society, where I wrote research summaries for their website, as well as completing an internship at Scientific American (a science magazine), where I pitched ideas for stories and produced website content that was provided by partners and bloggers. After graduating from journalism school, I acquired my first job as a Digital Health Editor for ABC News.

▶ **What was the most challenging aspect of your journey in the field of science communication?**

I think that the most challenging aspect was the degree of uncertainty and unknowingness that was associated with making a career change to science communication. For me, the idea of having to commit to a different program in a different city and not knowing what the benefits would be for my career was overwhelming. In hindsight, I believe that it was important to embrace these uncertainties by applying myself during the right moments and expanding my network of connections. Consequently, I was able to make the most out of these unknown factors and transform them into amazing opportunities. For instance, years ago, I never would have imagined that I would become a university professor for the program of Life Sciences. This speaks to the diversity of careers that this field offers and the idea that these transferable skills and qualifications lead to various different paths, if one keeps an open mind.

▶ **Despite the efforts of various courses, initiatives, and projects, many students remain uninformed about the opportunities in this field as viable career options. What exactly does a career in the field of science communication entail? Can you mention specific roles and/or projects that are typically carried out by experts in this profession?**

One career opportunity for science communicators is journalism, with which I had personal experience. This involves being able to write a report and provide objective perspectives from the field of science for different audiences, whether it is the general public, policymakers, or other scientists. Depending on the type of publication that is being created, the audience may vary in their level of scientific knowledge. Thus, the journalist must be flexible in how they create their narratives. While researchers use the scientific method to gather evidence, journalists use a similar process to collect information. This includes asking relevant questions, interviewing different people, and obtaining different perspectives that all contribute to the content of the manuscript.

Alternatively, science communicators can represent a certain institution, such as a hospital or university. In this case, there is a certain connection to the material being communicated because science communicators collaborate with the primary researchers or doctors to report scientific findings to different audiences on their behalf.

Another career route is advocacy, with which I also had experience with when I volunteered at the ALS Society. In the context of ALS advocacy, I focused mainly on introducing patients to different research projects and treatment options, framing and communicating research in meaningful ways, and connecting ALS patients with each other.

Education and outreach is another option, which involves sharing knowledge with a community, such as an elementary school, or a geographic region. Finally, there is the choice of policy revision and policy making. This involves translating scientific information specifically, from the public health sector, as well as lobbying for policy changes based on the most recent evidence that is available. For instance, pertaining to the issue of climate change, environmental scientists may suggest changes in waste management policies based on the research that they have conducted.

▶ **Since your appointment as an Assistant Professor in 2018, you have had a huge influence on the advocacy and undergraduate curriculum of science communication at McMaster University. What specific skills are emphasized within this discipline that differentiates it from others in the field of science?**

I think that the single aspect of science communication that differentiates it from other fields is the emphasis and focus on the audience. Other disciplines of science are more focused on the *content* of their research and building up their respective body of knowledge. Conversely, science communication is concerned with knowledge translation and the presentation of the information to audiences in ways that are relevant and accessible. As such, effective science com-

municators must be able to make clear points and statements, ask good questions, build relationships and connections with people, and showcase creativity and flexibility in how they communicate with different audiences. The overall goal is to get people excited about the work conducted by researchers and to invite audiences to join these conversations about science. In this sense, science communication can be thought of as a set of skills in itself.

▶ **Public perceptions of scientific discourse can often be negative due to gaps in knowledge and the feeling that science is 'inaccessible' or 'unimportant'. What techniques and/or philosophies do science communicators use to effectively present the research conducted by the scientific community, in ways that capture the interest of the general public?**

In order to capture the interest of the public, one must first decide what they want their audience to know and learn about. From there, one identifies the different ways of communicating this content effectively. Today, the audience's attention spans are shorter than ever, and when it comes to science articles or journalism, people often look for specific pieces of information. As a science communicator, one has to be able to deliver this captivating and important information in a timely manner, while also maintaining the integrity and accuracy of the work.

For more seasoned and long-term audiences who have been following a specific story, the challenge changes. Namely, the communicator asks: how can one ensure that the audience *stays* interested? How can these narratives be framed in a creative manner and continue to 'hook' the readers? Another fundamental aspect of effective communication is explicitly stating the significance of scientific projects in order to intrigue the reader. Even if a topic is not inherently interesting, there is always a way to relate the content to the audience. For example, people tend to have a natural interest in their own health, so these stories are easier to communicate. Conversely, many people may not find space exploration to be an interesting topic, so an effective communicator would be able to find a way to present this content in a relatable fashion. This may be done in different ways, such as covering interesting characters involved in the projects, emphasizing the plot of a story, or even explaining why these projects are an effective use of taxpayers' money.

"Science communication is concerned with knowledge translation"

▶ **Today, the transfer of information has become widespread and is now easily accessible through technological advancements, such as social media platforms. How have these developments in mass communication affected—both positively and negatively, the field of science communication? Accordingly, where do you see this field progressing over the next 10 years?**

Without a doubt, social media has changed *how* people acquire and share scientific information. In the past, the transmission of scientific knowledge used to follow a 'chain of command'— the only science available to the public was through subscriptions to journals. Furthermore, only selected studies gained a lot of initial interest and support, and would obtain media coverage. Overall, the transfer of this information was much more curated and restricted in the past.

Through the internet and social media, people now have instant access to online journals, news articles, and scientific content. Today, there is a move toward transparency and open access. Scientists now publish pre-prints of their research, in order for the public to see their work before it enters the review process of a journal. In addition, scientists may be active on social media platforms to promote and advocate for their work. However, a drawback to this open access of information is that individuals are at risk of drawing conclusions from information that is not necessarily verified or reviewed, thus, it might not be accurate.

Though the idea of widespread mass communication has negative effects, there are also some positive aspects, if applied properly and responsibly. For instance, the increased depth and diversity of research has allowed individuals to shift their interests and explore science to a greater degree. In addition, the internet and social media have created a sense of unity by connecting different audiences through forums and online groups. This permitted them to rally support for a particular research subject and raise awareness for different causes. Moving forward, the processes of information sharing will likely become even more prominent and the effects more pronounced. It is a powerful tool that can have very significant consequences if exploited. However, responsibility lies with both communicators and audiences in exercising discipline when sharing scientific information.

▶ **With increasing awareness for the field of science communication, it is likely that many more students will begin to express an interest in professions related to science journalism, media coverage of global health issues, and public health. Based on your experiences, what advice would you give to students who intend on pursuing a career in the field of science communication?**

My advice to these students is to be confident and app-

ly oneself whenever possible. Looking back on my own experiences, I would like to think that I often created my own opportunities. This might include reaching out to different organizations such as journals or newspapers, volunteering in science communication settings, and developing a portfolio of work that show-cases one's skills. One can refine their skills in this field by attending writing workshops, career information sessions, extracurricular clubs, or discussions and talks hosted by public health representatives or journalists. These opportunities should be seized because they provide a chance to not only develop one's skill set, but also to meet other people in the field.

Finally, one should read as much as possible! There are many effective science communicators in the field and following the work of these individuals is often the best— and in my opinion, the most enjoyable means of becoming a better writer and communicator. It is best to make an effort to stay informed about the latest developments in scientific research and to keep up with the conversations!

CONCLUSION

Science communication is a field that covers a plethora of different professions and creates unique opportunities for individuals that are proficient in storytelling, networking, and expressing creativity. A lack of knowledge and awareness of this particular field has led many individuals—particularly students, to remain uninformed and consequently, never consider science communication as a viable career option. From journalism to teaching, individuals like Dr. Katie Moisse exemplify the diverse array of careers that are obtainable through science communication. With the rise in advocacy and support for this field in recent years, science communication will continue to expand within the McMaster University community and beyond.

Newly Discovered Cell Shape Promoting Protein Complex Involved in the Maintenance of Distinct Helical Shape of *Helicobacter Pylori*

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ABSTRACT

The distinct helical shape of the bacterium *Helicobacter Pylori (H. pylori)* assists this organism in colonizing the digestive organs of its target host. It has been discovered that a key determinant of helical cell shape formation in *H. pylori* is the Csd5 protein, which engages in multiple cell shape promoting interactions with the cell wall and other various proteins. This finding has significant clinical implications, as it outlines Csd5 as a potential drug target for treating *H. pylori* infection in the future.

Keywords: *H. pylori*, Csd5, cell shape, protein complex, drug target

Bacterial infections of *H. pylori* in the stomach have been shown to greatly increase the likelihood of developing debilitating diseases such as stomach cancer.[1] A successful infection is dependent upon the organism's ability to colonize the stomach and the digestive tract.[2] In *H. pylori*, this process is aided through its unique helical cell shape which helps it penetrate the mucoid lining of the stomach to allow for successful colony formation.[3] Therefore, investigating the mechanisms that contribute to cell shape formation is of great interest to researchers as this knowledge has the potential to be applied in a therapeutic setting.

Bacterial cell shape is determined by the cell wall, which is predominately composed of carbohydrate molecules known as peptidoglycan (PG).[4] This molecule has a chain-like structure consisting of alternating N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) monomers.[4] In the cell wall, these chains are crosslinked together for added structural support (Figure 1A).[4] It has been observed that during bacterial growth, specific intracellular mechanisms are activated to successfully form the desired cell shape via chemical interactions with the PG cell wall.[5]

With regards to *H. pylori,* a fundamental component of helical cell shape formation is the protein Csd5, wh-

ich is a non-enzymatic protein unique to the species.[2] This protein was previously characterized in a study by Dr. Laura Sycuro et al. (2012), where it was discovered to be integral to the construction of helical cell shape in *H. pylori.* Specifically, it was found that cells containing a chromosomal deletion of the csd5 gene elicited a rod-shaped phenotype instead of the helical shape characteristic to *H. pylori.*[5] Additionally, PG levels within these cells remained unchanged, which suggested that alteration of cell shape stemmed from structural changes to existing PG within the cell. On the basis of these observations, it was thought that Csd5 acted alongside other Csd class proteins (namely Csd4), to promote the development of helical cell shape formation through the modification of PGs.[5] The specific mechanisms through which this occurred, however, remained uncharacterized. Consequently, this study laid the foundations for subsequent researchers to further investigate the properties of Csd5 in order to better understand the various mechanisms underlying structural phenotype in *H. pylori.*

This unique cell shape determining mechanism was the focus of a 2018 study by Dr. Kris Blair et al. from the University of Washington, who was assisted by Dr. John Whitney, a prominent researcher from Michael DeGroote Institute for Infectious Disease Research at

McMaster University. In this study, it was initially hypothesized that Csd5 was involved in the formation of helical cell shape through its ability to promote the alteration of crosslinking between PGs in the cell wall. Specifically, this enzyme was thought to be able to localize PG carboxypeptidases which could subsequently promote or inhibit downstream PG hydrolases to alter the crosslinking of PGs.[2] This hypothesis, however, was ultimately rejected due to the lack of reproducibility in *in vivo* studies.[2] Instead, it was found that Csd5 induces helical shape by interacting with the cell wall and various other proteins to form a cell shape promoting complex.[2]

To conduct this study, the researchers first analyzed Csd5 in order to identify any important structural features of the protein. This was done using Jackhammer, which is a computerized sequencing technology designed to predict functional domains and motifs.[2] The analysis uncovered the presence of highly conserved

N-terminal and C-terminal domains, as well as a less conserved, disordered domain within the middle of the protein.[2] Furthermore, it was discovered that the N-terminal region was responsible for mediating protein interactions while the C-terminal region was found to contain a unique SRC Homology 3 (SH3) domain that directly interacted with peptidoglycans.[2] Additionally, it was seen that the disordered domain contributed to the ability of the protein to maintain proper curvature and axis length of the membrane.[2]

After the characterization of the Csd5 protein, the researchers set out to further investigate the specific interactions at each domain. Upon analyzing the SH3 domain by homology modelling, a conserved functional motif within this domain, known as the Reverse Transcription (RT) loop, was discovered. Interestingly, this motif was found to be homologous to peptidoglycan binding motifs in other bacterial species.[2] Using this homology model, the researchers identified amino
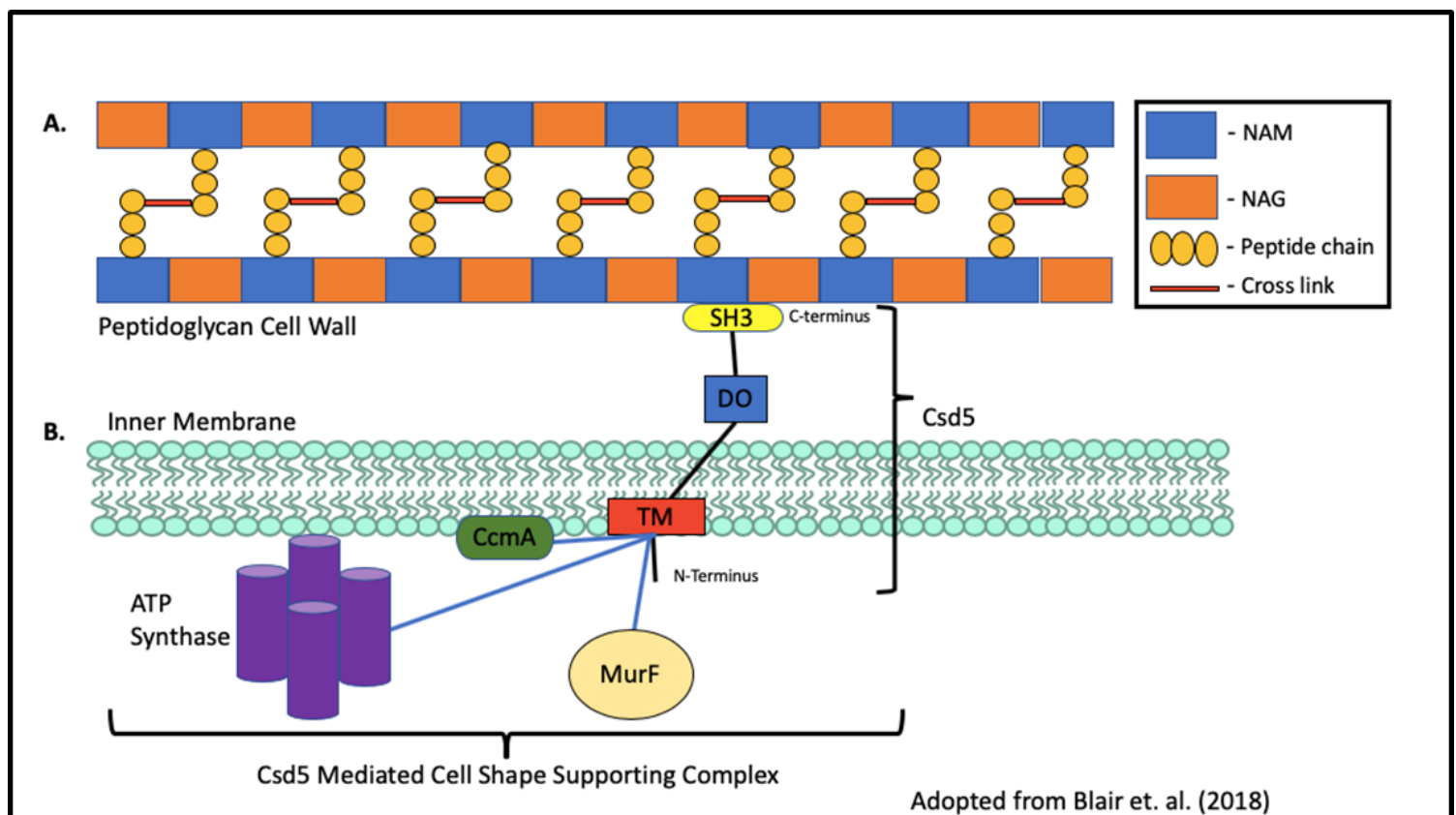
Figure 1A. **Schematic showing the structural composition of the bacterial cell wall.** The cell wall is positioned between the inner cell membrane and the outer cell membrane (not demonstrated). It is composed of alternating NAM and Nag residues that are crosslinked via peptide chains that extend from NAM residues. **Figure 1B.** **Hypothesized model for *H. pylori* cell shape promoting complex mediated by Csd5.** RT loop motif mediates peptidoglycan binding of the highly conserved C-terminal SH3 domain of Csd5. The disordered domain in the middle of Csd5, containing a characteristic glutamine-rich coiled-coil motif, helps to maintain cell axis length and curvature. The highly conserved N-terminal domain of Csd5 promotes interactions (represented by the blue lines) with the peptidoglycan synthesis enzyme MurF, the cell shape protein CcmA, and the bacterial ATP synthase. Overall, it is proposed that this multiprotein complex, composed of Csd5, MurF, CcmA and ATP synthase, is involved in maintaining the helical shape of *H. pylori*.

acids within the RT loop believed to be essential for binding, specifically, Arg146 and Thr151.[2] Consequently, when mutations were introduced at these sites, the helical cell structure was disrupted.[2] This confirmed the importance of this motif for interacting with peptidoglycans.

Next, the researchers used a proteomic screen to identify the distinct proteins interacting with Cds5 at its N-terminal domain. Through this screen, three candidate proteins were identified: MurF (a cytosolic peptidoglycan precursor enzyme), CcmA (an *H. pylori* cell shape protein), and ATP synthase.[2] In order to confirm these results, deletions were introduced into the N-terminal domain to verify that this region was, in fact, responsible for mediating interactions with the candidate proteins.

In the end, the researchers found that N-terminal deletions resulted in the disassociation of these proteins from Cds5, which evidently confirmed the function of this region.[2] Another notable finding that arose was the presence of a transmembrane domain at the N-terminus. It was observed that this domain helped to anchor the N-terminus of Csd5 into the inner membrane of the cell while simultaneously promoting interactions with the candidate proteins.[2]

Overall, the results from the study by Blair et al. (2018) clarified the role of Csd5 in mediating the formation of the helical shape of *H. pylori*. Specifically, these findings provide evidence for a multi-protein complex that exists within this microbe which induces and maintains helical shape.[2] A schematic of this proposed complex can be seen in Figure 1B.

The findings by Dr. Blair et. Al. are incredibly significant as they help to foster a more comprehensive understanding of the complicated mechanisms used by *H. pylori* to regulate cell shape. Consequently, this knowledge has the potential to be clinically beneficial as it could advance researchers to develop antibiotics against *H. pylori* that are designed to disrupt its cellular shape. As stated previously, the distinct helical shape of *H. pylori* facilitates the invasion and infection of host organisms.[2] Therefore, compounds which alter the shape of this bacterium may be effective in treating conditions related to *H. pylori* infection.

Currently, one of the frontline treatment methods for these types of infections is the antibiotic Clarithromycin, which is a ribosomal inhibitor in bacterial cells.[6] While this compound has been effective in the past, Clarithromycin-resistant *H. pylori* infections are becoming more common in patients and consequently pose a significant clinical challenge.[6] Based on the evidence put forward by Dr. Blair et al., a promising next step would be to investigate Csd5 as a drug target. Due to the role that Csd5 plays in determining cell shape,

successful inhibition of this protein may disrupt the ability of *H. pylori* to colonize the digestive tract and subsequently prevent infection. Ideally, more research investigating Csd5 and cell shape disrupting antibiotics should be conducted to improve the current treatment methods used to treat *H. pylori* infection.

ACKNOWLEDGEMENTS

REFERENCES

(1) Risk factors for stomach cancer [Internet]. Canadian Cancer Society, 2018 [cited 2019 Mar 4]. Available from: http://www.cancer.ca/en/cancer-information/cancer-type/stomach/risks/?region=on

(2) Blair K, Mears K, Taylor J, Fero J, Jones L, Gaafken P, Whitney J, Salama N. The Helicobacter pylori cell shape promoting protein Csd5 interacts with the cell wall, MurF, and the bacterial cytoskeleton. Mol Microbiol. 2018;114-127-110.

(3) Brown, L. Helicobacter pylori: epidemiology and routes of transmission. Epidemiol Rev. 2000;283-297-22.

(4) Peptidoglycan [Internet]. Essentials for Life Science, 2011. [cited 2019 Mar 4] Available from: http://www.els.net/WileyCDA/ElsArticle/refId-a0000702.html

(5) Sycuro L., Wyckoff T., Biboy J., Born P, Pincus Z, Waldemar V., Salama N. Multiple Peptidoglycan Modification Networks Modulate Helicobacter pylori's Cell Shape, Motility, and Colonization Potential. PLoS Pathog. 2012.

(6) Megraud, F. The challenge of Helicobacter pylori resistance to antibiotics: the comeback of bismuth-based quadruple therapy. Therap Adv Gastroenterol. 2012;103-109-5.

Could the Next Antibiotic Emerge from Bacteria?

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ABSTRACT

An intrinsic bacterial mechanism could play a fundamental role in the future of antibiotics. Using cryo-EM, the structural resolution of the effector protein complexed with its chaperone and other accessory proteins reveals the mechanism of action of type VI bacterial secretion system. The importance of the chaperone protein, used to prime the toxic effector protein, was previously identified. Future research efforts should encompass the immunity protein that may allow bacteria to evade the lethal effects of this mechanism.

Keywords: T6SS, Tse6, antibiotic resistance, antibiotics, antimicrobials, effector protein

The microscopic world is fascinating, with many variables that grant it much flexibility and a range of outcomes. Like in the macroscopic world, bacteria compete for resources, and in the process, utilize various mechanisms to outcompete and kill other bacteria. One such mechanism is the type VI secretion system (T6SS) as stated by Quentin and others.[1] Molecular insight into the various subcomplexes of this biochemical pathway have been elucidated by single particle electron cryomicroscopy (cryo-EM).[2,3] In this study, the structure of the chaperone paired with the effector protein, Tse6-EagT6, in complex with VgrG1 is resolved to further understand the T6SS mechanism of action in killing bacteria.[1]

T6SS Structure and Mechanism of Action

Bacterial pathogens commonly use protein secretion to facilitate interactions with their environment and other microorganisms.[4] T6SS is a protein secretion apparatus used by many gram-negative bacteria such as *Pseudomonas aeruginosa*. T6SS is encoded up to six times into the bacterial genome and is comprised of 13 core components and several accessory genes that can vary between bacteria, allowing for varying target specificity and firing modalities.[5,6] The apparatus is structurally homologous to a contractile phage tail attached to the prokaryotic membrane. It is composed of an inner tail tube complex made of stacked

hexameric rings of the hemolysin coregulated protein (Hcp). This is capped with a spike complex made of a valine-glycine repeat protein G (VgrG) trimer and a Pro-Ala-Ala-Arg (PAAR) repeat-containing protein.[5] This tube-like complex is engulfed by a sheath attached to a baseplate which is adhered to the membrane of the bacterium. The T6SS structure can be seen in Figure 1A. Similar to phages, T6SS functions by contracting and injecting an effector protein through the protrusion of the inner tube which is capped with the spike complex and has the effector attached to it.[1] Effector proteins can kill bacteria as the Tse6 exerts its functions in the cytoplasm through hydrolyzing nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$).[1] There is evidence that the effector proteins are injected into the periplasm of the target bacterium where a small subset moves to the cytoplasm.[7] The effector-producing bacterium and their sister bacteria are not affected by the toxicity of the effector protein due to the co-expression of another immunity protein that renders the effector inert.[1]

The effectors are shuttled to the target-competing bacteria through two methods. The smaller effectors that are less than 40 kDa are bound and stabilized by the lumen of the Hcp hexamers, while the larger multidomain effectors are bound to the spike, VgrG.[1]

In a 2019 study, Unterweger et al. determined that a

small subset of effector proteins require the assistance of chaperones to be loaded on VgrG.[8] EagT6 is one such example and is required for the loading of Tse6. Although the precise function of this chaperone is not yet elucidated, it is known to be essential for intracellular effector stability. Furthermore, Tse6 also requires interaction with the elongation factor Tu (EF-Tu), to be delivered to its target bacterium.[1]

Biochemical studies suggest that the association of the chaperone's homodimer to the transmembrane domain of Tse6 allows its protection from the cytoplasmic hydrophilic environment that might cause the effector to aggregate and degrade.[1] This chaperone-effector association also allows the PAAR domain on the effector to interact and associate with the VgrG, which is followed by its loading on the T6SS. A study conducted by Quentin et al. (2019), reported on the ability of Tse6 to translocate across a lipid bilayer. This led the research team to hypothesize that the effector is injected into the periplasm, where it passively crosses the inner membrane layer and translocate to the cytoplasm to exert its deleterious effects,[1] as shown in Figure 1. The effects of the association of the effector with EF-Tu is not yet fully understood. It was previously hypothesized that the elongation factor in the antagonist bacterium functions as a lock.[9] Through this mechanism of action, the effector binds to EF-Tu once it is in the cytoplasm, preventing it from being expelled to the extracellular space.

The experimental data also suggests a mechanism of membrane translocation, where the transmembrane hydrophobic domain (TMD) of Tse6 forms a small pore. This allows the unfolded large toxic domain of the Tse6 to cross the membrane where it folds again and kills the antagonist bacteria.

Impact on Antibiotic Resistance Research

Ever since the discovery of penicillin in 1928, this antibiotic has been used to treat serious bacterial infections. Following this scientific breakthrough, antibiotics have transformed modern medicine and saved millions of lives.[10] Penicillin was successful in treating infections during World War II but its efficacy was reduced shortly after, upon the emergence of antibiotic resistance. Antibiotic resistance became a growing global health concern, threatening penicillin's utility as
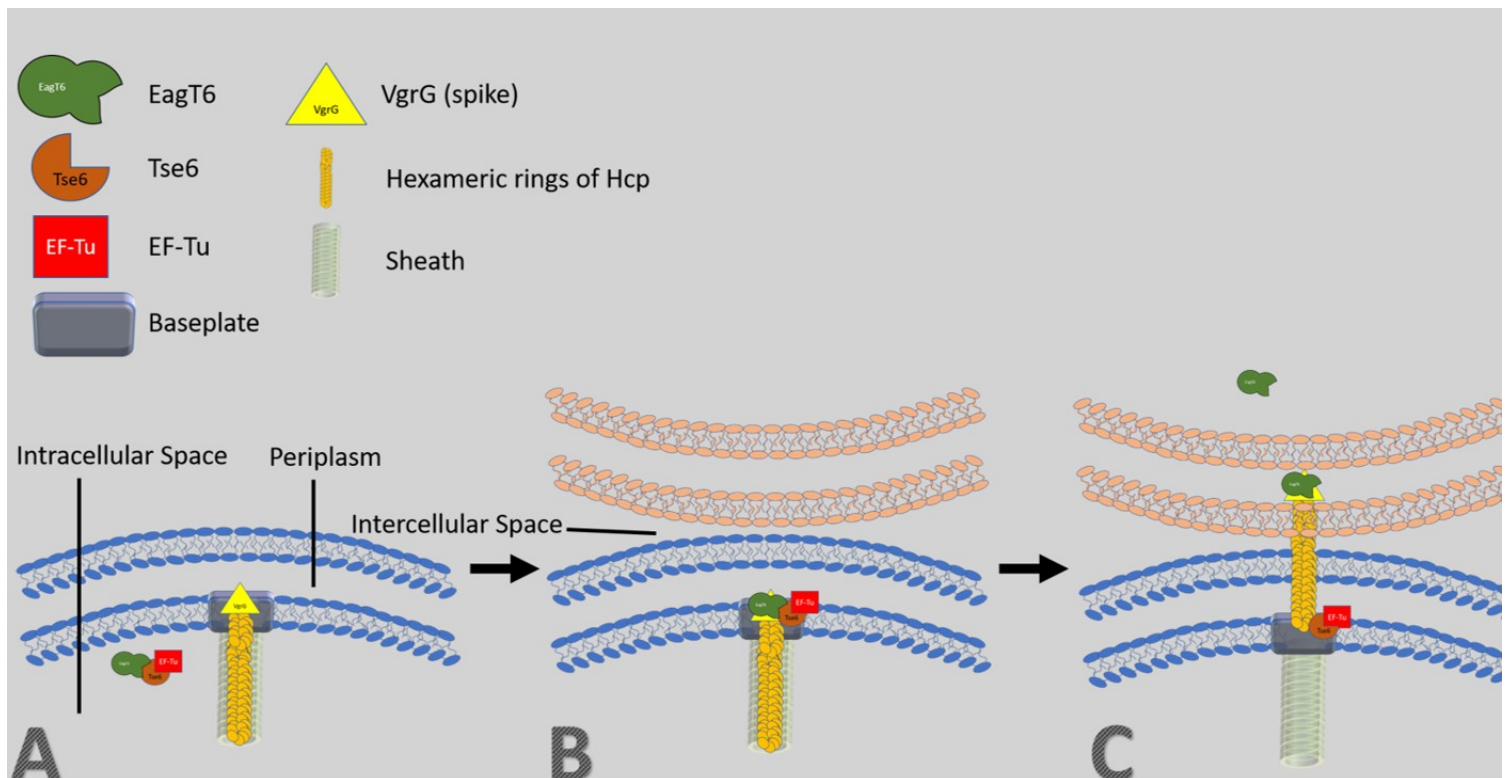
Figure 1. Interaction and loading of the Tse6-EagT6 complex onto the T6SS. The T6SS assembly can be seen in A-C. The mechanism of function of the T6SS is initiated by the association of the effector protein, Tse6, to the homodimer chaperone EagT6 (A). The assembly of the complex prevents the hydrophobic domain of the effector to aggregate with other Tse6 proteins and degrade in the hydrophilic environment of the cytoplasm (B).[1] The complex is then loaded on the T6SS spike formed by the VgrG protein and upon contact with an antagonist bacterium, the T6SS complex contracts and injects the effector into the periplasm of the antagonist bacterium (C).[1] The antagonist then crosses the inner membrane bilayer to the cytoplasm where it can exert its toxic effects, hydrolyzing NAD^+ and $NADP^+$.[1]

an effective treatment for infections.[10] This has created an "arms race" between modern medicine and bacterial infections, with continued development of new antibiotics which are rendered ineffective in a few generations due to bacterial resistance.

Today, any research that explores the mechanisms of targeting and killing bacteria is valuable, considering the continuing need for novel and unique antibiotics. Thus, the aim of the Quentin et al. paper is to study this lethal bacterial mechanism and its machinery, which may lead to novel therapeutics that are capable of conquering bacterial infections. One such novel therapeutic that may emerge could be in the form of a genetically engineered agent that exhibits the T6SS apparatus with lethal effectors to circumvent current antibiotic resistance to some antimicrobials.

Prospective Research and Considerations

The researchers of the study attempted -yet failed- to resolve the structure of the toxic domain of the Tse6-EF-Tu-Tsi (immunity protein that neutralizes the effector) complex, concluding that it is too flexible to be determined using cryo-EM. Future research should aim to identify the structure of the complex to further understand or confirm the researchers' hypothesis about the elongation factor Tu acting as a lock, in order to prevent the expulsion of the effector. This would be an essential characteristic of effective therapeutic. Additional studies must be conducted on the mechanism of action of the immunity protein, as it seems to be the most likely mechanism of antibiotic resistance that may arise if the effector is to be used as an antibiotic. Another potential avenue of this field of research could be to study whether this type VI secretion system is lethal against gram-positive bacteria in addition to its functionality against gram-negative bacteria.

ACKNOWLEDGEMENTS

REFERENCES

(1) Quentin D, Ahmad S, Shanthamoorthy P, Mougous JD, Whitney JC, Raunser S. Mechanism of loading and translocation of type VI secretion system effector Tse6. Nat Microbiol [Internet]. [cited 2019 Feb 17]; Available from: https://doi.org/10.1038/s41564-018-0238-z

(2) Kudryashev M, Yu-Ruei Wang R, Egelman EH, Basler Correspondence M, Brackmann M, Scherer S, et al. Structure of the Type VI Secretion System Contractile Sheath Article Structure of the Type VI Secretion System Contractile Sheath. Cell [Internet]. 2015 [cited 2019 Feb 17];160:952–62. Available from: http://dx.doi.org/10.1016/j.cell.2015.01.037http://dx.doi.org/10.1016/j.cell.2015.01.037

(3) Ge P, Scholl D, Leiman PG, Yu X, Miller JF, Zhou H, et al. Atomic structures of a bactericidal contractile nanotube in its pre-and postcontraction states HHS Public Access Author manuscript. Nat Struct Mol Biol [Internet]. 2015 [cited 2019 Feb 17];22(5):377–82. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4445970/pdf/nihms-692005.pdf

(4) Mougous JD, Cuff ME, Raunser S, Shen A, Zhou M, Gifford CA, Goodman AL, Joachimiak G, Ordoñez CL, Lory S, Walz T. A virulence locus of Pseudomonas aeruginosa encodes a protein secretion apparatus. Science. 2006 Jun 9;312(5779):1526-30.

(5) Chang Y-W, Rettberg LA, Ortega DR, Jensen GJ. In vivo structures of an intact type VI secretion system revealed by electron cryotomography. EMBO Rep [Internet]. 2017 [cited 2019 Feb 17];18:1090–9. Available from: http://embor.embopress.org/content/embor/18/7/1090.full.pdf

(6) Ho BT, Dong TG, Mekalanos JJ. A view to a kill: the bacterial type VI secretion system. Cell host & microbe. 2014 Jan 15;15(1):9-21.

(7) Russell AB, Hood RD, Bui NK, LeRoux M, Vollmer W, Mougous JD. Type VI secretion delivers bacteriolytic effectors to target cells. Nature. 2011 Jul;475 (7356):343.

(8) Unterweger D, Kostiuk B, Pukatzki S. Adaptor Proteins of Type VI Secretion System Effectors. 2017 [cited 2019 Feb 17]; Available from: http://dx.doi.org/10.1016/j.tim.2016.11.012

(9) Whitney JC, Quentin D, Sawai S, LeRoux M, Harding BN, Ledvina HE, Tran BQ, Robinson H, Goo YA, Goodlett DR, Raunser S. An interbacterial NAD (P)+ glycohydrolase toxin requires elongation factor Tu for delivery to target cells. Cell. 2015 Oct 22;163(3):607-19.

(10) Lee Ventola C. The Antibiotic Resistance Crisis Part 1: Causes and Threats [Internet]. Vol. 40. 2015 [cited 2019 Feb 16]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4378521/pdf/ptj4004277.pdf

Diagnosing Disorders of Consciousness

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ABSTRACT

The definition of consciousness has long been debated in a scientific and philosophical context due to its ambiguous nature. Recent developments in the concept of consciousness have contributed to a better understanding of associated Disorders of Consciousness (DOC). However, there has not been an equivalent rise in the accuracy of diagnostic measures for DOC. About half of the patients with DOC are incorrectly diagnosed due to significant reliance on subjective and inaccurate behavioural scales. Consequently, the misrepresentation of a patient's present residual consciousness severely affects the treatment and rehabilitation measures that they receive. These inaccurate diagnoses ultimately influence the patient's chance of survival. Thus, it is necessary to critique the current methods of evaluating consciousness. Neurophysiological scales are explored as a possible alternative method of evaluating consciousness, which is characterized by high sensitivity and objectivity. An understanding of the advantages and disadvantages of different consciousness-evaluating techniques can aid in the advocacy of their widespread use for DOC patients.

Keywords: Disorders of consciousness, electroencephalography, event-related potentials

DOC can be categorized into a coma, vegetative state (VS), and minimally conscious state (MCS).[1] Figure 1, adapted from Giacino et al. (2009), illustrates the variations of arousal and awareness in these DOC. Patients suffering from a coma experience complete loss of consciousness and present with their eyes closed and a lack of reactivity/arousal to external stimuli. A patient in a coma is not neurophysiologically aroused, thus, is not aware of the external environment.[1] A coma can be caused by several clinical conditions such as traumatic brain injury, cardiac arrest, stroke, metabolic or infectious diseases, or drugs.[1,2] This state can last anywhere between two weeks to a month, after which the patient may regain consciousness or potentially lapse into VS or MCS.[1,2] Patients in VS appear awake with eyes open but show no response to stimuli (i.e. no awareness).[1] Minimally conscious patients show complete arousal but transient awareness to stimuli, thus displaying inconsistent behavioural response signs.[1,2]

Limitations of Behavioural Scales

Due to the longstanding ambiguity in the understanding of consciousness and related disorders, DOC have primarily been assessed and diagnosed based on visible behavioural features, described by behavioural
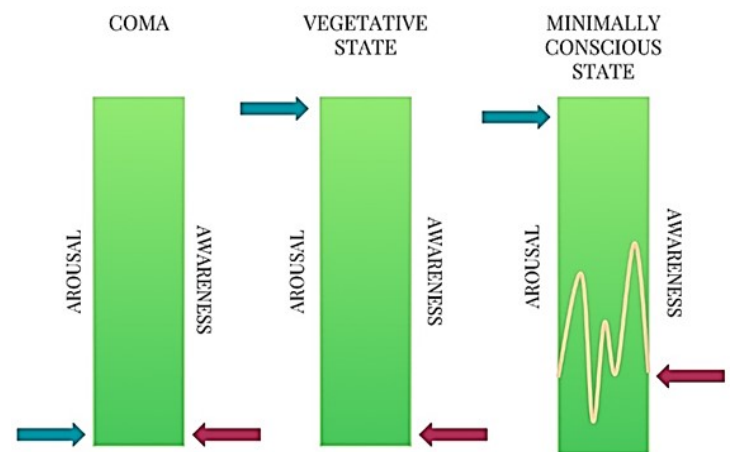
Figure 1. Two dimensions of consciousness: arousal and awareness.[3] For individuals suffering from coma or brain death, both arousal and awareness are absent. In the other two DOC (VS and MCS), arousal is relatively preserved, while awareness is absent in the VS and present in fluctuating quantities in the MCS. This figure was adapted from Giacino et al. (2009).

scales. These scales are based on subjective interpretations of behavioural signs at particular points in time

and are heavily reliant on visible signs to assess the integrity of various sensory and cognitive functions. According to a study by Morlet et al. (2013), 40% of MCS patients were misdiagnosed and incorrectly identified as being in the VS.[4] This paradigm is further complicated in intensive care units, where a patient's critical condition (e.g. intubation, tracheotomy, or immobilization) interferes with the process of identifying behaviours that fit the scope of these scales.[4]

The ideal consciousness measurement scale should provide an accurate analysis of a patient's state since this plays a role in predicting the patient's outcome and the treatment or rehabilitation measures that they receive. Clinical behavioural scales are insufficient to capture the subtle neurophysiological changes in a patient with DOC.[5] For instance, the Glasgow Coma Scale is a behavioural scale that merely assesses visual responses to stimuli, thus, is very limiting during a neurophysiological assessment. Moreover, in patients with DOC, assessment of their consciousness is confounded by the fluctuating states of their awareness and arousal, alongside possible sensorimotor impairment and sedation, all of which affect their responses to the administered stimulus.[3,5] In addition to this, there may be variability in a clinician's diagnosis due to the fluctuating nature of a patient's behavioural responses, if any are observed.[1]

Promising Novel Methods for Assessing Consciousness: Neurophysiological Scales

Neurophysiological scales are comparatively reliable due to their sensitivity for detecting residual consciousness in the form of cortical and brainstem activity without the need to assess visible behavioural responses to stimuli.[6] Electroencephalography (EEG) is highly preferred over other neurophysiological scales due to its feasibility, ease of manipulation, and reliability in measuring minor brain activity.[2] However, standard clinical EEG recordings on their own cannot be relied upon for accurate results due to the low spatial resolution of the brain activity. Thus, the use of EEGs has low diagnostic value.[1,7]

Event-related potentials (ERPs) resolve this limitation by averaging the data from several EEG recordings after stimulus presentation. ERPs can be further divided into two categories: Short Latency ERPs and Cognitive ERPs.[6]

Short-Latency ERPs are elicited between 0-100 ms after stimulus presentation.[1] An example of these ERPs is Brainstem Auditory Evoked Potentials (BAEPs), which are elicited in response to a variety of sound tonalities, pitches, and amplitudes. They are recorded in the first 10-15 ms as the auditory signals travel from the auditory nerve to the inferior colliculus of the brainstem.[1] According to a study by Fischer et al.

al. (2006), BAEPs have been used for over two decades due to their high predictive value for 100% of poor outcomes, i.e. the low probability of awakening from a coma in the absence of this activity.[8,9] Overall, BAEP activity seems to accurately predict structural damage to the brain and is effective in predicting survival based on the presence of these waveforms.[8,9]

Another subcategory of ERPs, termed Cognitive or Long Latency ERPs, are elicited in the cortical brain regions after 100 ms of the presentation of a stimulus.[1,4] One example of a sensitive long latency negative component is Mismatch Negativity (MMN), which is elicited in the primary auditory and prefrontal cortices. MMN is typically evoked 100-250 ms after a sound deviance is produced by a chain of infrequently interrupted repetitive auditory sequences.[7] There has been a significant positive correlation between the display of this component and positive outcomes of consciousness, with 90% of cases waking up from a coma if they displayed MMN.[10] Additionally, another study showed that 12 months after coma onset, MMN activity had a high positive predictive value of a healthy neurological function, measured at 87% accuracy.[6]

Current Limitations and Future Directions

Various studies have demonstrated the enhanced accuracy and sensitivity of neurophysiological scales, compared to the counterpart behavioural scales. As such, standard procedures for the diagnosis of DOC involve the use of both types of scales, behavioural and neurophysiological, to obtain more accurate conclusions.

While the efficacy of neurophysiological scales has been demonstrated through several clinical studies, there are multiple issues to address in order to consider their application for a long-term basis. ERP activity in patients with DOC is known to fluctuate [4] due to the sensitive nature of this scale, and to the variable nature of the residual consciousness observed in these patients. While MMN stands out as one of the most sensitive and reliable ERP components in terms of predicting an accurate functional outcome, more analysis needs to be performed on a long-term basis to prove the efficacy of this scale for widespread clinical application. BAEPs have typically been in use for a longer period of time, mainly to measure the intactness of the hearing pathway in individuals with hearing disabilities. The use of BAEPs to assess the functionality of the auditory pathway prior to EEG/ERP testing is a fairly recent experimental paradigm that has not shown much promise in related research findings. To conclude, extensive research needs to be conducted to assess the reliability of these scales due to their implications on the prognostication and therapy options for the patient, as well as the funding that the patient will receive, which will affect their odds of survival.[3] The grim future of such patients may be partial-

ly compensated for by the fast-paced development of research that is dedicated to designing evaluation tests. Additionally, many clinical research institutions around the world are actively exploring the field of cognitive neuroscience, including the Language, Memory and Brain Lab (LMB Lab) under the Centre for Advanced Research in Clinical and Experiential and Applied Linguistics (ARiEAL) at McMaster University.[11] One of LMB Lab's major areas of focus includes research on acquired brain injury and coma.[11] "VoxNeuro" was a program developed by the researchers from this lab in the 1990s that initiated the use of "ERPs in association with computer-adapted neuropsychological tests to assess people with neurologically-based communication problems".[11] This testing method has provided promising results for the assessment of various populations with significant communication impairments, such as those with acquired brain injuries.[11]

ACKNOWLEDGEMENTS

REFERENCES

(1) Giacino JT, Fins JJ, Laureys S, Schiff ND. Disorders of consciousness after acquired brain injury: the state of the science. Nature Reviews Neurology. 2014;10(2):99–114.

(2) Laureys S, Owen AM, Schiff ND. Brain function in coma, vegetative state, and related disorders. The Lancet Neurology. 2004;3(9):537–46.

(3) Giacino JT, Schnakers C, Rodriguez-Moreno D, Kalmar K, Schiff N, Hirsch J. Behavioral assessment in patients with disorders of consciousness: gold standard or fools gold? Progress in Brain Research Coma Science: Clinical and Ethical Implications. 2009;:33–48.

(4) Morlet D, Fischer C. MMN and Novelty P3 in Coma and Other Altered States of Consciousness: A Review. Brain Topography. 2013;27(4):467–79.

(5) Giacino JT, Smart CM. Recent advances in behavioral assessment of individuals with disorders of consciousness. Current Opinion in Neurology. 2007;20(6):614–9.

(6) Fischer C, Luaute J, Adeleine P, Morlet D. Predictive value of sensory and cognitive evoked potentials for awakening from coma. Neurology. 2004;63(4):669–73.

(7) Lehembre R, Gosseries O, Lugo Z, Jedidi Z, Chatelle C, Sadzot B, et al. Electrophysiological investigations of brain function in coma, vegetative and minimally conscious patients. Archives Italiennes de Biologie. 2012;150:122–39.

(8) Fischer C, Luauté J, Némoz C, Morlet D, Kirkorian G, Mauguière F. Improved prediction of awakening or nonawakening from severe anoxic coma using tree-based classification analysis*. Critical Care Medicine. 2006;34(5):1520–4.

(9) Petrova LD. Brainstem Auditory Evoked Potentials. American Journal of Electroneurodiagnostic Technology. 2009;49(4):317–32.

(10) Naccache L, Dehaene S, Cohen L, Habert M-O, Guichart-Gomez E, Galanaud D, et al. Effortless control: executive attention and conscious feeling of mental effort are dissociable. Neuropsychologia. 2005;43(9):1318–28.

(11) McMaster University [Internet]. Page Title. [cited 2019Mar24]. Available from: https://www.humanities.mcmaster.ca/~lmblab/current_research.html

Artificial Intelligence Can Improve the Health Care System

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ABSTRACT

Artificial intelligence (AI) is a computer system used to model human cognitive functions, intelligence, and behaviour. Components include both, a virtual and a physical aspect. Virtual aspects of AI include algorithms and neural networks instilled within the system to execute its assignments. Physical components include the entity in conjunction with a code. [1] AI is currently being developed by Nvidia Corporation, Alphabet, Twilio, Amazon, Micron Technology, Microsoft Corp., Baidu, Intel Corp., Facebook, and Tencent. [2] Expanding AI into the health care system can be beneficial for preventative care, patient safety, and reducing treatment costs for families. AI has proven to be useful in machine learning, thus, it can be programmed to complete specific tasks. By performing tasks such as data interpretation, the amount of time that it takes for a physician to consult patients regarding their results will be reduced. In addition, AI is capable of analyzing medical images to identify tumours and it has previously been used in various other branches of medicine such as neurology and cardiology. Overall, AI has great potential to improve the health care industry in North America and worldwide. However, potential violations while utilizing personal patient data must be addressed whilst modifying this technology.

Keywords: Artificial Intelligence, health care, disease evaluation, machine learning

The term "artificial intelligence" (AI) was first used in 1956 by a computer scientist, John McCarthy.[3] It was thought of as a proposed solution to solving problems using formal log-based models of reasoning.[4] AI involves a classifier, which identifies the set of categories or sub-populations that a new observation belongs to. These classifiers are built on data that has been inputted from previously systematized documents,[5] and is capable of natural language processing of data. The first Artificial Intelligence system was designed by Allen Newell at Carnegie Mellon University. It was called "Logic Theorist" and applied by J. Clifford Shaw.[4] Prior to its development, these individuals sought to understand how machines are capable of learning human functions such as visual perception and decision-making.

The physical aspect of AI refers to the software that is installed on a computer, whereas, the virtual aspect refers to machine learning, which is fulfilled through mathematical algorithms. Presently, artificial intelligence has experienced breakthroughs in deep learning, which refers to non-linear patterns in the data that have multiple layers. The technology is becoming increasingly popular since the volume and complexity of data is expanding.[6]

Using artificial intelligence represents a more efficient method of approaching issues such as diagnostic and organizational problems in the health care system, as machine models are applied in order to accurately make predictions and analyze data. Machine learning methods can be implemented to increase its technological performance since more information is systematically inputted into the machine's database. The application of these models offers many advantages, such as increased flexibility with changing conditions, lower costs, and compatibility with electrical sensing techniques, that are all rapidly advancing.[7] The use of AI will primarily be beneficial to health care practitioners since it is able to use the information from previously inputted health care data in order to enhance clinical practices.[6]

AI is capable of processing data more efficiently than humans due to robotic automation, which demonstrates a high degree of accuracy. Accordingly, it can be used to answer general questions regarding patient care, since it cultivates medical information that derives from resources such as journals and textbooks,[6] as well as previous archives. Furthermore, AI can be used to monitor large datasets in order to detect mistakes and potential threats to patient health. Lastly, AI can be trained to categorize physical examination notes that are taken by health care professionals, as well as clinical laboratory results and diagnostic images. Although in the prospect human assistance will not be required to complete such tasks, it is required to establish system automation.

Specific Uses for AI

The first successful application of AI was the clinical decision support systems (CDSS) established in the 1970s, in which a software named Pathfinder was used to help scientists identify lymph-node diseases.[8] The algorithms instilled in these systems allowed for the identification of abnormalities in medical images, including tumours and polyps associated with these diseases. AI has also helped clinical fellows in making decisions for treatment plans, as it is able to consider a plethora of treatment options and is also capable of "thinking like a doctor" in order to assess various health care problems.[9]

Previous exposure in the field of AI includes applications like MYCIN, AI/RHEUM, INTERNIST, SPE, and the TIA system.[10] MYCIN was developed in the early 1970s by Stanford University in order to perform diagnoses and provide treatment options for infectious diseases. This application was especially effective within the domain of meningitis, in which ten cases were selected and corresponding treatment recommendations were acquired using MYCIN. From the ten cases, in eight evaluations, 70% of MYCIN's were verified,[10] thus displaying moderate accuracy of the system.

AI/RHEUM is a system that computes rheumatic diagnoses, using the EXPERT system developed in the University of Missouri. In one study of 384 patients, the physician's diagnosis was compared with the diagnosis made by an AI system. Using raw data collected and presented to three rheumatologists from 48 cases, it was found that AI exhibited an accuracy rate of 94%. All rheumatologists agreed on 28 of the cases, and 96% agreed on 46 cases.[10]

The INTERNIST system uses clinical pathologic conferences from the New England Journal of Medicine as its source of data, and standards of data presentation are established by the performance of clinicians and discussants.[10] The Serum Protein Electrophoresis (SPE) system was created in order to analyze data that

is collected by a laboratory instrument. Using 256 cases, the study is unique to Artificial Intelligence in Medicine (AIM) since it was 100% acceptable, but warned scientists to expect differences of opinions, thus requiring further verifications.[10]

The Transient Ischemic Attacks (TIA) system is used in health care to help evaluate TIAs and suggest available therapeutic options. The system was held to the decision-making standards of stroke specialists at the University of Maryland. The participant group was made up of 103 patients and of these, the system was able to draw the same conclusions as an expert reviewer in all but 12 cases.

The Improvement of AI

New ways in which AI is being used to improve the health care industry is by building real-time inferences and models while accessing data from a large patient population. These developments can be used to provide alerts for patients who need immediate assistance, and to estimate the length of inpatients' stay.

Medical datasets are becoming increasingly accessible to the public, so AI can pick up on patterns used to predict treatments for patients while minimizing the risk of incorrect diagnoses.[9] In this way, AI techniques can also be combined with electronic health records (EHR), which are becoming increasingly popular in modern medicine to improve health outcomes for patients. By using EHR, AI will be able to classify any abnormalities in the health care records, thus aiding in the process of disease identification.

As previously described, the physical branch of AI refers to machines and robots, and the virtual aspect refers to the mathematical algorithms that are inputted into the systems and improved with experience. For instance, targeted nanorobots can be included as part of the physical branch of AI, in which a new drug delivery system is presented. The carriers are capable of performing a motor-based or pump-based drug delivery.[11] With this, it was discovered that medication can bypass neural networks which were previously known to be impossible for humans to accomplish.

In contrast to the successes of AI, there are various challenges associated with this technology. This includes any potential violations that overstep patients' private information, as well as ethical issues related to access to personal data. Other difficulties with using AI in hospitals include the size and complexity of data, which can be resolved with new algorithms and pattern detection approaches. Another current concern associated with AI is the extent of its regulation since there is no "gold standard" against which researchers can compare the system's performance.[12] There is a possibility that AI can alter the initial diagnosis or

treatment decision of the health care professional, which might deliver unforeseen consequences.[12] Lastly, it is important to consider the economic value of using AI systems, as health care costs in North America continue to rise. The initial cost of establishment would likely be high, however, upon its development and implementation, treatment costs for the affected individuals would drastically decrease.

CONCLUSION

AI can be used to cultivate the health care industry by advancing diagnostics and improving treatment propositions for patients. This technology proves to be accurate since it uses algorithms to analyze datasets from health-related data. It can also be enhanced with self-correcting abilities in order to improve accuracy after receiving feedback, which ultimately improves patient outcomes. In the future, AI can be used to personalize treatments for patients by evaluating their genetic makeup, which must be first considered for ethics board approval due to implications of having access to sensitive genetic data. Overall, further investigation into AI represents a valuable and multi-faceted avenue of research for the health care field.

ACKNOWLEDGEMENTS

REFERENCES

(1) Kumar D, & Meeden L. A robot laboratory for teaching artificial intelligence. ACM SIGCSE Bulletin. 1998;30(1), 341-344.

(2) Divine J. Artificial Intelligence Stocks: 10 of the Best AI Stocks to Buy [Internet]. 2018. Available from: https://money.usnews.com/investing/stock-market-news/slideshows/artificial-intelligence-stocks-the-10-best-ai-companies

(3) McCarthy J. Artificial intelligence, logic and formalizing common sense. Philosophical logic and artificial intelligence. 1989; 161-190.

(4) Flasiński M. Introduction to artificial intelligence. Krakow: Springer; 2016.

(5) Sebastiani F. Machine learning in automated text categorization. ACM computing surveys (CSUR). 2002; 34(1), 1-47.

(6) Jiang F, Jiang Y, Zhi H, Don Y, Li H, Ma S, et al. Artificial intelligence in health care: past, present and future. Stroke and vascular neurology. 2017; 2(4), 230-243.

(7) Zang Y, Zhang F, Di CA, & Zhu D. Advances of flexible pressure sensors toward artificial intelligence and health care applications. Materials Horizons. 2015; 2(2), 140-156.

(8) Neill DB. Using artificial intelligence to improve hospital inpatient care. IEEE Intelligent Systems. 2013; 28(2), 92-95.

(9) Bennett CC, & Hauser K. Artificial intelligence framework for simulating clinical decision-making: A Markov decision process approach. Artificial intelligence in medicine, 2013; 57(1), 9-19.

(10) Smirnov A. Artificial intelligence: Concepts and Applicable Uses. LAP LAMBERT Academic Publishing; 2013.

(11) Patra D, Sengupta S, Duan W, Zhang H, Pavlick R, & Sen A. Intelligent, self-powered, drug delivery systems. Nanoscale. 2013; 5(4), 1273-1283.

(12) Miller PL. The evaluation of artificial intelligence systems in medicine. Computer methods and programs in biomedicine. 1986; 22(1), 3-11.

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