DEAR READER,

Welcome to Issue 6 of Sciential! In the midst of a global pandemic, there has been a peak in interest in scientific research and some confusion regarding what that entails. As such, increasing accessibility has been our team’s continued priority this year. We are thrilled to continue the implementation of lay summaries preceding all of our pieces.

In this issue, we explore a variety of healthcare topics, ranging from diagnosis to social impacts. These topics include: the sex differences in the prevalence of obesity and hypertension, using electric source imaging to diagnose pediatric epilepsy, using biomarkers to diagnose myocardial injury in neonates, the importance of stathmin in Shigella infections, targeting leukemic stem cells in acute myeloid leukemia, and the growing field of urban health.

We hope that you will enjoy the pieces in this issue. This year has been different from preceding ones, but our team has worked tirelessly to embrace the online environment and provide you with high-quality student work.

We are incredibly grateful for the fantastic work ethic and dedication of our Senior Editors, Stefano Biasi and Reza Khorvash, and Creative Director, Simran Kaur. We appreciate the diligence and creativity of the Editors, Illustrators, Graphic Designers and the Communication Coordinator on our team. We would like to congratulate our incoming Editors-in-Chief and Senior Editors, Stefano Biasi and Naomi Suzuki, and Dalen Koncz and Lavanya Sinha, respectively. We also congratulate our incoming Creative Director, Angelina Lam.

We would also like to acknowledge the founders of Sciential, Aiman Shahid and Alisa Nykolayeva for their guidance, along with our Senior Advisor Team, Dr. Kimberly Dej, Dr. Veronica Rodriguez Moncalvo, Dr. Katie Moisse, and Science Librarian, Abeer Siddiqui, for their support.

As co-Editors-in-Chief, we were fortunate to have spent our graduating year supporting this phenomenal team and continuing to showcase undergraduate research. We look forward to the innovation that the new leadership will bring in the following years.
Sex Differences in the Association Between Obesity and Hypertension: A Systematic Review

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Introduction: Obesity is associated with multiple health-related complications, which together can deteriorate an individual's health. Some studies have demonstrated that sex influences the association between obesity and its related complications. However, no studies have directly looked at the effect of sex on the relationship between obesity and hypertension. This information is useful because it allows doctors to adapt patient care based on a patient's sex, which can increase efficiency and lessen economic healthcare burden. After reviewing related articles from three databases, eight articles were included in this study. Four articles showed that women with obesity are at a greater risk of developing hypertension, two articles showed that men with obesity are at a greater risk, and two articles showed no significant differences between the two sexes. Thus, the majority of the studies showed that women with obesity are at a greater risk than men of developing hypertension. However, due to the overall inconclusive results, it would be beneficial to perform a series of statistical tests in a meta-analysis. A meta-analysis will help to statistically conclude which sex is most at-risk based on these previous studies.

Objective: To evaluate current studies on the association between sex, obesity, and hypertension, so as to obtain an overall estimate of the effect of sex on the prevalence of hypertension in obese individuals.

Methods: A systematic search of EMBASE, MEDLINE, and PubMed was conducted. Search terms, such as “obesity”, “sex differences”, and “hypertension” were used to filter results. After reviewing 406 articles, eight articles were included.

Results: Four articles showed that obese women were at a greater risk of developing hypertension than obese men.⁵⁻⁸ Conversely, the results of two studies found that obese men are at a greater risk of developing hypertension.⁹,¹⁰ The remaining two studies showed that the difference between the sexes was insignificant.¹¹,¹²

Discussion/Limitations: Stronger evidence shows that obese women are at a greater risk of developing hypertension than obese men. The two studies that had contradictory conclusions had small sample sizes relative to the other studies. Additionally, the two studies that concluded that both sexes are at a similar risk highlighted that most other studies have determined that obese women are at a greater risk and that their limitations may have caused this discrepancy. Limitations of this review include the limited ethnicity of participants and the use of BMI to classify obesity, which can sometimes lead to misclassification due to varying muscle to fat ratios. These factors limit the generalizability of the results.
**Conclusion**: Obese women are seemingly at a greater risk of developing hypertension than obese men. However, this conclusion remains statistically inconclusive. Therefore, it would be beneficial to complete a meta-analysis in order to conclusively determine which sex is statistically more at risk of developing hypertension, when obese.

**Keywords**: Obesity, sex differences, hypertension

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The role of stathmin microtubule-destabilizing activity in *Shigella flexneri* motility and tunneling

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**SUMMARY**

Shigelloides, an infection by *Shigella* bacteria, causes many harmful and potentially dangerous symptoms. *Shigella* enter cells that line the human intestinal tract and travel through the cytosol. This environment contains long, thick obstacles called microtubules that form a dense network and provide structural support to the cell. In order to clear a path and tunnel through, *Shigella* use microtubule-destroying proteins. It is possible that the host protein stathmin may be involved in this process, since it is known to destabilize microtubules. This proposal outlines three experiments to determine stathmin’s role in tunneling, each involving the infection and comparison of normal and stathmin-lacking hosts. The experiments examine tunnel widths, microtubule densities and bacterial movement patterns in each strain. It is expected that microtubule destabilization and movement will be impaired when stathmin is absent. Since antibiotic resistance in *Shigella* is becoming more common and stathmin may be crucial for movement and subsequent spreading, the findings of this proposed study could provide an important, new treatment target.

**ABSTRACT**

The infection of the intestinal mucosa by *Shigella* bacteria is a global health issue resulting in a variety of potentially life-threatening gastrointestinal complications. Their unique method of intracellular motility depends on microtubule destabilization to clear the dense host cytoskeletal network in a process called tunneling. It is hypothesized that the host protein stathmin may play a role in this process, due to its tubulin-sequestering capability. This proposal aims to provide potential methodologies to elucidate the function of stathmin with respect to *Shigella flexneri* motility. Three experiments are proposed, involving comparisons between human intestinal epithelial cell strains under varying levels of stathmin expression, each infected with *S. flexneri*. Respectively, the experiments examine tunnel widths via electron microscopy, microtubule densities via imaging fluorescence correlation spectroscopy, and bacterial movement patterns via live fluorescence microscopy. If microtubule destabilization and movement is impaired in null stathmin strains, as predicted, such findings may inform a novel therapeutic target for shigellosis by preventing internal spreading. This is particularly significant in our current landscape, as antibiotic-resistant strains of *Shigella* are growing increasingly prevalent.

**Keywords:** *Shigella flexneri*, stathmin, microtubule destabilization, tunneling

**INTRODUCTION**

*Shigella* encompasses several intracellular bacteria, such as *Shigella flexneri*, that invade human intestinal epithelial cells (IECs) and cause inflammation and destruction of the intestinal mucosa.¹ Resulting symptoms include stomach cramps, ulcers, bleeding, and severe diarrhea.² The World Health Organization estimates that approximately 190 million cases of gastroenteritis were caused by shigellosis in 2010, making this infection a very serious global health concern.³ A unique feature of *Shigella* is their method of movement within infected cells, as they are capable of manipulating host-cell actin dynamics to form a comet tail at one pole (Figure 1).⁴ Here, the polymerization of host microfilaments propels them through the cytosol and contributes to dissemination.⁴

The host cytosol contains a dense network of microtubules (MT) that provide structural support to the cell.⁴ Intracellular microorganisms, such as *Shigella*, would be unable to navigate this mesh-like architecture if not for specialized proteins that prevent them from becoming trapped.⁵ In a process called tunneling, MT-destabilizing proteins destroy immediate MTs and clear a path that *Shigella* subsequently travel through (Figure 2).⁴

There have been several experiments examining *Shi-
gella motility and its ability to tunnel through cytoplasmic networks. Yoshida et al. demonstrated that Shigella in wild type (WT) nocodazole-treated hosts display linear movement patterns, whereas in untreated hosts, they display movement that involves occasional changes in direction and a slight zigzag pattern. Due to the MT-destabilizing activity of nocodazole, the experiment signifies that MTs serve as barriers to intracellular Shigella motility. Furthermore, when the same procedure was repeated for null VirA bacteria, Shigella movement halted, as VirA is a MT destabilizer. This demonstrates the necessity of tunneling and MT destabilization for adequate S. flexneri motility.

The bacterial protease, VirA, modulates host cell cytoarchitectural remodeling during infection. Tunneling may be similarly influenced by other MT destabilizers, including the host protein stathmin, which is known to sequester free tubulin dimers needed for polymerization. Additionally, stathmin is recruited to the bacterial surface during Shigella infection, suggesting it as a plausible contributor to tunneling. In the host, the normal function of stathmin is to enhance cell differentiation, growth, and cell mobility.

Listeria monocytogenes is a bacterium with a similar mechanism of infection and motility that also recruits stathmin. Despite not exhibiting tunneling, MT density surrounding L. monocytogenes has been shown to increase in stathmin-depleted human colorectal cells. In addition, these bacteria exhibited significantly impeded speed. This provides evidence that stathmin destabilizes MTs and facilitates Listeria movement through the cytoskeletal matrix, but whether it is involved in the same process and tunneling during S. flexneri infection requires elucidation.

An estimated 164,000 fatalities, associated with shigellosis, are recorded annually. Treatment of bacterial infections rely on antibiotics; we are also acutely aware of growing concerns associated with antimicrobial resistance (AMR). Limitations in treatment exist due to the increasing prevalence of multi-drug resistant strains of Shigella. Alternative therapies may be required if AMR renders standard treatments inefficacious. Characterization of stathmin’s influence on S. flexneri infection may provide an alternative treatment for shigellosis. If expressional differences in stathmin influence bacterial motility and follow-up experimentation provides evidence of associated implications in pathogenesis, stathmin may provide a novel therapeutic target for shigellosis. Specifically, modulation of stathmin activity intracellularly may preclude efficient motility, which is necessary for the dissemination of S. flexneri within tissues and the internal spread of infection.

We propose three experiments below to study stathmin’s role in tunneling. Each examines factors related to MT destabilization and S. flexneri motility under differing levels of stathmin expression. Experiments one and two analyze tunnel widths and local MT density, respectively, to establish stathmin’s role in MT clearance and destabilization. Experiment three investigates movement patterns at large, to identify whether stathmin facilitates movement through MT obstacles.

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*RESEARCH QUESTION*

Does stathmin play a role in microtubule destabilization and tunneling during *S. flexneri* infection?
HYPOTHESIS

Stathmin contributes to microtubule destabilization and tunnel formation during S. flexneri infection.

EXPERIMENT 1

Prediction:

Narrower tunnels will form in null stathmin human IECs infected with S. flexneri.

Stathmin’s influence on MT stability and its broader role in the modulation of host-cell cytoskeletal dynamics is understood; however, its influence on Shigella tunneling requires further study. The first method that will be used involves measuring tunnel widths in infected cells under differing levels of stathmin expression, in vitro. We predict that narrower tunnels will form in cultured null stathmin human IECs infected with S. flexneri.

Three groups of human IECs will be cultured and infected with S. flexneri— one control group and two experimental groups. The control group will consist of cultured WT human IECs. The first experimental group will consist of cultured human IEC stathmin null mutants and the second will consist of mutants with a stathmin gene duplication. Gene duplication will represent upregulated stathmin levels. After infection, cultures will be incubated in a sufficient medium at 37°C for five hours, prior to assessment.

After the incubation period, a JEOL 1200EX TEM-SCAN electron microscope will be used to capture images of the cultures. Ten images of each group will be captured during three successive intervals, 20 minutes apart. During each of these intervals, images of a microscopic ruler will be captured at the same magnification to be used to calibrate the image analysis software’s measurement tools. Post-image analysis will make use of the software ImageJ to measure the average tunnel widths. ImageJ will be calibrated for each set of images. Using the ten images of each group, collected during each interval, the tunnels’ widths will be measured. The average tunnel widths will be calculated for each group during each interval. Comparisons will be made between each of the groups’ respective average tunnel widths for each interval and analyzed for statistical significance using Prism GraphPad software. We will make use of a two-tailed Student’s t-test between each group and calibrate significance with a p-value less than 0.05.

It is expected that statistically significant differences in average tunnel widths will be observed between all three groups. The ascending order of expected average tunnel widths is: null, WT, duplication. Stathmin destabilizes MTs, therefore, human IEC stathmin null mutants should exhibit narrower tunnels (i.e., less clearance) than cultured cells with a stathmin gene duplication and WT. Conversely, increased expression of a MT destabilizer in the gene duplication group should exhibit wider tunnels (i.e., more clearance) than cultured cells with a stathmin null mutation and WT.

This is a reasonable expectation given previous research involving S. flexneri and L. monocytogenes. Yoshida et al. demonstrated that the presence of MT destabilizers — VirA and Nocodazole — resulted in the clearance of MTs and VirA-implicated tunnel widths in S. flexneri-infected cells. Stathmin, with MT-destabilizing properties as well, was theorized to exhibit similar effects. Furthermore, Costa et al. demonstrated that stathmin’s sequestration of tubulin leads to depolymerization of MTs surrounding L. monocytogenes. Therefore, increased stathmin expression is expected to further destabilize bacterial-surrounding MTs and form wider tunnels.

EXPERIMENT 2

Prediction:

Null stathmin human IECs will have a higher density of MTs near S. flexneri during infection, when compared to WT cells.

Stathmin contributes to MT destabilization during Listeria infection; however, whether stathmin plays the same role during Shigella infection is not well studied. Therefore, examining the MT density near Shigella under varying levels of stathmin expression is a valid next step. Higher densities would signify impaired MT destabilization and implicate stathmin’s role in this process. The same control and experimental groups will be used as in experiment one; however this time, all IECs will additionally contain a tubulin-GFP fusion gene construct for MT visualization.

For visualization of the bacteria, direct immunofluorescence will be performed. Each group will be infected with Shigella bacteria and treated with a rhodamine-tagged antibody for an O-antigen (O-Ag), a component of lipopolysaccharides found exclusively in gram-negative bacteria such as Shigella. All host cultures infected with Shigella will be incubated at 37°C for five hours before assessment.

After preparation of the experimental groups, the density of MTs will be measured via imaging fluorescence correlation spectroscopy (ICS), using a Stellaris 5 confocal microscope. A focused laser beam will excite fluorescent molecules near the bacteria and the microscope will capture emitted photons, generating a two-dimensional image to represent the fluctuation of fluorescence intensity. Autocorrelation software will then be used to calculate the MT density from these fluctua-
tions. Statistical analyses to determine significance in the results would be conducted using Prism GraphPad software. A two-tailed Student’s t-test and a p-value of less than 0.05 would indicate significance.

If stathmin is involved in MT destabilization during infection, then cells with stathmin will contain a lower density of MTs near Shigella, providing evidence for its role in tunneling. It is expected that experimental group two, mutants with stathmin gene duplications, would have the lowest density of MTs. Comparatively, experimental group one, which lacks stathmin, would be expected to have the highest density. The WT control is expected to have a value in between experimental group one and two.

**EXPERIMENT 3**

**Prediction:**
In untreated null stathmin IECs, *S. flexneri* will exhibit jagged movement patterns, with more frequent changes in direction compared to the WT.

Dysfunctional movement in null stathmin hosts would provide additional evidence for the hypothesis. Yoshida et al. examined Shigella motility in WT hosts and discovered a relatively linear movement pattern upon nocodazole treatment. However, in untreated hosts, there were occasional changes in direction. Since nocodazole is a MT-desstabilizer, these findings signify that MTs behave as obstacles and that collisions result in deflections. If stathmin does in fact behave as a MT-desstabilizer like nocodazole, then stathmin’s absence will result in a zigzag, non-linear movement pattern as Shigella collide with MTs more often. This would also demonstrate that stathmin plays a role in tunneling.

The preceding experiments have the objective of elucidating the function of stathmin with respect to MT destabilization during *S. flexneri* infection. However, demonstrating stathmin’s activity in this way is not sufficient to determine its implications on motility at large. Therefore, experiment three’s examination of Shigella movement patterns is necessary. Furthermore, stathmin’s overall potential as a therapeutic target may be better understood if motility is truly impaired in null strains, since adequate mobility is required for dissemination and the spread of infection within tissues.

This experiment will include two strains of cultured human IECs to serve as hosts, one of which will be a WT and the other a null stathmin mutant. Additionally, *S. flexneri*, an antibody against an O-Ag tagged with rhodamine, nocodazole, and the Zeiss Axio Imager Z1 fluorescent microscope will be required. Controls will include nocodazole-treated and untreated WT hosts and the experimental groups will consist of nocodazole -treated and untreated null stathmin strains (Figure 3). Nocodazole is a MT-desstabilizing drug that significantly reduces the number and density of MTs. Wild-type IECs treated with nocodazole are a control, since previous studies have shown that Shigella movement is linear under these conditions from a lack of MT barriers. Null stathmin IECs treated with nocodazole must be compared to the equivalent WT treatment in order to verify that movement patterns are only different due to differences in MT destabilization. This would be evident if no significant differences are observed between the two, as neither will have MT obstacles. Independent variables will include the presence of stathmin and nocodazole. The dependent variable will be the average frequency of direction changes for the Shigella in each group.

Throughout the experiment, all incubations and video recordings will occur at 37°C. Initially, a culture of WT and null stathmin IECs will each be treated with a nocodazole solution and incubated for ten hours. This will be followed by the infection of all groups with *S. flexneri* and an additional incubation of five hours. Next, each group will be treated with rhodamine-anti-O-Ag and incubated for a period of 120 minutes. This will be done with the objective of visualizing individual Shigella via direct immunofluorescence. Lastly, live Shigella motility will be captured and recorded using the Zeiss Axio Imager Z1 fluorescent microscope for 120 minutes under 2500x magnification.

Frequencies of *S. flexneri* direction changes for each group will be recorded using the Manual Tracking ImageJ plug-in, which allows for path tracing and the measurement of angles. Direction changes are to be classified as deviations from the bacterium’s course by a minimum of 15° under 2500x magnification. This is to only account for large changes due to collisions with obstacles and ignoring minor random changes in motion. Each direction-change frequency will then be tested for statistical significance using Prism GraphPad software and the two-tailed Student’s t-test. A p-value smaller than 0.05 will be considered statistically significant.

Due to the MT destabilizing activity of nocodazole, it is expected that both treated groups will have few MTs acting as obstacles. Therefore, they will likely yield linear *S. flexneri* movement and infrequent changes in direction, regardless of whether stathmin is present or not. This would confirm that differences in movement in the other groups are solely due to stathmin’s effect on tunneling since movement patterns are not influenced by other factors aside from MT destabilization. The untreated WT control is expected to have slightly more frequent direction changes compared to the nocodazole groups as seen in the study by Yoshida et al.,...
but still remain relatively linear due to the presence of stathmin.4 The untreated null stathmin mutants are expected to have an impaired ability to degrade MTs, thus presenting Shigella with zigzag movement patterns and significantly more frequent direction changes compared to all other groups. Movement is not expected to halt completely because Shigella are still able to take advantage of other MT-destabilizers, such as the secreted VirA.4

In the third experiment, quantifying direction changes with a minimum deviation angle poses a potential limitation, as this method assumes that collisions with MTs always result in large deflections. A number of smaller diversions from MTs still behaving as barriers to movement may be missed and slightly obscure validity. This is, however, a necessary sacrifice to discount random deviations, as S. flexneri seldom travel in a perfectly straight line.14

Stathmin has also been found to indirectly contribute to comet tail integrity and movement by activating coflin.8 Coflin is an actin-severing protein that prevents unnecessary growth of actin filaments in the comet tail.8,15 It has been demonstrated that Listeria in null stathmin host cells move slower and have longer comet tails than the WT, due to inactive coflin.8 This may be a possible limitation of the study, as it is not known whether a difference in speed will also be present in Shigella infection or if it will affect movement patterns, tunneling, or MT destabilization. Regarding movement, similarities in motility between the two nocodazole-treated groups in the third experiment would demonstrate that this is not a significant concern. It is still unclear, however, whether speed might affect the other two processes in a way that cannot be accounted for.

Lastly, as with all in vitro experiments, the results of this study cannot be readily extrapolated within the human body, where many additional elements are at play.

**CONCLUSION**

There are several gaps in our current understanding of S. flexneri’s interactions with host cell machinery and the proposed experiments will address these areas. It has been shown that similar to VirA — a bacterial protease — host proteins may influence S. flexneri motility and promote pathogenesis. Interactions between stathmin and L. monocytogenes influence bacterial movement and dissemination.8 Yoshida et al. have demonstrated that VirA’s MT destabilizing behaviour promotes S. flexneri tunneling and dissemination. Therefore, we hypothesize that stathmin may function similarly.4 Currently, stathmin’s interactions with S. flexneri remain uncharacterized. Unlike L. monocytogenes, S. flexneri motility is reliant on tunneling — the clearance of dense MT networks; however, Shigella tunneling is not fully understood and requires further investigation. This study seeks to broaden our understanding of S. flexneri motility, host-cell interactions, and infection, at large.

With the growing concern of AMR in the treatment of shigellosis, it is imperative that alternative treatments are sought after. The findings of this proposed study can pave the way for a novel therapeutic target in the
form of stathmin, whose inhibition may result in compromised Shigella motility. Further research may then suggest that this impairment prevents its spread from cell to cell within tissues, thus limiting infection.

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The Role of Electrical Source Imaging in Pediatric Epilepsy and Pre-Surgical Evaluation

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SUMMARY

Epilepsy is a neurological disorder involving frequent and involuntary seizures. Caused by an imbalance of electrical activity in the brain, epilepsy is typically treated with anti-epileptic drugs to reduce or cure symptoms. Unfortunately, there are individuals who do not respond to medication. When all other treatment options have been exhausted, doctors might recommend surgery to remove the area of the brain thought to be the cause of seizures (the epileptogenic zone). This area is determined by a thorough pre-surgical evaluation employing a variety of diagnostic imaging technologies. The goal of this evaluation is to identify the epileptogenic zone. Unfortunately, the use of invasive tests can potentially scar brain tissue and traditional methods of determining the epileptogenic zone can potentially be inaccurate. Electrical source imaging (ESI) is a non-invasive imaging software proposed as an additional tool to be included within the pre-surgical evaluation of children with epilepsy. ESI combines the patient’s brain scans and recordings of electrical activity to identify the potential sources of seizures. Despite evidence supporting its use, ESI is underutilized in clinical settings and within pediatric research. The following review looks into the literature surrounding ESI and advocates for its inclusion within the pre-surgical evaluation of children with epilepsy.

ABSTRACT

Children with drug-resistant epilepsy undergo an extensive pre-surgical evaluation to determine the part of the brain thought to be the cause of seizures. The employment of non-invasive diagnostic imaging tools plays an important role in establishing surgical candidacy, preventing the need for invasive procedures. Electrical source imaging (ESI) has been explored as a modern alternative to traditional diagnostic techniques in pre-surgical workup. Through computational analysis of recorded electric potentials and individualized head scans, ESI provides a non-invasive method of obtaining more accurate localizations. However, its use within the clinical setting is limited. The following review looks to examine the literature surrounding ESI and advocates for its inclusion within the pre-surgical workup of children.

Keywords: Electrical source imaging (ESI), electrical source localization, epilepsy, pediatrics, pre-surgical evaluation, epileptogenic zone

INTRODUCTION

Epilepsy is a neurological disorder that is commonly treated with anti-epileptic drugs to control seizures and improve quality of life. A large proportion of children diagnosed with epilepsy do not respond to medication and are often referred for surgery as a curative measure. This involves a detailed and extensive pre-surgical evaluation to identify the epileptogenic zone, a hypothetical region of the cortex that, once resected, provides the patient with seizure freedom. However, this area is only confirmed retroactively if the resection provides seizure freedom.

Non-invasive imaging techniques are used to determine this region via the presence of anatomical or physiological anomalies. Clinicians are able to infer the location of the epileptogenic zone and identify surgical candidates if these tests show concordant information and there is no overlap with the parts of the brain responsible for sensation, movement, language, or speech.

The electroencephalogram (EEG) is one such diagnostic tool that plays a pivotal role in understanding the abnormal neuronal activity observed in epilepsy. EEGs measure and record the
synchronous firing of cortical neurons using electrodes placed on the scalp. Video analysis of EEG recordings is a crucial first step during the presurgical evaluation of children. Traditionally, these recordings are visually analyzed for distinctive waveforms and spike patterns to approximate sources of epileptic activity within the brain. The ictal onset and irritative zone are two regions of interest that can be defined through EEG analysis. The ictal onset zone is defined by the EEG waves that occur at the beginning of the seizure (the ictal phase), while the irritative zone is identified by the spikes that occur between seizures (the interictal phase).

EEGs play a pivotal role during the early stages of presurgical evaluation, helping neurologists build towards a general understanding of the epileptogenic zone. However, visual analysis of these recordings lacks the accuracy required to define the resection margins. Computational analysis, known as electrical, or EEG, source imaging (ESI), has shown promise in overcoming these limitations. Through a set of mathematical algorithms, ESI maps potential sources of EEG activity onto a magnetic resonance imaging (MRI) head model of the patient.

The field of ESI has expanded exponentially in recent years. Advancements in software technology have allowed for more robust and rapid source localization of EEG recordings. Utilizing readily accessible information in MRI scans and EEG recordings, ESI presents itself as a safe and effective tool within presurgical workup. Ictal ESI determines the ictal onset zone from ictal EEG waves, while interictal ESI determines the irritative zone using interictal EEG spikes. In conjunction with other functional imaging techniques, ESI is a highly recommended modality that can improve the localization of the epileptogenic zone.

In spite of a growing body of evidence and a need for non-invasive diagnostic tools, ESI is underutilized within the field of pediatric epilepsy. EEGs used for ESI analysis are typically recorded with large electrode arrays containing 100 to 200 electrodes. These high-density (HD) recordings have shown to yield more accurate results and provide greater spatial resolution with increased electrodes. However, their use is impractical and cumbersome for long-term EEG monitoring. Low-density (LD) recordings employing 20 to 30 electrodes, demonstrate similar accuracy to HD-EEG and presents an encouraging alternative for pediatric epileptology.

There is a lack of studies looking at the use of low-density ESI in children during presurgical workup and, to date, none comparing ictal to interictal ESI.

The following review aims to examine the current role of electrical source imaging in clinical epileptology and its practicalities as a diagnostic tool in pediatric surgery.

FUNDAMENTALS OF ESI

The concept of electrical source localization has been explored since the inception of the EEG. With recent advances in ESI, researchers have gained a better understanding of how electric fields are generated and propagated deep within the cortex. ESI attempts to localize the source of scalp-recorded potentials by solving for a set of forward and inverse problems.

The inverse problem addresses the guiding question behind source localization: determining the source of electrical activity in the brain from a given EEG recording. The answer to this problem is theoretically impossible as there can be an infinite number of potential sources for the recorded electrical potential. In light of this, a forward problem is created where the scalp potential is determined from a hypothetical source in the brain. As a single, unique potential can be calculated, the forward problem is solved to address the inverse.

The forward problem accounts for the conduction of electrical signals towards the scalp via the use of a spherical shell or realistic head model. The spherical shell model is a simpler model using a sphere to represent the skull and the brain, ignoring more complex factors that might affect the spread of electrical activity towards the scalp. Realistic head models are based on the individual MRI scans of each patient and are preferred for their increased complexity and specificity. Popular techniques include the boundary element method, the finite element method, and the finite difference method. These methods vary technically but have all shown to provide more accurate localization results than spherical models.

The inverse problem further restricts the possibility of solutions by using a set of models. Single dipole, multiple dipole, and distributed source models are most prevalent within clinical ESI. Dipole models draw from the assumption that recorded potentials derive from either a single source or multiple sources in the brain. Dipoles are fitted using different computer algorithms to a location in the brain that is calculated to most likely be the epileptogenic focus. A single dipole or dipole cluster indicates where the epileptogenic zone is likely to be. In contrast, distributed source models assume that each potential
The use of interictal HD-ESI in epilepsy analysis and post-operative success is two commonly used standards in ESI research. iEEG is an invasive test that measures the spikes recorded from electrodes inserted directly over or into the brain. iEEG recordings can localize both the irritative and ictal onset zone, demonstrating greater spatial resolution than EEG. One study retrospectively compared iEEG localization of the irritative and epileptogenic zone to ESI in 38 patients. ESI accurately localized the irritative zone to within 15 mm of iEEG analysis and was within the resection margins for 80% of seizure-free patients, demonstrating strong overlap with the epileptogenic region. However, it should be noted that iEEG is an imperfect diagnostic standard and can run the risk of infection or hemorrhage. In rare situations, the epileptogenic zone can be missed entirely if this region is located deeper within the brain.

Comparisons to post-operative outcomes provide clinically relevant information regarding the efficacy of ESI. Seizure freedom following surgery indicates definitive proof of the epileptogenic zone. Correct localization is observed if the ESI result is within the resected region for seizure-free patients or the ESI result is outside of the resected region in patients with persistent seizures. Conversely, a false ESI localization is indicated by ESI results outside of the resected region in seizure-free patients or results within the resected region of unsuccessful surgeries. Several studies have looked at the effectiveness of ictal and interictal ESI when compared to the resected zone. A prospective study, conducted by Brodbeck et al., examined the specificity and sensitivity of ESI in a cohort of 152 patients. The use of interictal HD-ESI in combination with individual MRI head models compared favourably with established diagnostic tools. These included structural MRI, ictal single-photon emission-computerized tomography (SPECT), and positron emission tomography (PET) results. In fact, ESI was found to be more accurate than both SPECT and PET. Additional studies conducted have further depicted the strong predictive value of HD-ESI and its strong concordance to other diagnostic measures. Magnetoencephalography (MEG) is another important non-invasive modality worth mentioning. MEG is a resource-intensive test measuring the magnetic fields produced by electrical activity in the brain. MEG results are considered an accurate measure of the epileptogenic zone, acting as a strong predictor of post-operative seizure freedom. While less accurate than MEG, the majority of ESI epileptogenic zone localizations were concordant with iEEG findings and clinical localizations.

Most of these studies highlight the use of ESI with interictal waveforms to determine the irritative zone. This is in large part due to the difficulty in analyzing ictal patterns and the presence of artifacts disrupting EEG signals. Despite the preference for interictal ESI, there is some debate surrounding its overlap with the epileptogenic region. This has led to a growing body of research towards the use of ESI in ictal onset zone localization. Studies have demonstrated the accuracy of both LD- and HD-ictal ESI with post-operative outcomes. When combined with a functional connectivity analysis, a tool used to determine the spread of seizures and connectivity of pathological networks in the brain, there is a significant improvement to ictal source localization. Comparative studies of ictal to interictal ESI depict similar accuracy in source localization and present both approaches as viable diagnostic methods. The evidence points to the positive predictive value of both ictal and interictal ESI and their ability to provide clinically relevant information.

Despite evidence indicating the reliability of ESI in adults, there are few studies assessing the predictive value in a pediatric population. Two papers examining the interictal ESI analysis of LD-EEG recordings in children saw correct localization of the epileptogenic zone in a majority of patients. ESI localizations compared favourably with PET and ictal SPECT and, in fact, displayed a higher accuracy in MRI-negative cases, which are arguably more difficult to diagnose. Using large cohorts of 30 to 60 patients, these studies demonstrate the specificity of ESI and the feasibility of LD-EEG recordings in ESI analysis. Additional comparisons of interictal ESI to MEG in a pediatric population observed a strong correlation between HD-ESI localization and the resected region, suggesting that HD-ESI might provide similar accuracy to MEG data.

There are a few underlying concerns regarding ESI research, preventing its widespread utilization within clinical epilepsy. Particularly, there appears to be a lack of standardization across clinical research.
A recent systematic review assessed the clinical validity of papers relating to ESI and MEG source imaging (MSI). They determined studies were a reliable indicator of epileptogenic source localization if they utilized HD-ESI, included more than ten patients, and utilized post-surgical outcomes of at least ten months after operation as the reference standard. From 51 studies assessing MEG or ESI, 11 met the aforementioned criteria.

Additionally, they observed a lack of standardization with the use of inverse and forward models and what is considered an accurate ESI localization.33 The authors address a need for standardization across clinical studies and to explore the efficacy of source localization in long-term prospective studies.32

Regarding pediatrics, a great deal of the literature surrounding ESI focuses on the localization of HD-EEG recordings utilizing 128 to 256 electrodes.30 As previously mentioned, this method of recording is impractical for children and presents itself as a major detractor toward the implication of ESI in epileptogenic centers. Additional studies related to LD-ESI can work to address this concern and to warrant the widespread use of ESI in clinical settings.

CONCLUSION

Within children, there is an apparent need for non-invasive measures that effectively localize the epileptogenic zone. In conjunction with other diagnostic measures, ESI can work to guide the use of invasive modalities and improve the general understanding of the epileptogenic zone in patients. There is already a large body of evidence supporting the inclusion of source localization within the presurgical workup of children. ESI exhibits strong concordance with other established imaging techniques, namely SPECT, PET, and MRI scans, demonstrating high localization values. Taking advantage of readily accessible information in EEG recordings, ESI presents itself as an efficient method of predicting sources of abnormal neuronal activity in epilepsy. However, there is a lack of research for pediatric populations and for comparisons of ESI localization values to different epilepsy subtypes. Future studies must continue to examine the localization of both the irritative and ictal onset zone and create a clinical standard for conducting ESI.

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Evaluating the use of biomarkers for the diagnosis of myocardial injury in neonates

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SUMMARY

When most individuals think of a heart attack patient, they picture a middle-aged man or woman. While heart attacks are not as common in babies, they do occur. Currently, there are limited diagnostic strategies for cardiac complications and heart attacks in babies, which has resulted in high mortality rates. This literature review explores the use of specific proteins found in the blood, referred to as biomarkers, as a diagnostic tool for neonatal myocardial infarction. The review concludes that one particular biomarker, cardiac troponin, shows great promise in diagnosing cardiac injury in babies. This knowledge will help reduce the mortality rates of heart injury in babies. Future steps that need to be taken are exploring the limitations of cardiac troponin and improving diagnostic accuracy by using high sensitivity assays and umbilical cord blood to test biomarker levels.

ABSTRACT

Myocardial infarction is defined as the obstruction of blood flow to the heart, resulting in oxygen deprivation. While myocardial infarction in adults is common and has sufficient diagnostic strategies, there remain gaps in the diagnostic strategies for myocardial infarction in neonates. Presently, biomarkers such as creatine kinase-MB, brain natriuretic peptide, myoglobin, and troponin are believed to be potential diagnostic tools for neonatal myocardial infarction. This literature review explores the efficacy of biomarkers for early diagnosis of neonatal myocardial infarction. The review concludes that creatine kinase-MB, brain natriuretic peptide, and myoglobin do not serve as accurate biomarkers for myocardial infarction in neonates. However, cardiac troponins, in particular cardiac troponin I, have high sensitivity and specificity for diagnosing myocardial injury. Cardiac troponins experience rapid elevation upon myocardial injury, and they remain unaffected by gestational age and birth weight. In addition, they do not cross the placenta and are therefore intrinsic to the neonate. Future research should be conducted to verify the accuracy, sensitivity, and specificity of cardiac troponins as myocardial infarction biomarkers.

Keywords: Myocardial infarction, biomarkers, neonates, creatine kinase-MB, brain natriuretic peptide, myoglobin, troponin

INTRODUCTION

Myocardial injury, ischemia, and infarction occur when there is a lack of blood flow and oxygen supply to the myocardium (heart muscle) due to a coronary artery occlusion (Figure 1).¹ This can lead to necrosis and damage to the myocardium tissue.¹ Myocardial injury, ischemia, and infarction represent different durations of myocardium oxygen deprivation.² In particular, less than 20 minutes of myocardium oxygen deprivation results in myocardial injury, between 20 minutes and two hours results in myocardial ischemia, and over two hours results in myocardial infarction.² While myocardial injury, ischemia, and infarction are common among adults, they are rare among neonates.³ Myocardial infarction, injury, and ischemia have high mortality rates in neonates, with myocardial infarction having the highest mortality rate at 40 to 50%.³ Early diagnosis of neonatal myocardial complications is challenging because the clinical symptoms are nonspecific.⁴ Also, many of the diagnostic methods that are used for adult myocardial injury, ischemia, and infarction are invasive and inconclusive, and thus not sufficient for neonates.⁴ For example, myocardial infarction in adults can be detected with electrocardiographic (ECG) changes.⁵ In particular, the ECG readings of adults with myocardial
Infarction have a pathological Q-wave, which is non-existent in neonates. Q-waves in an ECG represent the left to right ventricular depolarization of the heart. When the Q-wave is abnormally deep and wide, it reflects myocardial infarction. Therefore, further research needs to be conducted so the appropriate diagnostic and management strategies can be developed for myocardial injury, ischemia, and infarction in neonates.

Figure 1. Blockage of the coronary artery leading to myocardial infarction. (A) Normal Coronary Artery. Oxygen and nutrients can flow to the heart tissue. (B) Blockage of Coronary Artery. Inflammatory and fatty substances adhere to the artery walls which form plaque. Plaque can form a clot (yellow) in the coronary artery, resulting in an insufficient supply of oxygen to the heart tissue. This can result in severe damage and even death to that portion of the heart. [Image made with BioRender]

Presently, biomarkers such as cardiac troponin, serve as a diagnostic tool for myocardial infarction in adults. However, only a few studies have been conducted on the use of biomarkers for myocardial infarction in neonates. This literature review will analyze the accuracy, sensitivity, and specificity of four biomarkers that have been used in previous studies for the diagnosis of myocardial injury in neonates: creatine kinase-MB, natriuretic peptides, myoglobin, and troponin.

CREATINE KINASE-MB

Creatine kinase is a protein found in many tissues of the body, such as skeletal, cardiac, and brain tissues. Creatine kinase-MB (CK-MB) is an isoenzyme of creatine kinase and is found mostly in the heart muscle. Following injury to the heart, CK-MB in the myocardium is released into the bloodstream and can be measured. However, based on a thorough analysis of previous studies, it is evident that serum CK-MB should not be used as a biomarker for myocardial injury in neonates. This can be attributed to a few reasons.

The primary reason is that CK-MB elevation has low cardiac specificity as neonatal CK-MB is derived from both myocardial and skeletal muscle. Hence, it is difficult to determine if serum CK-MB is high due to skeletal or myocardial injury. In addition to this, CK-MB has been shown to be elevated in healthy neonates. Almeida et al. illustrated that healthy neonates had elevated CK-MB levels in their first 24 hours of living, followed by a significant decline to approximately half their initial values. This result suggests that high neonatal levels are due to stress or injury from delivery and skeletal involvement. CK-MB is also affected by gestational age, birth weight, and mode of delivery. In particular, CK-MB levels increase with decreasing gestational age and birth weight. Furthermore, neonates who are delivered vaginally have significantly higher levels of CK-MB than neonates who are delivered by cesarean section.

While CK-MB was previously believed to be a specific biomarker for myocardial injury, many studies show contradictory and interesting results. For example, myocardial injury and infarction in neonates have been shown to be caused by asphyxia. In particular, myocardial damage occurs in up to 73% of asphyxiated neonates. Asphyxia can also cause renal failure, which causes CK-MB to lose its specificity for myocardial injury when renal problems arise, resulting in false positive rates of 20 to 30% for myocardial injury. This is supported by the results of a study performed by Sadoh et al. The study included a control group of asphyxiated neonates without myocardial or renal injury and three experimental groups: a group of neonates with myocardial injury, a group with renal injury, and a group with combined myocardial and renal injury. The results illustrated that CK-MB was significantly higher in the renal injury group and combined injury group than in the control group (P<0.0001 and P=0.006, respectively). However, CK-MB was not significantly higher in the myocardial injury group than in the control group (P=0.55). The results of this study suggest that CK-MB is an effective biomarker for renal injury, but not myocardial injury.
CK-MB should not be used as a neonatal myocardial injury biomarker because the elevation of serum CK-MB concentration takes longer compared to other biomarkers. For example, CK-MB takes three to eight hours to rise after myocardial injury, whereas troponin I typically rises within two to three hours of myocardial injury.

Overall, CK-MB is not an accurate biomarker for myocardial injury in neonates because it has low cardiac specificity, it is affected by gestational age and birth weight, and its elevation is delayed compared to other biomarkers.

**BRAIN NATRIURETIC PEPTIDE**

Brain natriuretic peptide (BNP) is a myocardium hormone that is released into the bloodstream upon ventricular filling and myocardium stretching following injury to the heart. It is believed that BNP does not cross the placenta and, therefore, neonatal BNP levels are intrinsic. Despite the advantages of using an intrinsic biomarker, there are certain downfalls to the diagnostic use of BNP as highlighted below.

A study conducted by Jiang et al. demonstrated that BNP is not a predictor of myocardial injury in neonates. This study used the current Chinese diagnostic criteria to identify myocardial injury in the neonates. The criteria includes perinatal hypoxia, abnormal electrocardiogram readings for the ST-T wave for two to three days, and clinical manifestations such as bradycardia, low blunt heart sounds, and signs of poor circulation. This criteria differs from other studies as other studies focus only on clinical manifestations. Focusing solely on clinical manifestations has limitations because atypical cases, in which the neonate does not display clinical symptoms, can occur and result in delayed diagnosis.

There was no significant difference in serum BNP levels 12 hours after birth between the myocardial injury group and non-myocardial injury group (P=0.398). These results suggest that serum BNP levels do not rise following cardiac injury and therefore cannot be used as a cardiac biomarker. In addition to its lack of elevation following cardiac injury, BNP has a couple more limitations. Neonates who develop myocardial dysfunction from asphyxia are often exposed to hypoxia. A hypoxic environment can increase the expression of the ventricular BNP gene, which can lead to increased levels of plasma BNP that mimic acute myocardial injury. Therefore, increased BNP levels can be a reflection of hypoxia rather than myocardial injury. That being said, if the hypoxic environment is controlled for, researchers can overcome this limitation and BNP can be used as an indicator of neonatal myocardial injury. This is shown by a clinical study performed by Zhu and Nie. The researchers found that serum NT-proBNP was significantly higher in the asphyxia with myocardial injury group than the asphyxia with non-myocardial injury or control groups (P<0.01). These results suggest that, when a hypoxic environment is controlled for, BNP can be used as a biomarker for neonatal myocardial injury.

Furthermore, BNP has low specificity to myocardial injury as BNP levels rise in healthy neonates. Almeida et al. showed that healthy neonates have a significant rise in BNP levels during the first 24 hours of their life. This rise is attributed to the ventricular overload during the transition from fetus to neonate. In addition, the left and right ventricles release BNP to reduce the work done by the heart. This release is known to help with the adaptation to extrauterine life and to achieve physiological homeostasis. Thus, the elevation of BNP in neonates occurs to aid with growth, and may not be reflective of a myocardial injury.

Overall, BNP is not an accurate biomarker for myocardial injury. BNP serum levels do not always rise following myocardial injury, BNP is limited in hypoxic environments, and it lacks specificity for myocardial injury as levels also rise in healthy neonates.

**MYOGLOBIN**

Myoglobin is a protein that binds oxygen and is located in the heart and skeletal muscles. Upon injury to the muscle, myoglobin is released into the bloodstream and can be measured. Very few studies have focused on the use of myoglobin for the diagnosis of myocardial injury in neonates. One study, conducted by Jiang et al., showed that there was no significant difference in myoglobin levels between neonates with and without myocardial injury. The study concluded that myoglobin is found in a wide range of muscle tissues and is therefore not a specific marker for myocardial injury.

In addition to this, a study performed by Kaur et al. showed that myoglobin levels are strongly correlated with renal failure and renal tubular damage. This further reduces the specificity of myoglobin because asphyxia, previously noted as a common cause of myocardial injury, can also cause renal failure. As a result, this can lead to false positives of myocardial injury in neonates with asphyxia.
Overall, myoglobin lacks specificity for myocardial injury as it is found in many muscle tissues of the body and is correlated with renal injury.

**TROPRONIN**

Troponin I, C, and T are regulatory proteins found in the troponin-tropomyosin complex and play an essential role in skeletal and cardiac muscle contraction. Troponin C has one isoform that is found in both skeletal and cardiac muscle, whereas troponin I and T have isoforms that are distinct between the skeletal and cardiac muscle. As a result, cardiac troponin I and T (cTnI and cTnT) are often used in the diagnosis of myocardial damage in adults. If cTnI and cTnT are found in the extracellular space, such as the serum, it is reflective of myocardial injury (Figure 2).

Figure 2. Structure of troponin complex in the myocardium. (A) Normal cardiomyocyte (B) Damaged cardiomyocyte resulting in the release of cardiac troponin T (yellow) and cardiac troponin I (purple) into the bloodstream. [Image made with BioRender]

Jiang et al. conducted a study to test the diagnostic ability of cTnI. High sensitivity cardiac troponin I (hs-cTnI) was measured using a chemiluminescence immunoassay (Abbott Laboratories) with a detection limit of 1.1-1.9 ng/L. At 12 hours postnatal, hs-cTnI was significantly higher in the myocardial injury group than the non-asphyxia control group (P<0.001) and the non-myocardial injury with asphyxia group (P=0.016). It is important to note that seven days after birth, there were no significant differences in serum hs-cTnI among the three groups. This indicates that hs-cTnI should be used for the diagnosis of myocardial injury closer to 12 hours after birth. Jiang et al. also determined that the sensitivity and specificity of hs-cTnI, using a cut-off value of >0.087 ug/L, was 55.6% and 95.5%, respectively.

Overall, troponins are preferred over other biomarkers for a few reasons. First, cardiac troponins have a large diagnostic window due to their intracellular compartmentalization. They rapidly elevate in the serum two to four hours after the initial injury, peak after 12 hours, and can remain elevated for up to ten days. Second, they are not affected by gestational age, mode of delivery, sex, or birth weight. Finally, cardiac troponins have large molecular masses that are unable to freely diffuse across the placenta. In particular, cTnT and cTnI have molecular masses of 37 kDa and 24 kDa, respectively. As a result, the neonatal cardiac troponin levels that are measured are not affected by maternal levels.

**CARDIAC TROPONIN I VS. CARDIAC TROPONIN T**

While cardiac troponin I and T are both regulatory proteins of the troponin-tropomyosin complex, they differ in many ways, such as their molecular weight, half-life, and intracellular compartments. There seems to be controversy surrounding which troponin is more accurate. One reason why cTnT may be preferred is that cTnI is more sensitive to covalent and enzymatic modifications (i.e., phosphorylation and methylation), which decrease its binding capacity to specific antibodies in the assay system. This could lead to reduced signals in the assay and under-
estimation of cTnI levels. However, most studies have a preference for cTnI as it is believed to have better diagnostic ability for neonatal myocardial injury than cTnT. This is because cTnT levels are affected by adrenaline administration during cardiopulmonary resuscitation. Hence, this reduces the value of cTnT as a biomarker for myocardial injury in neonates as resuscitation is often necessary postnatally. A second reason is that the expression of the cTnT gene is more complex, as it contains four alternatively spliced transcripts. This makes it difficult to determine the immunoassay to use for the detection of the protein. Finally, Immer et al. illustrated that cTnT levels were higher in neonates with postoperative renal failure, which can lead to false positives of myocardial injury. Therefore, cTnI has the advantage of not being affected by renal problems.

FUTURE STEPS

After thorough analysis of all biomarkers, it is evident that cardiac troponins, in particular cTnI, have high sensitivity and specificity for diagnosing myocardial injury. Future steps that need to be taken include finding methods to enhance its diagnostic ability. For example, using the most appropriate assay and blood sample type to measure troponin levels. With new technological advances, enhanced assays are being developed which allow for more accurate results. Presently, venous blood samples are the most common blood sample type; however, recent advances in the field have shown promise for other blood sample types such as umbilical cord blood. Improving diagnostic ability will enable early diagnosis of myocardial injury so preventative measures can be taken to reduce cardiac complications.

ASSAY TYPE

Currently, there are many different assays available for measuring cardiac troponins. The first assay was developed by Cummins et al. in 1987 and, since then, cTnI assays have become 1000 times more sensitive. Assays are classified based on the sample percentage of healthy subjects that exceed the assay’s limit of detection (LOD). Low sensitivity assays have the highest LOD, whereas high sensitivity assays can have an LOD that is an order of magnitude lower than low sensitivity assays. High sensitivity assays are a recent development; therefore, more studies need to be performed using these assays to determine and verify diagnostic ability.

BLOOD SAMPLE TYPE

In addition to the assay type, the blood sample type also affects diagnostic ability. Neonates are often subject to acute renal failure and, therefore, have limited renal clearance of biomarkers. This can result in the elevation of specific biomarkers using venous blood samples. However, umbilical cord blood samples do not have this issue with renal clearance and is therefore a more accurate indicator of perinatal injuries. For example, a recent study in press by Mondal et al. was conducted to determine a reference interval for hs-cTnI in the umbilical cord blood of neonates using a hs-cTnI assay. Mondal et al. concluded that hs-cTnI levels in the umbilical cord blood of neonates are comparable to those of an adult reference population. These findings contradict the results of current literature which state that troponin I levels in neonates are higher than adult troponin levels. This result can be attributed to the difference in the type of blood sample that was collected in the Mondal et al. study.

Furthermore, the studies referred to in this literature review were conducted at later stages in neonatal life, resulting in elevation of specific biomarkers due to other neonatal-related injuries. However, using umbilical cord blood largely avoids this. Overall, it is important to consider the type of blood sample taken.

CONCLUSION

To conclude, CK-MB, BNP, and myoglobin do not serve as accurate biomarkers for myocardial injury in neonates. CK-MB is limited due to its low specificity for myocardial injury, as well as its delayed elevation following myocardial injury. BNP has low specificity for myocardial injury and levels can be affected by a hypoxic environment. Myoglobin lacks specificity for myocardial injury as it is found in numerous muscle tissues of the body. Overall, studies have shown that cardiac troponins are the most accurate biomarkers for myocardial injury. Troponins experience rapid elevation upon myocardial injury, they remain unaffected by gestational age and birth weight, and they do not cross the placenta and are therefore intrinsic to the neonate. In particular, cTnI shows great promise as it has high specificity and a large range of immunoassays can be used for its detection. However, further research needs to be conducted using high sensitivity assays and umbilical cord blood. Also, the limitations of cardiac troponins need to be further explored, and their specificity and sensitivity need to be verified.
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The Selective Targeting of Unique Metabolic Properties of Leukemic Stem Cells in Acute Myeloid Leukemia

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SUMMARY

Acute myeloid leukemia is currently treated with chemotherapy and radiotherapy, effectively eradicating most cancerous cells. However, shortly after the depletion of these cells, the re-emergence of cancer often occurs. This has been attributed to the presence of leukemic stem cells, a small population of cells capable of resisting chemotherapy and radiotherapy. Further, the stem-like properties of such cells allow them to regenerate the cancerous cells, which the disease primarily consists of, limiting our treatments and greatly increasing death and suffering. Attempts have been made to target leukemic stem cells; however, no standard therapies have been approved for this purpose. One method of targeting these cells may be through their metabolic differences from healthy cells. Recently, several unique metabolic properties of leukemic stem cells have been discovered and targeted, successfully reducing the ability of leukemia to return after treatment.

ABSTRACT

Current therapeutic options in the treatment of acute myeloid leukemia often succumb to high instances of relapse and subsequent mortality. Chemotherapy and radiotherapy have long been used as the standard treatment for this disease, remaining stagnant over the past few decades. Recently, a small self-renewing population of leukemic stem cells have been identified as drivers of cancer relapse and progression due to their increased resistance to anticancer therapeutics. This enables these cells to maintain a minimal residual disease and results in downstream differentiation, leading to relapse. Targeting these cells may lead to effective therapies that reduce relapse and mortality. Recently, the metabolic properties of leukemic stem cells have begun to be elucidated. Here, we discuss recent discoveries regarding the metabolism of leukemic stem cells and approaches to targeting their unique metabolic properties.

Keywords: Acute myeloid leukemia, leukemic stem cells, cancer stem cells, stem cell metabolism, cancer relapse

INTRODUCTION

Acute leukemias are a rare set of cancers with disproportionately low rates of survival. Although these illnesses make up less than three percent of all cancers, they result in more deaths than any other cancer in those under 39 years of age.1 Acute myeloid leukemia (AML) is the most common leukemia diagnosed in adults and carries with it the highest mortality rate among all leukemias.2 AML occurs when immature leukocytes, called blast cells, begin to occupy the bone marrow and prevent normal blood formation. Blast cells originate from myeloid progenitor stem cells, which differentiate from hematopoietic stem cells and further differentiate into leukocytes under normal circumstances.

Standard treatment of AML consists of a combination of chemotherapy and radiotherapy. Treatment standards have remained stagnant over the past few decades and are often followed by damage to healthy tissue and a high incidence of relapse. Despite achieving complete remission in a majority of AML patients, over 70 percent of adults and 30 percent of children will relapse and succumb to the disease within five years of their initial diagnosis.3 A unique subset of cancerous cells called cancer stem cells (CSC) have been implicated in relapse. These cells have been shown to resist typical anticancer therapies used in AML treatment and can remain in a quiescent state and maintain a
minimal residual disease. While chemotherapy is effective against the bulk of cancer cells, the persistence of CSCs after chemotherapy is thought to be responsible for the emergence of relapse, where CSCs differentiate into various cancer cells and the disease regenerates. This regenerating ability makes the eradication of CSCs necessary to effectively cure AML. The specific CSCs involved in AML have been identified as leukemic stem cells (LSCs). Several treatments have been developed which target leukemic stem cells; however, no drug has been able to eradicate LSCs. One method of doing so is through metabolic differences. LSC metabolism has been somewhat understood until now, with recent breakthroughs showing key metabolic differences capable of being targeted in an LSC-specific manner.

### TARGETING LSC AMINO ACID DEPENDENCY

Several drugs have been developed to address the need for a more effective, well-tolerated therapy of AML. One therapeutic option is B-cell lymphoma 2 (BCL-2) inhibitors. The BCL-2 protein inhibits cancer cell apoptosis and is highly expressed in LSCs. BCL-2 inhibitors such as Venetoclax reduce the expression of BCL-2 in AML patients, inducing apoptosis and reducing oxidative phosphorylation. Venetoclax has shown promise in clinical trials, but once again is not sufficient to cure AML. Hypomethylating agents, such as azacitidine, have also been developed as an alternative to chemotherapy for elderly AML patients. Gene hypermutation is extensive in AML and contributes to its progression; hypomethylating agents reverse this state and slow the disease. Preclinical models have shown BCL-2 inhibitors to work synergistically with azacitidine, leading to a clinical study of the venetoclax-azacitidine (ven/aza) combination therapy. The combination was able to elicit high rates of remission and durable responses in patients with AML, suggesting that these effects were due to the selective targeting of LSCs.

In a recent study by Pollyea et al. in 2018, the metabolic state of LSCs was compared to blast cells, which make up the bulk of cancerous cells. The study found decreased oxygen consumption after ven/aza treatment in LSCs and metabolite levels consistent with decreased oxidative phosphorylation. Following this, decreased energy production was found in the ven/aza-treated LSCs, aligning with previous findings that LSCs rely selectively on oxidative phosphorylation for energy production, and alluding to the mechanism behind ven/aza treatment. Importantly, these effects were specific to LSCs and were not seen in blast cells or normal hematopoietic stem cells isolated from these same patients, showing selectivity of ven/aza towards LSCs.

In a later study by Jones et al. in 2018, amino acid metabolism was found to be a large contributor to LSC energy production. Global metabolic profiling revealed increased amino acid content and uptake in LSCs compared to blast cells, suggesting that amino acid metabolism plays a key role in LSC survival. This was supported by decreased LSC viability and colony-forming potential when cultured in amino acid-depleted media, while blast cells saw minimal changes. LSCs grown in amino acid-depleted media further showed decreased oxidative phosphorylation, revealing that these amino acids are needed for oxidative phosphorylation and suggesting that LSCs lack metabolic flexibility, being unable to compensate using other fuel sources. This dependency was confirmed when these amino acid-depleted LSCs and blast cells were supplemented with fatty acids; while blast cells increased fatty acid uptake and metabolism, LSCs did not.

Ven/aza treatment was then investigated and found to lower amino acid levels in LSCs, indicating that the drug targeted this dependence. Supporting this finding, gene expression of common amino acid transporters was significantly reduced in LSCs after ven/aza treatment, indicating the involvement of amino acid uptake inhibition in the ven/aza-induced reduction of oxidative phosphorylation. A causal relationship was established by flooding these LSCs with high concentrations of amino acids prior to ven/aza treatment. This pretreatment elevated amino acid levels remained post-ven/aza and rescued LSC viability. The amino acid reduction was further shown to cause a reduction in LSC oxidative phosphorylation, which the amino acid pretreatment was also able to rescue. This confirmed that the reduction of amino acids caused by ven/aza treatment reduced oxidative phosphorylation in LSCs.

### METABOLIC FLEXIBILITY OF RELAPSED LSCs

Recently, reduced responses to ven/aza treatment in relapsed or refractory (R/R) AML patients after previous chemotherapy have been found, suggesting resistance among these LSCs. With ven/aza inhibiting oxidative phosphorylation of amino acids, Jones et al. in 2018 suggested that altered metabolic properties and dependencies may exist among R/R LSCs. To test this, the metabolic properties of pre-chemotherapy (de novo) and R/R LSCs from AML patients were compared. The study found that while oxygen consumption was decreased by ven/aza in de novo LSCs, R/R LSCs were not affected and were resistant to the ven/aza-induced depletion of oxidative...
phosphorylation. R/R LSCs cultured in amino acid-depleted media further showed higher cell viability and oxygen consumption rates than de novo LSCs, revealing a loss of dependency on amino acid metabolism and suggesting a gained metabolic flexibility and shift to other sources of energy. Indeed, amino acid-depleted R/R LSCs showed increased fatty acid levels followed by elevated citrate, indicating that R/R LSCs could switch to fatty acid metabolism and explaining their resistance to ven/aza. By treating R/R LSCs with a fatty acid uptake inhibitor, the cells were re-sensitized to the treatment. These findings pose the combination of amino acid and fatty acid metabolism as a potential therapeutic target in reducing relapse in AML.15

NICOTINAMIDE MEDIATES VEN/AZA RESISTANCE

Another study published by Jones et al. in late 2020 observed global metabolite levels of de novo and R/R LSCs, identifying increased nicotinamide levels after relapse.17 Nicotinamide is a substrate for NAD+ production, which was also elevated in R/R LSCs. Stemming from the role of NAD+ as an essential coenzyme in various energy production pathways, R/R LSCs generated higher levels of ATP compared to de novo LSCs. This NAD+-mediated increase in energy production was shown to occur through both increased amino acid metabolism and fatty acid oxidation, explaining how R/R LSCs gain metabolic flexibility and compensate for amino acid depletion to resist ven/aza treatment. To confirm the causal relationship between increased nicotinamide and resistance to ven/aza treatment, de novo LSCs were pre-treated with concentrated nicotinamide and subsequently treated with ven/aza. Compared to those without the nicotinamide pre-treatment, pre-treated LSCs showed rescued viability and supported the finding that increased nicotinamide levels mediate ven/aza resistance.17

The researchers then inhibited NAMPT, the enzyme responsible for synthesizing NAD+ from nicotinamide, which selectively decreased R/R LSC viability and revealed a reliance on nicotinamide.17 In paired de novo and R/R LSCs, standard chemotherapy had little effect on either sample, while ven/aza treatment caused a decrease in de novo LSC viability only. NAMPT inhibitors, APO866 and KPT-9274, both selectively decreased R/R LSC viability, showing efficacy and selectivity of NAMPT inhibition for targeting R/R LSCs. A reduction in oxygen consumption was found after NAMPT inhibition, suggesting it targets oxidative phosphorylation.17 Supporting this, a decrease in NAD+-dependent TCA cycle enzyme activity was seen in R/R LSCs, while not in NAD+-independent enzymes, suggesting NAMPT inhibition targets R/R LSCs through reducing nicotinamide levels and NAD+ production. NAMPT inhibition in R/R LSCs further showed decreased amino acid levels, confirming that the nicotinamide increase in R/R LSCs increases amino acid metabolism. Fatty acid metabolism was also reduced, indicating that increased nicotinamide allows R/R LSCs to become more metabolically flexible and use fatty acids for energy production.17

CONCLUSION

The high rate of relapse accompanying chemotherapy and radiotherapy has led to the development of many drugs; however, no effective therapies have been able to replace the standard treatment or overcome relapse. Ven/aza therapy has effectively targeted LSCs, but remission is still not achieved in many de novo AML patients and relapsed LSCs resist the treatment through shifting their metabolic dependencies. NAMPT and other factors related to nicotinamide metabolism have shown potential as therapeutic targets for reducing AML relapse; however, more studies are needed to fully characterize the metabolic properties of LSCs before and after relapse. Future efforts targeting metabolic processes of both de novo and relapsed LSCs would benefit from considering the possibility of nicotinamide-mediated therapy resistance and the involved metabolic pathways. The processes behind relapse in AML have important implications in the design of therapeutics targeting LSCs. For future drug development, therapeutics targeting de novo LSCs could benefit from avoiding selective pressures which may induce metabolic flexibility and lead to resistance. Additionally, drug development targeting relapsed LSCs should consider the process by which these cells have become resistant and their resulting differences, which could lead to more selective and effective targeting.

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REFERENCES


Urban health — What it is and Why We Should Care

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SUMMARY

Urban health studies show how two interdependent factors, urbanicity and urbanization, impact the overall health and wellbeing of individuals who reside in cities. As an emerging discipline in the world of academia, urban health holds many potential opportunities for practice and research, including forming the foundation of new and innovative subject areas. It has promise to tackle health challenges in an urban context, including growing disparities and inequitable access to care. This piece concludes with a discussion of some recent advances in this area, which aims to shed light on future directions in the field of urban health.

ABSTRACT

Urban health is a field of study that draws upon multiple disciplines including sociology, public health, epidemiology, and geography among others. This piece argues for the further development and prioritization of urban health as an area of research. This is discussed with respect to structural health inequalities, urbanization and urbanicity, and demographic change. Urban health is inherently complex and needs a multifaceted approach to tackle unique public health problems. This complexity, alongside its potential to inform emerging areas of scientific research such as neurourbanism, makes developing urban health of utmost priority.

Keywords: Urban health, vulnerable populations, social determinants of health, public health, health disparities

Urbanization is arguably the single most significant demographic shift in our history.¹ It represents a substantial change in how most of the world’s population has lived over the past several millennia.¹ While over 55% of the world’s population lived in urban areas as of 2018, this proportion is expected to approach 70% by 2050, largely due to increasing urbanization in developing nations.²⁻⁶

It has long been appreciated that cities can impact community health.² When Europe was rapidly urbanizing, not only was there an increase in population density, but there was also an increase in the prevalence of marginalized populations, pollution, and crime.¹ In many cases, the effects of urbanization resulted in worse health outcomes for urban residents relative to those living outside of cities.¹ This makes it apparent that urban environments are unique health contexts, worthy of further study.²

Urban health accounts for how two complementary dimensions, urbanization and urbanicity, affect health and wellbeing.³ Urbanization refers to the change in a location’s composition over time (i.e., to a denser built form), whereas urbanicity refers to the impact associated with residing in urban centres at any given time.³ As an area of study, urban health considers the relationship(s) between characteristics of the urban environment and population health through studying the physical environment, the social environment, and the often-inequitable access to health and social services.¹⁻³

Due to limited research thus far and the inherent complexity of this topic, urban health faces many methodological and conceptual obstacles.¹ There is no common language; instead, there are inconsistent definitions of key subject-area terms.¹⁻⁴ Further, it is difficult to conclusively identify causation in the urban context and, thus, to choose an appropriate study design.¹ Despite these barriers, urban health has the potential to bring together scholars from various schools of thought and methodological backgrounds.⁴

Urban health is generally deemed a multidisciplinary field of study that is united by a common topic rather than a shared methodology; however, some propose classifying it as a standalone discipline which may have implications for its identity as an area of research.⁴ Regardless, as it is already being studied by experts of various backgrounds including urban planning, sociology, and epidemiology, urban health will continue to be a fertile ground for cross-disciplinary research.²⁻³ Deeming urban health as an area of focus may facilitate greater research advances through contributions such as new infrastructure and frameworks,
which are elements this emerging field has already started to see.³

The importance of studying urban health is exemplified in past epidemics where millions of deaths occurred due to poor sanitation and living conditions in cities.²⁵ Even in wealthier countries, crowding was associated with a higher risk of infection.² By the mid-20th century, health disparities were increasingly observed between urban and rural or suburban residents.² Years later, the HIV epidemic and rise of violence led to a greater burden of disease in city centres, bringing forth the notion of an “urban penalty”—the idea that urban residents experience worse health outcomes than others.² Nowadays, urban centres are home to growing disparities, illuminated by higher HIV rates and illicit drug use. Such disparities became more apparent in the 1990s when experts declared that, despite neighbouring affluent districts, the mortality rate in low-income urban neighbourhoods in developed countries was greater than the mortality rate in some developing nations.³

The face of cities is changing worldwide, and future research needs to account for this. Urban health tackles systemic health disparities fuelled by social determinants of health including race, ethnicity, socioeconomic status (SES), sex, gender, and geography among others. These disparities are population-level differences with respect to access to care, procedure of care, and health outcomes. Urbanization, particularly the resulting population growth, has contributed to a strain on available jobs, thereby devaluing the hourly wage and sparking higher unemployment.³ The resulting dynamic SES composition of urban centres can be considered a unique determinant of urban health, where a lower SES is generally associated with limited access to healthcare and worse health outcomes.³ Further, growing immigrant and minority populations face different stresses and barriers than dominant groups.³ Crime is more prevalent in cities, with homicide and substance use more commonplace than in rural areas.³ Coupled with a 38% increase in the likelihood of having a mental disorder and more substantial effects of pollution on respiratory health in cities than in non-urban areas, it quickly becomes clear that there is a growing need to study urban health and to develop this area of research.¹³

Urban health has many potential directions for research, practice, and related subjects.¹³ Consider neurourbanism which, as an emerging academic discipline, draws on areas ranging from epidemiology to urban prevention and therapy research to study the relationship between the urban environment and neurosciences.⁵ It has far-reaching implications on how we plan our cities, improve the mental health of communities, and support high-risk individuals.⁵ Urban health can inspire and inform the foundation of new disciplines such as neurourbanism, potentially reshaping how we study public health and medicine in an urban context.

Developing urban health as an area of research would bring together experts from a variety of backgrounds, each with their own methodological and conceptual frameworks. The ability to develop innovative and unique ideas to combat modern urban health concerns may effectively solve these challenges in the future. Prioritizing the development of this field is crucial; after all, urban health is an important extension of public health, arguably worthy of a dedicated suite of academics. Cities provide a unique context for many urban health determinants. Thus, the development of this area may help researchers better analyze and interpret health disparities to encourage the formulation of appropriate solutions through more robust discipline-specific data collection and analysis methodologies. More awareness, accompanied by research advancements made into the relationship between urban environments and population health, may indirectly contribute to the improved health and wellbeing of urban communities. The further study of urban health determinants may inform public health policy, shape social programs, and change the rhetoric behind society’s most vulnerable populations. It brings forth and solidifies the notion of intersectionality in medicine, from access to care to clinical outcomes. Now, it is time for the scientific community to prioritize urban health as an area of research and work towards a brighter future.

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