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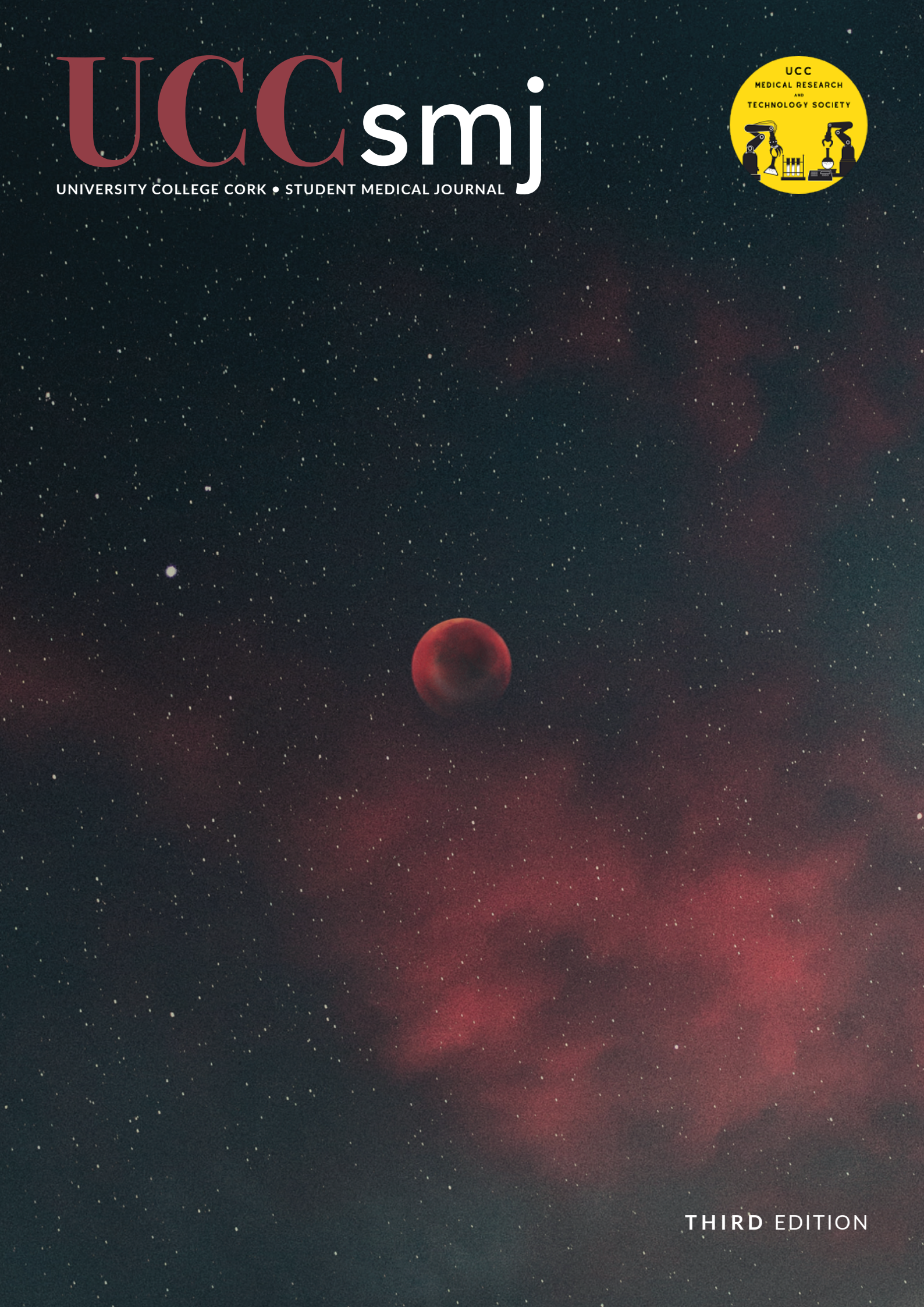

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FOREWORD

Decades ago, mankind looked up into the celestial sphere, immensely curious about what lies above and beyond. The innate curiosity, coupled with venturing on the quest for answers, paved the pathway for sequential space exploration and the commonplace phrases such as "outer space" and "the cosmos". It is paramount to flag out that the avenue via which this transcending process occurred was via the intricate and meticulous methodology of research and technology.

Spaceflight was born out of space exploration, with the revolutionary first artificial earth satellite, Sputnik 1, being launched by Soviet Union in 1957. This was followed in suit by the first man to leave mankind's footprint and mark on the moon in 1969 – Neil Armstrong. Such riveting achievements would not have been possible, if not for the underlying concept of research, where brilliant minds worked together to go above and beyond to manipulate, innovate and create technologies that were already in existence and reproduce it with new modalities.

Curiosity, innovation, research, and failures with eventual success are concepts which have proven themselves to be cardinal, especially in the "COVID-19" times we live in. Rules and regulations which once dictated our everyday movements have now been lifted with international bodies collectively working towards restoring and establishing normality. Vaccinations borne out of tedious and scrupulous methodologies, owing to various bodies, have enabled us to see light at the end of the tunnel. Curiosity and a need for answers yet again played a pivotal role.

Our successful 1st edition was in 2020, which piqued the minds of our students who thought outside of the box, beyond what was disseminated in classrooms and lecture halls during COVID-19, and our sequentially successful 2nd edition was in 2021, where scholars of medicine celebrated coexistence of science and art in dealing with the human body. After such immense success in displaying and sharing students' hard work and effort, UCC Medical Research and Technology society is very pleased and proud to present to you our 3rd edition, extending our appreciation to all contributing students who never fail to scale the stratosphere.

With life seamlessly transitioning into a novel post COVID-19 era, we have put together the diligent and preserved effort of our students who have stepped out of their comfort zone, ventured into the vast and endless space of research, innovated and produced remarkable findings and results. They unceasingly stand true to the famous saying of "One small step for man, one giant leap for mankind".

We would like to extend our sincere gratitude and appreciation to the School of Medicine for their endless support for the formation and continuation of this student led and run journal. We whole-heartedly thank our peer-reviewers who were challenged with critical questions to aid their fellow peers and the realm of science. Our medical journal will not have been successful without the contributions of our students and the faculty members who took time off their precious schedule to provide invaluable advice. Last but not least, we thank our readers whose support keeps us going.

With yet another successful publication, we earnestly hope that as a society we have contributed in enabling research to be within a students' reach instead of an unexplored abyss. UCC MRT and the Student Medical Journal team wish you a joyous and intriguing read and we hope to see you at our next publication.

Kind regards,

Ramya Baskaran

RAMYA BASKARAN
Co-Editor-in-Chief

address

In medicine, you face continuous problems in every aspect of health and disease. Finding solutions to these problems is research. Occupying oneself with discovering novel solutions to individual ill health and suffering or to population health thus helping society is a unique opportunity that is available in every corner of medicine.

"Somewhere, something incredible is waiting to be known" – DR CARL SAGAN

The quest can start, and often is best started, as a young medical student. Pursuing this quest over a lifelong career is a compelling way to avoid the inevitable 'burn-out'. Being part of an original research manuscript as a student or an early career trainee is such a fulfilment and joy that it drives seeking 'more and more' new challenges, each more difficult than the last and yet ever so fulfilling.

"Every great advance in science has issued from a new audacity of the imagination." – JOHN DEWEY

Research is a challenge and needs preparation, mentorship, skills, persistence and resilience. The three pillars of success are: Priority setting, Time management and Mentorship. Fame is not the goal; fulfilment is the goal though success does opens up new opportunities.

How to create a priority list?
My golden rule is creating lists:

- LIST 1: Things I'm doing that I want to quit.
- LIST 1A: Things I've just been asked to do that I don't want to do.
- LIST 2: Things I'm not doing that I want to start.
- LIST 3: Things I want to keep doing.
- LIST 4: How I plan to shorten List 1 and lengthen List 2 over the next 6-12 months.

What are the key steps in setting up a research career? I give a few personal tips below that has helped me, but a most important advice is to remember that as a clinician you have a great advantage in understanding the problems your patients face or the society faces. These drive formulating your questions and challenges. So, my tips are:

- Identify unique problems.
    - Clinical problems
    - Scientific problems
    - Both (Translational Research)
- Work with strong Research Groups.
    - Interdisciplinary and transdisciplinary
    - Identify your own unique area
- Raise funds.
    - Start with baby steps.
    - Remember that you will need a track record.

It is important to have a plan for success. High level research career is a steep mountain to climb. Therefore, careful planning is required and search for opportunities should be constant. The planning strategy should think about:

- Aim to have career defining publications.
- Diversify gradually – do not lose focus but keep sight of the problem.
- Be bold enough to explore and take some risks.
- Remain curious and excited.
- Give credit to your team.
- Remain patient.

Finally, research is a rigorous career, but it is important to have some fun with research and laugh with your colleagues. It is important to have a life outside career and interesting pastimes and hobbies to spend time with friends. Here are my final 10 thoughts which always guides me:

- Have a good team.
- Have good collaborators.
- Have diversity of skills around you
- Start with a finish in mind.
- Visit other centres.
- Host a vibrant academic culture.
- Plan well ahead
- Have good mentors.
- Celebrate successes.
- Be prepared to fail.

Subrata Ghosh

Chair and Head of Medicine
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contents

A five-year retrospective review of fatalities involving novel psychoactive substances in Southern Ireland

ANDREW MAZUREK[1], MARGOT BOLSTER[2]

Abstract

INTRODUCTION: Novel psychoactive substances (NPS) are rapidly emerging and being reformulated to evade legislative controls, creating unpredictability in drug markets and ineffective drug policies. Given that Ireland has the highest self-reported NPS use within Europe and literature regarding health risks is lacking, the *National Advisory Committee on Drugs* has advocated for the surveillance of regional trends.

OBJECTIVES: To elucidate the demographic and autopsy findings of fatal cases involving NPS in Southern Ireland as compared to deaths involving traditional drugs of abuse (TDOA).

METHODS: Post-mortem reports from the Cork-Kerry region with positive toxicology for illicit substances between 2012-2016 were retrospectively analyzed to compare NPS and TDOA cases with respect to circumstances surrounding death, toxicological results, and pathological findings.

RESULTS: Between 2012-2016, there were 164 cases involving illicit substances in the Cork-Kerry region. NPS accounted for 17 (10.4%) cases, with an average annual mortality rate of 34.8 per 100,000 population. NPS contributed to the cause of death in 70.6% of cases where detected, compared to 43.5% for TDOA only. In both cohorts, fatal abusers were predominantly young males. Importantly, cases involving NPS showed a higher proportion of mono-drug intoxication and presentation to hospital prior to death. Autopsy findings were non-specific but commonly featured pulmonary congestion, aspiration, and cerebral oedema.

CONCLUSION: NPS are detected in a small proportion of medicolegal autopsies but contribute significantly to acute intoxication deaths. This study highlights the need for effective drug monitoring and enforcement strategies, along with improved management of drug toxicities presenting to hospital.

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Introduction

Novel psychoactive substances (NPS) encompass those psychoactive substances not prohibited under the *United Nations Convention on Narcotic Drugs*, designed to mimic the effects of illicit drugs[1]. To circumvent legal restrictions, they are often mislabeled as 'research chemicals' or 'bath salts'. NPS are emerging and being reformulated at increasingly rapid rates, creating unpredictable drug markets and ineffective drug policies[1]. By 2021, the *EU Early Warning System on NPS* was monitoring over 830 substances with synthetic cannabinoids, synthetic cathinones and phenylethylamines among the most commonly reported[2].

EPIDEMIOLOGY

Major European monitoring centres report a low incidence of NPS in drug-related emergency presentations, between 0-2.8% monthly, with higher frequencies for traditional recreational drugs and misused prescription medications[3]. Synthetic cathinones were the most frequently encountered, particularly mephedrone and methedrone. Highly concordant across major geographic zones was the demographic profile of NPS users reporting acute toxicity: male (>75%) and under 30 years old[4-5]. Clinical presentation upon admission was predominantly neurological and psychological, with cardiovascular symptoms also present in a significant proportion of cases[4-6]. The authors have previously reviewed the common clinical symptoms associated with NPS toxicity and proposed mechanisms of action for these psychoactive substances[7].

The leading cause of death reported in forensic casework studies was acute drug toxicity, particularly with synthetic cathinones[8]. Pathological findings commonly reported were cardiac ischaemia and cerebral hypoxia, which may be explained by chronic vasoconstriction[5,8]. Traumatic injuries, including hangings and homicidal cases, were also important contributors to fatalities. Post-mortem drug concentrations vary widely in the literature, prohibiting the establishment of 'fatal ranges' for commonly encountered NPS.

IRISH PERSPECTIVE

The emergence of NPS in Ireland was noted in 2005 with the rise of 'legal highs' sold in headshops, which at the time complied with Irish law[9]. In response, the Irish government implemented two legislative controls in 2010 by i) amending the *Misuse of Drugs Act* to include NPS, and ii) introducing the *Criminal (Psychoactive Substances) Act*[10]. While regulations succeeded in curtailing headshops, availability through street and online markets has continued[11]. 'Darknet' cryptomarkets have particularly changed the model of illegal drug distribution by utilizing anonymized online transactions[12].

While significant changes to lifetime prevalence were not detected post-legislation, this period demonstrated lower rates of recent NPS use and problematic practices[13], consistent with short-term results observed elsewhere[14]. While use of all NPS categories remains higher than desired by legislators, small-scale post-legislative studies in Ireland have reported reductions in various NPS detection rates and shifts in attitudes regarding the perceived safety of these products[11,15].

Despite reduced detection rates, levels of NPS use in Ireland remain disproportionately high. In the *Youth Attitude on Drugs* report[16], Ireland had the highest self-reported NPS use at 16% prevalence, whereas most European countries reported levels under 5%. Furthermore, the national drug-induced mortality rate for adults was 71 deaths per million, over three times the European average[17]. Current literature regarding drug fatalities linked to NPS, however, is limited mainly to case reports. Despite anecdotal accounts, published data on NPS toxicity remain scarce due to the rapid emergence of novel formulations[18]. Consequently, the *National Advisory Committee on Drugs* has recommended further research into shifting patterns of drug consumption, with particular focus on the surveillance of local trends[18]. These steps would contribute to a pragmatic public health approach, critical for the development of appropriate evidence-based responses.

AIMS AND OBJECTIVES

The aim of this study was to elucidate demographic and autopsy findings of fatalities involving consumption of NPS in Southern Ireland compared with traditional illicit drugs of abuse (TDOA). Specific objectives were to: i) compare the fatality rates involving NPS toxicity with TDOA; ii) analyze demographic characteristics of fatal abusers within both cohorts; and iii) explore the common autopsy findings in NPS overdoses.

Methodology

STUDY DESIGN

A retrospective cohort analysis was conducted, reviewing all post-mortem reports completed by the Assistant State Pathologist between 2012-2016. The geographic area covered by the Assistant State Pathologist corresponds to the *Health Service Executive* Community Healthcare Organization Area 4, covering counties Cork and Kerry[19]. A total of 4129 cases were completed by the Assistant State Pathologist between 2012-2016.

Given that drug toxicity deaths are rare within the general caseload of the forensic service, with NPS constituting an even smaller fraction, the study aimed to include all deaths involving illicit substances over the 5-year period. All reports for 2012-2016 were screened to identify cases involving illicit substances, using toxicology reports and cause of death statements. Cases positive for illicit substances were reviewed in full to extract study variables.

STUDY MEASURES

Case histories, post-mortem findings and toxicological analyses were reviewed from each case to ascertain trends in demographic data and pathological findings. NPS were identified according to classification by the *European Monitoring Centre for Drugs and Drug Addiction*[20]. TDOA included cocaine, heroin, and

amphetamines/methamphetamines. Other co-ingested drugs were also recorded, however ethanol was not recorded where the report indicated decomposition as the likely source.

Demographic measures included year, sex, age, location of death, and history of drug addiction. Pathological findings included cause of death, comorbidities, and major autopsy findings. A drug-related death was defined as one in which the forensic pathologist concluded that the drug contributed to the death, directly or indirectly[21]. A direct drug death was defined as an acute overdose with the drug reported as the primary cause of death, whereas an indirect drug death was a death in which the drug significantly contributed to death but was not the primary cause.

STUDY ETHICS

This study was approved by the Clinical Research Ethics Committee of the Cork Teaching Hospitals. Written permission to access post-mortem reports was obtained from relevant coroners. Data was extracted in aggregate and identifying information pertaining to decedents were not retained.

DATA ANALYSIS

The primary analysis was descriptive, calculating the proportion of positive cases between NPS and TDOA for each categorical study variable. The mean age and standard deviation for the two groups was calculated. The average annual fatality rate was calculated using census data for Cork and Kerry counties from the Central Statistics Office[22].

Results

Between January 1, 2012 and December 31, 2016, there were 164 cases with positive toxicology for illicit substances. NPS were detected in 17 (10.4%) cases while 147 (89.6%) were positive for TDOA only. The psychoactive substances detected varied each year (*Table 1*). NPS were implicated in the cause of death in 70.6% (12/17) of cases where these substances were detected; in comparison to 43.5% (64/147) for TDOA only. The proportion of drug-related deaths for the NPS cohort (range 50-100%) was indeed equal to or higher than the TDOA cohort (range 33-50%) for each given year (*Figure 1*). All NPS cases were direct drug deaths, while 12.5% of cases with TDOA were indirect drug deaths.

Figure 1. Proportion of cases with positive toxicology for illicit substances deemed to be a drug-related death (novel psychoactive substances (NPS) vs traditional drugs of abuse (TDOA)), per year.

DEMOGRAPHIC TRENDS

There were 68 male and 8 female drug-related deaths from 2012-2016 in the Cork-Kerry region. The gender disparity was similar in NPS and TDOA groups, with males representing 91.7% and 89.1% of cases, respectively (*Table 2*). Age at death ranged from 18-54 years (NPS: 18-42 years, TDOA: 19-54 years). The NPS group had a slightly lower mean age of 28.8 years compared to TDOA with a mean of 32.1 years.

A known history of drug addiction was recorded in 25.0% of NPS fatalities and 35.9% of TDOA fatalities. Regarding location of death, rates of death occurring in a home environment were similar between the two groups but the rate of presentation to hospital prior to death was higher in the NPS cohort (33.3%) versus the TDOA cohort (14.1%).

Table 2. Demographic characteristics of fatal abusers (NPS vs TDOA).

	NPS (n=12)	TDOA (n=64)
Gender, male	91.7%	89.1%
Age at death (years), mean ± SD	28.8 ± 8.3	32.1 ± 8.2
History of drug addiction	25.0%	35.9%
Place of death		
Home	66.7%	59.4%
Hospital	33.3%	14.1
Outdoor	--	4.7%
Other	--	20.3%
Unknown	--	1.6%

POLYSUBSTANCE USE

Polydrug intoxication was the most common scenario in both NPS and TDOA fatalities (Table 3). There was a higher rate of mono-drug overdose in the NPS group (29.4%) than for TDOA (12.5%). Most commonly co-ingested with NPS were benzodiazepines (47.1%) and amphetamines (41.2%). Amphetamines and alcohol were also found to be mixed with other drugs, though at lower frequencies.

Table 1: Novel psychoactive substances detected by toxicological analysis by year.

2012	2013	2014	2015	2016
Mephedrone PMA PMMA	MDEA Methylone PMMA	4-MEC Benzylpiperazine Mephedrone MDVP Methylone PMA PMMA	Butylone	25I-NBOMe "N-Bomb" Acetylfentanyl Fluorofentanyl Mitragynine

4-MEC: 4-methylethcathionine; MDEA: Methylenedioxyethylamphetamine; MDVP: Methylenedioxypyrovalerone; PMA: Paramethoxyamphetamine; PMMA: Paramethoxymetaphetamine

Table 3. Proportion of mono-drug intoxications and polydrug combinations detected, per drug class

Drug	Total (n)	Mono-drug overdose (%)	Proportions of mixed drugs by type (%)						
			NPS	Heroin	Cocaine	AMP	BDZ	Alcohol	Methadone
NPS	17	29.4	--	5.9	11.8	41.2	47.1	11.8	5.9
Heroin	50	4.0	2.0	--	18.0	0	88.0	42.0	28.0
Cocaine	22	18.2	9.1	40.9	--	31.8	68.2	31.8	18.2
AMP	14	7.1	50.0	0	50.0	--	64.3	--	14.3
BDZ	60		13.3	7.3	25.0	15.0	--	15.0	25.0
Alcohol	28		3.6	75.0	25.0	14.3	67.9	14.3	10.7
Methadone	17		5.9	82.4	23.5	11.8	88.2	11.8	--

AMP = amphetamines; BDZ = benzodiazepines

Table 4. Proportion of major autopsy findings in NPS overdose deaths, by organ (n=12).

Organ	Autopsy Findings	Cases (%)
Brain	Cerebral oedema	75.0
	Acute hypoxic changes	41.7
Lungs	Lungs heavy, congested and oedematous	100.0
	Trachea and major bronchi contain blood or aspirate	75.0
	No evidence of pneumonia or pleural effusion	91.7
Heart	Heart normal size and shape	91.7
	Sectioning of myocardium shows no gross evidence of disease	91.7
	Coronary arteries show no evidence of disease	100.0
Liver	Centrilobular congestion	33.3
	Mild or extensive fatty change	33.3
Overall Autopsy	No evidence of significant natural disease	100.0
	No evidence of significant injuries or trauma	91.7

AUTOPSY FINDINGS IN NPS OVERDOSE FATALITIES

A general pattern of autopsy findings emerged within NPS overdose fatalities (*Table 4*). The brain commonly displayed generalized oedema while lungs were congested and oedematous. The trachea and major bronchi often contained blood or other aspirated material. The heart was grossly and histologically normal in almost all cases, while the overall autopsy failed to demonstrate any significant natural disease or trauma.

Discussion

Despite an overall low number of 12 NPS cases detected in post-mortem toxicology screens over the five-year period, there was great variability in the classes of NPS detected. This reflects the accelerated speed at which NPS are emerging, resulting in legislation which struggles to adapt. There have been positive developments, however, with 2016 marking a significant decrease in new synthetic cannabinoids and cathinones reported[20]. This may reflect the sustained deterrent efforts by European states and efforts abroad to close laboratories producing these substances.

While the emergence of new compounds may have slowed, 2016-2017 saw a large increase in the availability of synthetic opioids, particularly fentanils, throughout Europe[23]. These trends are reflected in the Cork-Kerry data with the emergence of acetylfentanyl and fluorofentanyl in this period. This is a concerning trend identified in the region given the highly potent nature of synthetic opioids and risk of respiratory depression[24].

According to a systematic review of synthetic cathinones and

cannabinoids, acute toxicity is the leading cause of NPS fatalities[8]. This risk profile is reflected in the region as 70.6% of NPS detected were implicated in the cause of death. Furthermore, all cases were deemed a direct drug death, unlike for TDOA where 12.5% were indirect drug deaths. The acute risk of death linked to NPS has not yet been fully explained in the literature. Proposed explanations involve their increased potential for monoamine uptake blockade, which significantly increases the risk of respiratory depression[24]. This increased risk of mortality may also explain why NPS represented 10.4% of illicit substance detections when general population surveys report prevalence rates around 1%[8]. Of course, concomitant use of other drugs in this and other studies limits any definitive implication as to the exact risk profile of NPS. There is also potential that TDOA are more prevalent within the general population, making individuals better aware of non-toxic consumption levels.

Polysubstance use was implicated in 75% of NPS-related fatalities, a figure concordant with national Irish data[25]. International studies likewise demonstrate polysubstance rates of 71-92% in fatal overdoses[26]. High rates of benzodiazepine co-ingestion with all other drug categories are a concerning trend which has been consistently raised. In fact, benzodiazepine use in opioid-dependent patients has been correlated with more severe drug abuse[26]. This study thus supports continued efforts to address benzodiazepine abuse, particularly in at-risk individuals with a history of drug addiction.

DEMOGRAPHIC PROFILE OF NPS USE IN IRELAND

The 9:1 male-to-female ratio within both NPS- and TDOA-related deaths is in keeping with published literature[28]. The

underlying reason is one which begs further explanation as Irish data indicates the proportion of females entering treatment for illicit substances ranges from 19-37%[28]. Theories that male drug abusers are more likely to engage in harmful behaviours do not fully explain this study as all NPS-related deaths were direct drug deaths, without traumatic injuries.

While the age range for fatal NPS abusers in this study ranged from 18-42 years, recent data indicates that NPS use remains prevalent among adolescents and further educational efforts are required for this vulnerable population. NPS use by Irish teenagers aged 15-16 was 5% in a recent survey, placing Ireland within the top five European countries[29].

The finding that a greater proportion of NPS overdoses initially present to hospital indicates there may be opportunity for better recognition and/or management of NPS toxicities in emergency departments. Utilization of urine dipstick screens which detect commonly encountered NPS is recommended, especially when the history or physical examination is unclear, uncorroborated, or raises suspicion of drug intoxication. Such dipstick screens are available commercially but not currently implemented in the Cork-Kerry region. While antidote medications are not currently available for NPS toxicity, early recognition of NPS involvement can help prioritize the initiation of supportive measures or admission to intensive care, steps which require timely implementation to prevent irreversible or fatal consequences.

THE ROLE OF TOXICOLOGY IN NPS-RELATED FATALITIES

This study emphasizes the importance of toxicology testing, including NPS screening, in autopsies without significant natural disease or trauma. Recognition of the constellation of autopsy findings most often observed in drug-related fatalities is important for forensic pathologists as it can highlight the role of toxicological analysis, even if other co-morbidities are present. Pertinent demographic factors including male gender, young age, and history of drug addiction can further strengthen the rationale for toxicology.

While literature pertaining to NPS fatalities is limited mainly to case reports, a retrospective analysis of 61 autopsy cases involving synthetic cathinones and cannabinoids was conducted by Ezaki and colleagues[30]. Similar findings of cerebral hypoxia and pulmonary aspiration were recorded although at lower rates than for the present study. Other retrospective studies involving NPS autopsy cases have tended to focus on the methodological principles of toxicological testing used for NPS detection[31].

STRENGTHS AND LIMITATIONS

The use of post-mortem reports as the source of data confers certain limitations which need to be considered. Fatalities are a limited measure of overall health risk, representing a small, but serious, part of the spectrum of adverse effects which can occur with illicit substance use. The data analyzed relies upon the interpretation of the forensic pathologist in providing an accurate assessment as to the cause of death, an area which can be challenging when NPS are involved. Furthermore, the occurrence of post-mortem effects such as drug degradation and redistribution, can vary between cases and is not fully understood for NPS. The high level of polysubstance use was a confounding variable which prevented statistical analysis and creates difficulty in determining which agents directly contributed to death. Further, the inference of causality is restricted by the retrospective nature of the study. The small number of NPS cases retrieved in the study period limits the generalizability of the findings outside of the geographic focus of the study.

It is important to recognize that due to economic restraints, toxicological testing is not performed on all post-mortem cases but selected according to case history and autopsy findings. It is possible the number of NPS cases is underestimated in this study, as some cases without toxicological analysis could potentially reveal NPS involvement. Further, toxicology screens are only able to detect substances for which the State Laboratory has reference samples, so certain formulations may elude these drug screens.

Allowing for these limitations, the study is nonetheless able to provide regionally-focused data on the health risks of NPS abuse. The geographic focus allows for detection of trends specific to the Cork-Kerry region and inclusion of all cases with positive toxicology results for illicit substances allows for a representative picture of the prevalence and variety of NPS and TDOA being fatally abused by Cork-Kerry residents without unintended sampling bias. The reports were completed by one forensic pathologist, limiting variability in reporting and toxicological interpretations. Additionally, the State Laboratory has implemented an NPS Analytical Strategy – for which it was awarded a Civil Service Excellence and Innovation Award – lending credibility to the toxicological analyses. This project required minimal cost and labour, making it feasible for future replication.

Conclusions

Knowledge of trends in NPS prevalence and toxicity are important considerations to the development of effective and proactive drug monitoring or enforcement strategies. Dissemination of this data is important for healthcare professionals, particularly emergency physicians and forensic pathologists, in order to make accurate medical assessments. Future directions for research include establishment of the pathological processes linked to individual NPS and addressing the possibility of genetic vulnerability to NPS overdose.

This study has provided an improved understanding of the vulnerable populations and health risks specific to this demographic region. NPS are involved in a small fraction of autopsy cases but contribute significantly to acute intoxication fatalities, especially when polydrug ingestion is implicated. It remains to be seen what effects targeted efforts at drug enforcement may have on the prevalence of NPS use in Ireland and rates of drug-related fatalities.

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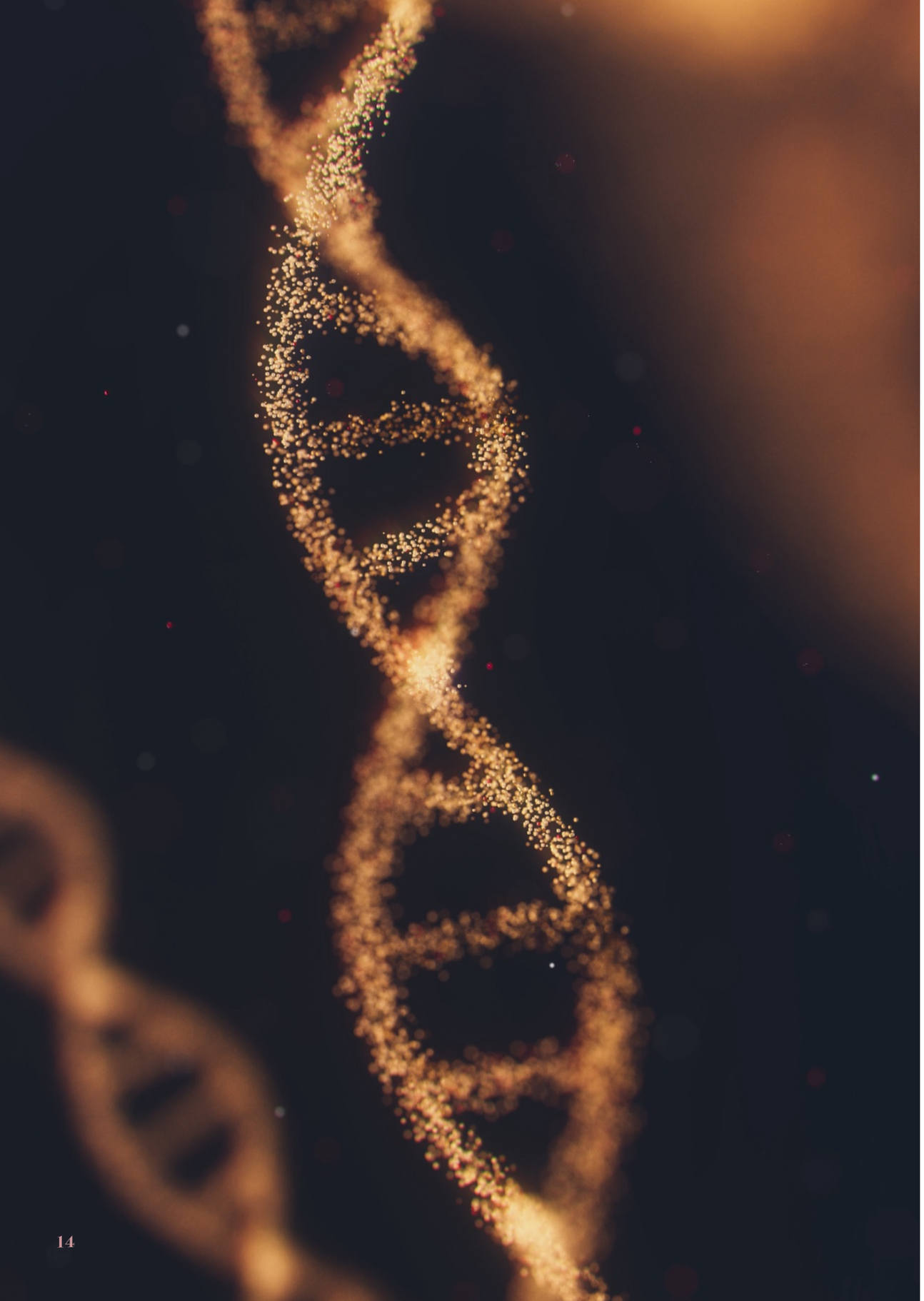


Design and Construction of a GAP-43 Reporter System for Potential Identification of Effective Therapeutics for Peripheral Nerve Regeneration

NINA CARLOS-DE CLERCQ, LISA BREEN, TOMMIE MCCARTHY

Abstract

BACKGROUND: Peripheral nerve injury (PNI) is a condition that can result in muscle paralysis and sensory disturbances. Electrical stimulation and/or the application of exogenous neurotrophic factors and cytokines are effective at enhancing nerve regeneration and is mediated via the expression of regeneration associated genes (RAGs) such as the growth associated protein GAP-43. Therapeutic upregulation of GAP-43 has potential use as a treatment for improving recovery from PNI. Few studies have investigated the potential of increasing GAP-43 for PNI therapeutic purposes and current methods for measuring GAP-43 expression are limited.

AIMS AND OBJECTIVES: The broader aim of this work was to construct a motor neuron-like cell model with a GAP-43 reporter system. Such a model would have potential use in screening for novel therapeutics that upregulate GAP-43 and in the optimisation of electrical stimulation treatment in combination with these therapies.

The key aim of the work was to design and construct a Cas9 expressing plasmid bearing a gRNA that targets GAP-43 cleavage and a donor plasmid bearing a reporter GFP or Neo cassette flanked with 5' and 3' GAP-43 homology arms (HAs) to facilitate the insertion of the reporter immediately 3' of the GAP-43 promoter via CRISPR/Cas9 homology directed repair (HDR). Such an insertion would enable quantitative measurement of endogenous expression of the GAP-43 gene.

METHODS AND RESULTS: To guide the Cas9 nuclease to the target location, GAP-43 gRNA oligomers were designed and cloned downstream of the U6 promoter in the Cas9 expression plasmid px330, which also expresses the Cas9 gene and the cloned gRNA when transfected into cells.

For CRISPR/Cas9 HDR, the 5' and 3' GAP-43 HAs were amplified from mouse genomic DNA and cloned into the donor plasmid using Gibson Assembly so that they flanked the reporter cassette.

RESULTS: This work successfully constructed the Cas9 gRNA expressing plasmid to target cleavage of the GAP-43 gene in mouse cell lines and has provided the complete design and construction foundation for generation of the reporter system for the endogenous GAP-43 gene in mice.

Introduction

Peripheral nerve injury is a condition that may result in muscle paralysis as well as sensory disturbances. In a study of 60,422 leg injuries, 1.8% of these patients suffered additional nerve trauma (1). Nerve injury has the highest level of socioeconomic expense due to the need for extensive rehabilitation (1). Peripheral nerves have the capability of repairing themselves after injury. However, natural nerve regeneration is slow (1-3mm/day) and has an increased risk of muscle atrophy leading to possible withdrawal of central nerve synapses (2).

In the case of neurotmesis, the axolemma (nerve cytoplasm) is exposed for a brief period to the extracellular environment. During this interval, ions are free to travel down their concentration gradients, leading to a calcium influx in both the proximal and distal stumps (5). In the proximal portion this calcium influx has a positive effect. It aids in the repair of the damaged axon membrane and sends a wave of depolarization toward the cell body (6). Once this wave reaches the cell body it causes an upregulation in the transcription of regeneration associated genes (RAGs) (2). In the distal stump, the calcium influx has the opposite effect. This portion is disconnected from the cell body, which is its source of energy and proteins. The lack of ATP means the sodium ATPases can no longer maintain equilibrium (7). A change in concentration gradient ensues and causes a reversal in the Na+/Ca++ exchanger channel, causing a further influx in calcium (5). This triggers Wallerian Degeneration in the nerve segment distal to the nerve injury (5). This type of degeneration clears the way for the regenerating neurites of the proximal stump to grow

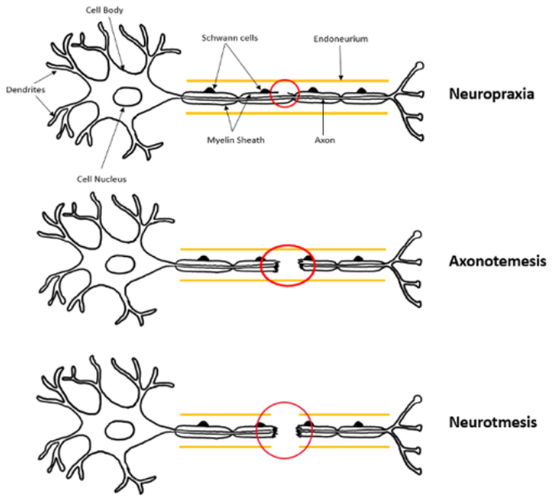


Figure 1. Illustration of Seddon's classification of nerve injury. In neuropraxia there is only mild demyelination, hence the nerve is capable of spontaneous recovery. Axonotmesis involves moderate demyelination and a severed axon. However, since the endoneurium is intact the nerve is capable of repair without treatment. Neurotmesis is a complete transection of the nerve where the myelin, axon, and endoneurium are all interrupted. An intact endoneurium is key to guiding the regenerating neurons, hence surgical repair is required to achieve adequate recovery. (4)

into the remaining endoneurium of the distal stump, eventually reinnervating the target organ (5).

The use of electrical stimulation at the time of surgical repair further aids the process of regeneration. In a study by Al-Majed et al. a transected nerve was given electrical stimulation succeeding surgical repair. The electrically stimulated nerve regenerated over a 25mm gap 5-7 weeks faster than the sham stimulated nerve (8). Electrical stimulation is thought to mimic the intracellular calcium wave that occurs in the proximal segment of the neuron after nerve injury (9).

Following nerve injury, several RAGs are upregulated (10). GAP-43 is an example of one of these genes (11). It is essential in nerve regeneration through the regulation of the nerve's cytoskeleton. The cytoskeleton functions as the internal scaffold of the nerve, maintaining its shape (12). A type of cytoskeleton involved in nerve growth are microfilaments, composed of actin monomers. GAP-43 plays a role in regulating these actin monomers. In the process of cytoskeleton assembly, GAP-43 promotes the clustering of phosphatidylinositol-4,5-bisphosphate (PIP2) (11). PIP2 is a phospholipid component of the cell membrane that is involved in cell signalling (12). Its accumulation leads to a recruitment of proteins that carry out actin polymerisation, increasing the nerve length (11).

Following nerve injury, GAP-43 expression is upregulated with a strong correlation to nerve regeneration (11, 13, 16, 17). Knockdown studies of GAP-43 in neurons resulted in reduced neurite outgrowth and impaired neuronal path finding (14, 16). Furthermore, in studies where GAP-43 was overexpressed nerve regeneration was enhanced (18). Hence, GAP-43 is a suitable indicator of successful peripheral nerve regeneration and can be used to measure the effectiveness of various therapies treating peripheral nerve regeneration.

One particular methodology that can be used to measure gene expression is a reporter system. Rojas et al. (2015) successfully engineered a reporter system downstream of a target gene using CRISPR/Cas9 (20). This method used a Cas9/gRNA nuclease complex to make a double stranded break in the endogenous genomic DNA, immediately 5' to the start codon (ATG) of the gene. The DNA is repaired via homology directed repair (HDR) using a donor vector with homology arms (HAs) flanking the reporter cassette as a template. There were two different reporter cassettes used throughout, one containing the 717bp Green Fluorescent Protein (GFP) and the other 792bp Neomycin resistance gene (Neo). The plasmids containing these cassettes were named pLucGFP and pLucNeo, respectively. GFP can be used to select for positive clones via Fluorescence-Activated Cell Sorting (FACS). Neo positive clones can be selected using G418 antibiotic selection. Both cassettes also contained the 558bp Gaussia luciferase (gLuc) reporter gene, the 587bp

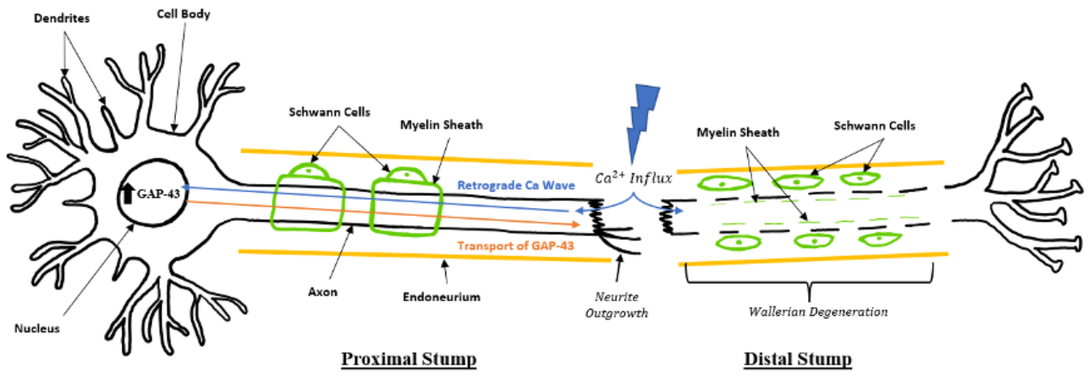


Figure 2. Schematic overview of the calcium influx effect following PNI. In the proximal stump the calcium influx aids in the repair of the membrane and sends a retrograde wave of depolarisation toward the cell body. This signals for the upregulation of GAP-43 which is sorted into vesicles for fast axonal transport to the injury site to aid in neurite outgrowth. In the distal stump an excessive influx of calcium triggers Wallerian Degeneration. This section of the nerve breaks down into its various components: Schwann cells, myelin sheath and axon. Macrophages are recruited to break down the myelin and axon debris, leaving the Schwann cells intact. The neurites continue to regenerate throughout the distal stump until the target organ is reached.

IRES element and the 54 bp 2a peptide. The gLuc is secreted from transfected cells, allowing changes in gene expression to be quantitatively measured by determining the luciferase activity in the cell culture media. IRES ensures separate translation of GFP/Neo and luciferase protein. The 2a peptide cleaves the GFP/Neo from the downstream GAP-43 protein, ensuring the proteins are produced independently.

The overall aim of this study is to serve as the first step in the development of a motor neuron-like cell model allowing efficient measurement of GAP-43 promoter activity. Such a model has potential use in screening for nerve repair therapeutics that upregulate GAP-43 and in the optimisation of electrical stimulation treatment in combination with these therapies. The key objectives are to design and construct both a Cas9 plasmid bearing gRNA that targets GAP-43 cleavage and a donor plasmid comprising of a reporter cassette to be inserted immediately 3' of the endogenous GAP-43 promoter using CRISPR Cas9 HDR. This work will focus on the mouse GAP-43 gene, as mouse motor neuron-like cell models are well developed in comparison with human cell model.

Materials and Methods

EXPERIMENTAL STRATEGY

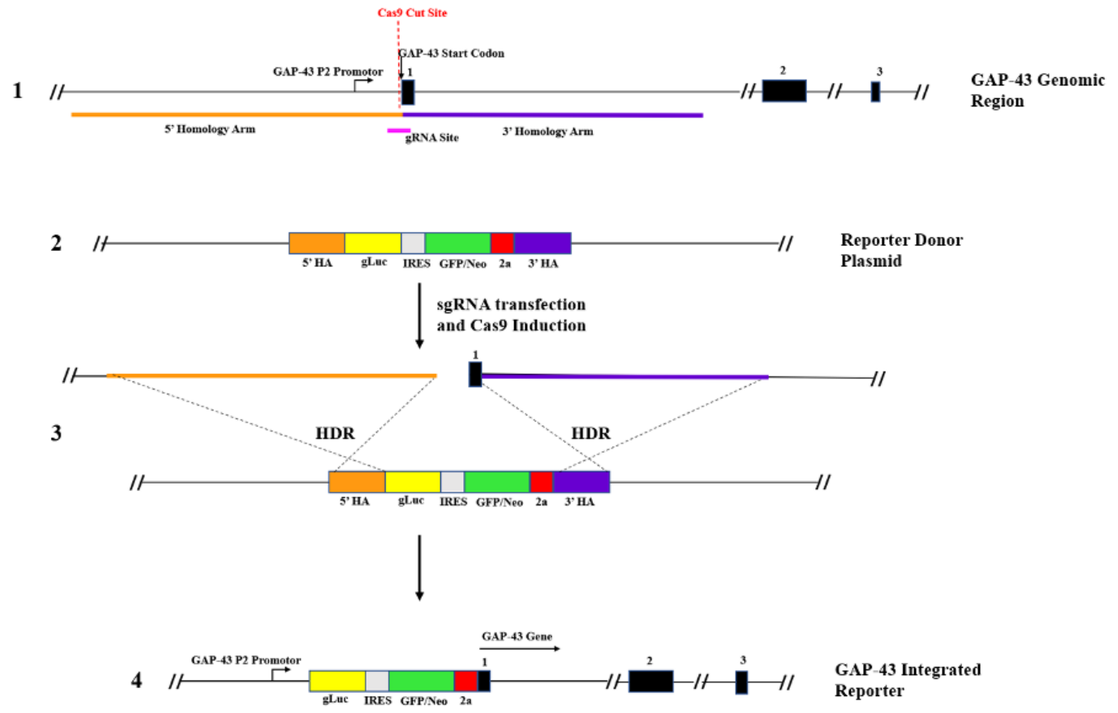


Figure 3. A schematic representation of the Rojas et. al. experimental strategy. (1) Genomic GAP-43 gene has three exons represented as black boxes. 5' and 3' GAP-43 HAs in the genome are represented by orange and purple lines, respectively. The targeted insertion site is directly 5' of the GAP-43 start codon. The gRNA site, in pink, overlaps the junction between the 5' and 3' GAP-43 HAs. The Cas9 cut site is located between the A and T of the ATG start codon. (2) The reporter donor vector including the 5' and 3' GAP-43 HAs, luciferase (Luc), Internal Ribosomal Entry Site (IRES), Green Fluorescent Protein (GFP)/Neomycin (Neo), and 2a peptide. (3) HDR of the DNA after cleavage via the gRNA/Cas9 nuclease complex, using the reporter donor plasmid as a template. (4) Reporter integrated into the GAP-43 locus.

Ligation of GAP-43 gRNA Sequence into the px330 Vector

ANNEALING OF GAP-43 gRNA OLIGOMERS

The GAP-43 gRNA target site was selected as close as possible to the reporter cassette insertion site (immediately 5' of the GAP-43 ATG start codon). The Benchling Design tool (https://www.benchling.com/) was used to design the gRNA site. The GAP-43 gRNA complimentary single strand oligomers were designed with BbsI overhangs to facilitate ligation to the BbsI digest px330 plasmid (Fig. 18.) The oligomers were ordered using IDT DNA oligo service (https://eu.idtdna.com/pages/products/custom-dna-rna/dna-oligos).

The gRNA complimentary oligomers (Table 1) were annealed by adding 1.5µL of each of the sense and antisense oligomers along with 5.0µL of 10X NEB T4 ligase buffer. The mixture was incubated in a thermocycler, at a setting of 95 degree celcius for 4 minutes. This was followed by a reduction in temperature of 0.1 degree celcius every second until room temperature was reached (26 degree celcius) and repeated for 8 cycles. Annealing reaction mixtures were analysed on a 1.5% agarose sodium borate gel, with a 1x sodium borate buffer at 200V for 10 minutes and a Solis BioDyne 100bp DNA ladder.

Table 1. Sequence of the GAP-43 gRNAs as oligomers. BbsI overhangs are highlighted in blue.

Name of RNA	RNA Sequence
gRNA-sense-Gap43	5'- CACCCCATGCTGTGCTGTATGAGA -3'
gRNA-antisense-Gap43	5'- AAACTCTCATACAGCACAGCATGG -3'

PREPARATION OF px330 VECTOR

1µg of the px330 plasmid was cut with 1.0µL BbsI-HF restriction enzyme in 5.0µL of 10X rCutSmartBuffer. The mixture was incubated at 37 degree celcius for 15 minutes. The cut px330 vector was analysed on a 0.8% agarose sodium borate gel, with a 1x sodium borate buffer at 100V for 40 minutes and a Solis BioDyne 1kbp ladder.

GOLDEN GATE ASSEMBLY OF GAP-43 gRNA DS OLIGO AND PX330 VECTOR

A ligation reaction of the annealed oligomers (1µL), the open px330 vector (3µL), 2µL T4 ligase and 2µL 10X ligase buffer was carried out. The mixture was incubated at 16 degree celcius for 3 hours.

TRANSFORMATION OF THE GAP-43 gRNA px330 RECOMBINANT PLASMID

The gRNA-px330 plasmid was then transformed into DH5alpha cells for amplification. Using 2µL ligation reaction with 25µL of DH5α cells. (Control was 2µL of cleaved px330 plasmid in place of the ligation reaction.) The cells were heat shocked at 42 degree celcius in a water bath for 90 seconds, followed by an incubation on ice for 2 minutes. Cells were recovered with 400µL of LB (Lysogeny Broth) and incubated for 1 hour at 37 degree celcius. The px330 backbone has ampicillin resistance allowing for the selection of successfully transformed cells. The transformants (150µL) were pipetted onto LB/Ampicillin plates and incubated at 37 degree celcius for 15 hours.

A miniprep kit (Thermofisher Scientific Genejet Miniprep Kit) was used to purify the gRNA-px330 plasmid from the cells and confirmed via sequencing, using the 'Eurofins Mix-2-Seq kit.'

Design and Assembly of the Reporter Donor Plasmid

DESIGN OF THE GAP-43 HOMOLOGY ARMS

To incorporate the GAP-43 HAs into the donor plasmid, Gibson Assembly was used. These HAs were designed to facilitate insertion of a reporter cassette immediately 5' of the GAP-43 start codon. The optimal HA length is 500-1000 base pairs (22), hence there was a long and short variation designed for each of the 5' and 3' HAs. The long and short 5' HAs designed were 969bp and 595bp respectively, and the 3' HAs 725bp and 530bp respectively.

Amplification of the 5' and 3' Homology Arms: the 5' and 3' GAP-43 homology arms forward and reverse primers were designed homologous to the insertion site (immediately 5' to the ATG start codon of GAP-43) (Table 2). The 5' GAP-43 homology arm forward and reverse primers had incorporated Gibson tails compatible with the BclI site of pLucGFP/pLucNeo plasmid and the 5' luciferase side of the reporter cassette, respectively. The 3' GAP-43 homology arm forward and reverse primers had incorporated Gibson tails compatible with the 3' T2A side of the reporter cassette and the BglII site of pLucGFP/pLucNeo plasmid, respectively. For PCR amplification 4 µL HOT FIREPol Blend Master Mix (HOT FIREPol DNA polymerase, proofreading enzyme, 5x Blend Master Mix Buffer, 1mM dNTPs of each, Bovine serum albumin) was used with 100ng mouse genomic DNA for the template. The PCR conditions were carried out as recommended by the HOT FIREPol protocol.

Table 2. Sequence of the homology arm primers for GAP-43. The lower case represents the Gibson assembly overlap. The upper case represents the annealing region primer

Name of Homology Arm Primers	Sequence
5' Homology Arm Forward	5′ gacggccagtgaattcacttTGCAGTGCGGAAAGTCAG 3′
5' Homology Arm Reverse	5′ aacagaactttgactcccatGGTGGTATCTTCCCCTGC 3′
3' Homology Arm Forward	5′ tggaggagaatcccggcccaATGCTGTGCTGTATGAGAAG 3′
3' Homology Arm Reverse	5′ cgactctagaggatccagtaCGAGCACGAAATCAGGTATC 3′

GIBSON ASSEMBLY OF THE REPORTER DONOR PLASMID

The donor plasmid was made by assembling the multi-use reporter donor vectors pLucGFP and pLucNeo. The vectors are comprised of a pUC19 backbone and either the Luc-IRES-GFP-T2a or Luc-IRES-Neo-T2a reporter cassette flanked by the GAP-43 5' and 3' homology arms. Gibson assembly primers were designed using the NEBuilder Gibson Assembly Fragment Calculator.

The pLucNeo and the pLucGFP plasmids were prepared using a BclI and BglII double restriction enzyme digest. Both the pLucNeo and the pLucGFP plasmids were first cut with 1µL Bgl-II restriction enzyme in 5µL 10X NE buffer. The pLucGFP was incubated with the enzyme for 2 hours, the pLucNeo for 2 hours 30 minutes. Restriction enzyme Bcl-I was then added to both mixtures and incubated for a further 15 minutes at 50 degree celcius. Restriction enzyme digest mixtures were analysed on a 0.8% agarose sodium borate gel, with a 1x sodium borate buffer at 100V for 40 minutes and a Solis BioDyne 1kbp DNA ladder.

These fragments were assembled by adding the relevant segments (5' and 3' homology arms of GFP/Neo) in equimolar amounts to a 15µL Gibson Assembly Master Mix (5x isothermal Master Mix, T5 exonuclease, Phusion DNA Pol, Taq DNA ligase) and incubated for 1 hour at 50 degree celcius.

TRANSFORMATION OF pLucGFP/NEO PLASMID

The Gibson Mixtures of the pLucNeo and pLucGFP were then transformed into DH5α cells. Each of the amounts in Fig 20 were added to separate Eppendorf's. The cells were heat shocked at 42 degree celcius in a water bath for 90 seconds, followed by an incubation on ice for 2 minutes. The cells were recovered using 400µL LB, and 150µL of the transformants were pipetted onto LB/Amp plates. The plates were incubated at 37 degree celcius for 15 hours.

COLONY PCR OF THE PLUCGFP/NEO TRANSFORMANTS

A successful Gibson assembly was indicated in select colonies by amplifying the 5' GAP-43 homology arm using each colony as a template in colony PCR (61.2 degree celcius annealing temperature). The PCR product for select plasmid colonies were analysed using a 1% agarose sodium borate gel, with a 1x sodium borate buffer at 100V for 40 minutes and a Solis BioDyne 100bp DNA ladder. Based on this result 3 colonies were swabbed and grown overnight in 5ml LB and 5µL Ampicilin for 17 hours. Followed by plasmid purification using 'Thermofisher Scientific Genejet Miniprep Kit'. A successful Gibson assembly was confirmed via sequencing using the 'Eurofins Mix-2-Seq kit.'

Results

1. Successful Construction of the GAP-43 gRNA px330 Recombinant Plasmid

AMPLIFICATION OF THE GAP-43 gRNA AND CLONING INTO THE px330 VECTOR PLASMID

GAP-43 gRNA sense and antisense oligomers were amplified and then annealed to generate double stranded gRNA. Results were assessed by agarose gel electrophoresis. Annealed oligomers migrated as a higher molecular weight (Fig. 4, lane 4).

The px330 plasmid was prepared for cloning of the double stranded GAP-43 gRNA by cutting with BbsI restriction enzyme (Fig. 5).

Ligation of the GAP-43 gRNA and px330 plasmid was performed successfully. The ligation mixture was transformed into DH5α E. coli cells. The GAP-43 gRNA px330 plasmid was prepared from 3 positive colonies. DNA sequencing of these plasmids confirmed the presence of the correct GAP-43-gRNA sequence in the px330 plasmid.

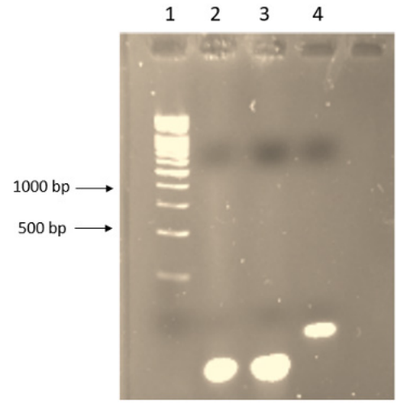


Figure 4. Analysis of GAP-43 gRNA annealed oligomers. Annealing was carried out by incubation of sense and antisense gRNA oligomers at 95 degrees celcius for 4 minutes followed by a reduction in temperature of 0.1 degrees celcius every second until 26 degrees celcius was reached. The mixture was analysed using agarose gel electrophoresis (1.5%) and stained with SafeView to allow visualisation. Lane 1, 100bp Solis BioDyne molecular weight ladder. Lane 2 and 3, 24bp sense gRNA oligomer and 24bp antisense gRNA oligomer. Lane 4, annealed gRNA oligomers.

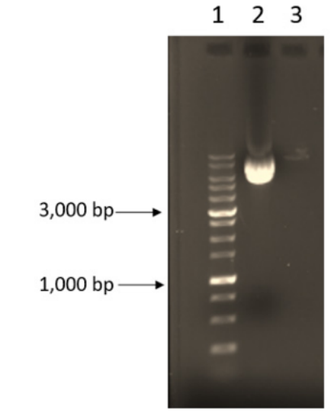


Figure 5. Analysis of px330 restriction enzyme digest. The 8484bp px330 plasmid was incubated with BbsI at 37 degrees celcius for 15 minutes. The mixture was analysed using agarose gel electrophoresis (0.8%) and stained with Safeview to allow for visualisation. Lane 1, 1kbp Solis BioDyne molecular weight ladder. Lane 2, px330 plasmid prior to BbsI restriction digest Lane 3, BbsI digested px330 plasmid.

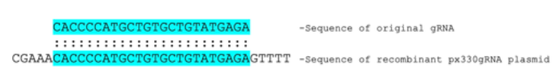


Figure 6. Sequencing results of GAP-43 gRNA cloned into the px330 vector plasmid.

2. Construction of the Reporter Donor Plasmid

AMPLIFICATION OF THE GAP-43 HOMOLOGY ARMS AND CLONING INTO THE PLUCGFP/PLUCNEO DONOR PLASMID

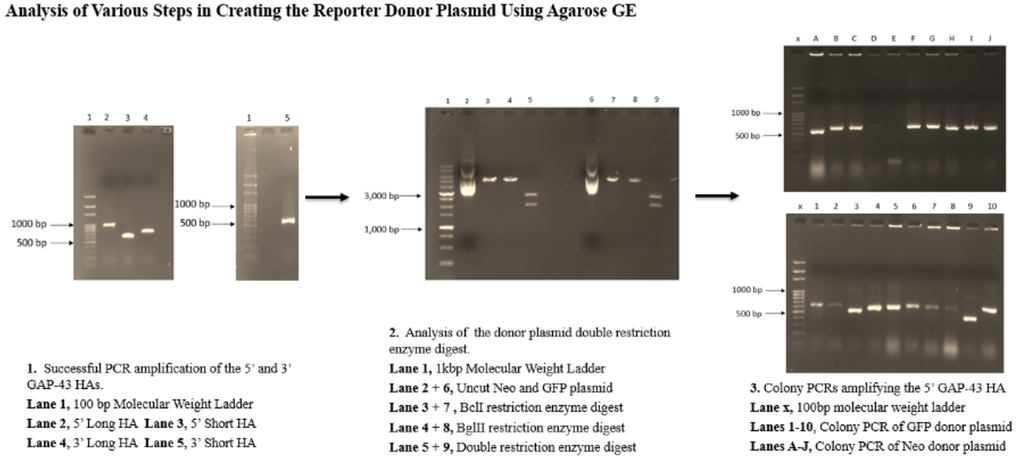
All of the 5' and 3' GAP-43 HAs were successfully amplified (Fig 4.1). This was followed by a double restriction enzyme digest of the pLucGFP/pLucNeo reporter cassette plasmids with the BclI and BglII restriction enzymes that was verified by gel electrophoresis (Fig. 4.2). For Gibson Assembly of the digested pLucGFP/pLucNeo plasmids were incubated with 5' and 3' GAP-43 HAs in the presence of the Gibson Assembly master mix. While the long HAs were amplified successfully, their use in Gibson Assembly was unsuccessful, hence the short 5' and 3' GAP HAs were used throughout.

Following Gibson assembly, the master mix was transformed into DH5α E. coli cells and were plated on LB/ampicillin agar plates. Ten ampicillin resistant colonies were selected from the each of the pLucGFP and pLucNeo Gibson assembly product plates. Subsequently, colony PCRs amplifying the 5' GAP-43 HA were carried out to indicate positive clones. (Fig. 4.3). DNA sequencing of 3 selected colonies from each reaction confirmed the presence of the final the 5' and 3' GAP-43 HA in the pLucGFP/pLucNeo GAP-43 reporter donor plasmid. These plasmids were named p5G43pLucGFP3G43 and p5G43pLucNeo3G43 (Appendix B, Fig. 14, 15).

SEQUENCE ALIGNMENT OF THE 5' HA FOR P5G43PLUCGFP3G43 AND P5G43PLUCNEO3G43

DNA sequence alignment of the cloned HAs with the reference sequence was carried out using CLUSTAL Omega. Sequence changes were observed in the 5' HA differed from the mouse genomic reference sequence. These are highlighted in yellow (Fig. 5). In the 5' GAP-43 HA of the p5G43pLucNeo3G43 plasmid there were four point mutations observed along with several mutations around position 360-420 where the pattern of bases was repeated a number of times (Fig. 5). However, in the p5G43pLucGFP3G43 plasmid there were only 2 point mutations noted.

Analysis of Various Steps in Creating the Reporter Donor Plasmid Using Agarose GE



1. Successful PCR amplification of the 5' and 3' GAP-43 HAs.
Lane 1, 100 bp Molecular Weight Ladder
Lane 2, 5' Long HA **Lane 3,** 5' Short HA
Lane 4, 3' Long HA **Lane 5,** 3' Short HA

2. Analysis of the donor plasmid double restriction enzyme digest.
Lane 1, 1kbp Molecular Weight Ladder
Lane 2 + 6, Uncut Neo and GFP plasmid
Lane 3 + 7 , BclI restriction enzyme digest
Lane 4 + 8, BglII restriction enzyme digest
Lane 5 + 9, Double restriction enzyme digest

3. Colony PCRs amplifying the 5' GAP-43 HA
Lane x, 100bp molecular weight ladder
Lanes 1-10, Colony PCR of GFP donor plasmid
Lanes A-J, Colony PCR of Neo donor plasmid

Figure 7. Analysis of the steps in the construction of the Donor Plasmid using Agarose Gel Electrophoresis

Sequence Differences Observed in the 5' GAP-43 HA of the Reporter Donor Plasmids

```
GFP GGACGAGAGGGAGAGAGAGGGGGAGAGAGGGAGAGAGAGAGAGAGAGAGAGAGAGA 360
Ref GGACGAGAGGGAGAGAGAGGGGGAGAGAGGGAGAGAGAGAGAGAGAGAGAGAGAGA 360
Neo GGACGAGAGGGAGAGAGA---------------------------------------- 360
    ******************

GFP GAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG--AGAGAGAGAATGCGCG--------- 420
Ref GAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAATGTGCGTGTGCGGTG 420
Neo ----------------GGGGGAGAGAGGGAGAGAGAGAGAGAATGTGCGTGTGCGGTG 420
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Figure 8. Sequence differences observed in the 5' GAP-43 HA of the GAP-43 GFP and Neo reporter donor plasmids.

Sequence Differences Observed in the 3' GAP-43 HA of the Reporter Donor Plasmids

```
GFP ACAGGGTTTCCTCGTGAAAGGCAGAAAAAAAAT--TTTTTTTTTTAAAAAAAAACTGCTT 180
Ref ACAGGGTTTCCTCGTGAAAGGCAGAAAAAAAAT-TTTTTTTTTTAAAAAAAAACTGCTT 180
Neo ACAGGGTTTCCTCGTGAAAGGCAGAAAAAAAAATTTTTTTTTTTAAAAAAAAACTGCTT 180
    ********************************  ************************

GFP CCACGGAAGATACCTGATTTCGTGCTCGTACTGGATCCTCTAGAGTCGACCTGCAGGCAT 600
Ref CCGCGGAAGATACCTGATTTCGTGCTCG------------------------------- 600
Neo CCGCGGAAGATACCTGATTTCGTGCTCGTACTGGATCCTCTAGAGTCGACCTGCAGGCAT 600
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Figure 9. Sequence differences observed in the 3' GAP-43 HA of the GAP-43 GFP and Neo reporter donor plasmid

Discussion and Conclusions

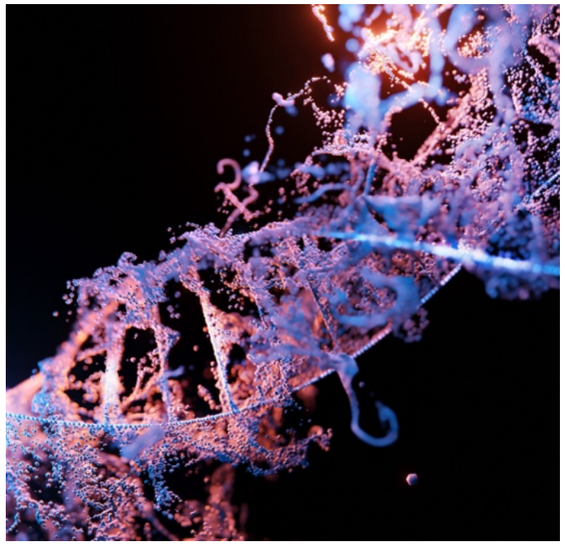
Recombinant plasmids containing the key elements required for Cas9 mediated insertion of a reporter into the GAP-43 gene were successfully generated in this project. The assembly of the Cas9 gRNA component for the cleavage of GAP-43 into px330 plasmid was achieved and the sequence of gRNA in the px330 plasmid was also validated. The GAP-43-gRNA px330 plasmid contained no sequence errors. Hence, it is ready for use in transfection into the mouse cell line to generate the GAP-43 target cleavage necessary for the insertion of the reporter cassette by CRISPR/Cas9 HDR.

Following the construction of the reporter donor plasmid, sequencing errors were observed in the cloned 5' and 3' GAP-43 HAs. They are believed to have occurred in the amplification process and reflect DNA replication errors. These errors should be readily amended by repeating this work using a high-fidelity PCR amplification protocol. Most mutations observed occurred in regions with runs of As and repeated sequences, where PCR amplification errors are common (23). The polymerase used for the work reported here was a standard type of Taq polymerase with an approximate error rate of 1 base in 4000 amplified. The amplification of the GAP-43 HAs should be repeated using a DNA polymerase such as Q5 polymerase which has a higher fidelity with a 10-20 fold reduced error rate. Regardless of the sequence errors, this project has successfully provided the design of DNA primers for the amplification of the 5' and 3' GAP-43 HAs and has validated that these primers successfully amplify the HAs selected. It has also demonstrated that these HAs can be successfully incorporated into the pLucGFP and pLucNeo plasmids.

The design of this work is aimed at measuring GAP-43 expression from its P2 promotor, as it is 10 times more active than P1 (14). To further investigate the promoter activity of GAP-43, a replica of the project design with relevant modifications has the potential for insertion of the reporter under the control of the GAP-43 P1 promoter.

Potential cell lines for the next stage of this project were reviewed. A promising primary cell line is the NSC-34 cell line. The cell line was recommended by S. Geuna et al. who reviewed various in vitro models for peripheral nerve regeneration (24). The NSC-34 cells are consistent with motor neuron properties such as generating action potentials and the production of acetyl choline (24). In addition, the NSC-34 cells express GAP-43 making the cell line an ideal candidate for the insertion of the reporter system (25) for use in screening novel therapeutics that upregulate GAP-43.

The approach outline has high prospects for identifying novel therapeutics for upregulating GAP-43 for enhancing peripheral nerve regeneration. Electrical stimulation (ES) is an accessible therapy that can be used following surgical repair of a damaged nerve (2, 8, 11). It encourages earlier and more sustained upregulation of GAP-43 (2, 8). This reporter system model could be used for the optimisation of ES treatment on PNI. The dose dependent application of exogenous factors such as BDNF and IL-6 to injured nerves has exhibited improvements in nerve regeneration in previous studies (27, 28, 29). The reporter system of GAP-43 may be used to investigate the optimal dosage of these factors to treat PNI. Finally, there are limited studies investigating the combination of electrical stimulation and the application of exogenous factors such as BDNF and IL-6 to treat PNIs. The GAP-43 reporter system has the potential to be used to discover multimodal treatments producing optimal GAP-43 upregulation for nerve regeneration.



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Appendix 1: Sequence Alignment Results of the GAP-43 Homology Arms

SEQUENCE ALIGNMENT OF THE 5' HA FOR p5G43pLucGFP3G43 AND p5G43pLucNeo3G43

DNA sequence alignment of the cloned HAs with the reference sequence was carried out using CLUSTAL Omega. Sequence changes were observed in the 5' HA differed from the mouse genomic reference sequence. These are highlighted in yellow (Fig. 14, 15). In the 5' GAP-43 HA four point mutations were observed along with several mutations around position 360-420 where the pattern of bases was repeated a number of times.

```
GFP GGGGTTTCAGTCACGACGTTGTAAACGACGGCCAGTGAATTCACTTTGCAGTGCGGAAAG 60
Ref ------------------------------------------------TGCAGTGCGGAAAG 60
Neo --------GGTCACGACGTTGTAAACGACGGCCAGTGAATTCACTTTGCAGTGCGGAAAG 60
.                                                   **************

GFP TCAGTGGGTAATTGGGTCCAGATTGGAGGTGTTTAAATATTCATGAGGCTGGCAGGGGAC 120
Ref TCAGTGGGTAATTGGGTCCAGATTGGAGGTGTTTAAATATTCATGAGGCTGGCAGGGGAC 120
Neo TCAGTGGGTAATTGGGTCCAGATTGGAGGTGTTTAAATATTCATGAGGCTGGCAGGGGAC 120
.   ************************************************************

GFP TGGGAGGGGGTGACTGTCTAGAAATGGGGGTAGGGGCTACGGGAAGTGATTAGTCACTGG 180
Ref TGGGAGGGGGTGACTGTCTAGAAATGGGGGTAGGGGCTACGGGAAGTGATTAGTCACTGG 180
Neo TGGGAGGGGGTGACTGTCTAGAAATGGGGGTAGGGGCTACGGGAAGTGATTAGTCACTGG 180
.   ************************************************************

GFP AAGCTAGCAAACAATTCTGAGAAAGGGACCCAGGGAGAAGGAAGAAAAAGATTGGGTGGG 240
Ref AAGCTAGCAAACAATTCTGAGAAAGGGACCCAGGGAGAAGGAAGAAAAAGATTGGGTGGG 240
Neo AAGCTAGCAAACAATTCTGAGAAAGGGACCCAGGGAGAAGGAAGAAAAAGATTGGGTGGG 240
.   ************************************************************

GFP GAGTGGAGGAAAGAGGAGAAGGAAGGAAGGAAAAGGAGAGAGGAAGGAAAGAGGAGGAAG 300
Ref GAGTGGAGGAAAGAGGAGAAGGAAGGAAGGAAAAGGAGAGAGGAAGGAAAGAGGAGGAAG 300
Neo GAGTGGAGGAAAGAGGAGAAGGAAGGAAGGAAAAGGAGAGAGGAAGGAAAGAGGAGGAAG 300
.   ************************************************************

GFP GGACGAGAGGGAGAGAGAGGGGGAGAGAGGGAGAGAGAGAGAGAGAGAGAGAGAGAGA 360
Ref GGACGAGAGGGAGAGAGAGGGGGAGAGAGGGAGAGAGAGAGAGAGAGAGAGAGAGAGA 360
Neo GGACGAGAGGGAGAGAGA----------------------------------------- 360
.   ******************

GFP GAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG--AGAGAGAGAATGCGCG--------- 420
Ref GAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAATGTGCGTGTGCGGTG 420
Neo ------------------GGGGGAGAGAGGGAGAGAGAGAGAGAATGTGCGTGTGCGGTG 420
.                     * * ******* ***  ************ ***

GFP ------------------------------------------------------------ 480
Ref AGCAATAGCTGTGGACCTTACAGTTGCTGCTAACTGCCCTGGTGTGTGTGAGGGAGAGAG 480
Neo AGCAATAGCTGTGGACCTTACAGTTGCTGCTAACTGCCCTGGTGTGTGTGAGGGAGAGAG 480

GFP ------------------------------------------------------------ 540
Ref AGAGAGAGAGGGAGAGGGAGGGAGAGGGAGGGAGGGAGGGAGAGCGCGCTAGCGCGAGAG 540
Neo AG---------------------------------------------------------- 540
```

Figure 10. Sequencing results of the amplified 5' forward GAP-43 HA from the reporter donor plasmids (5' reverse was the same). The 5' HA sequence obtained from the p5G43LucGFP3G43 (GFP) and p5G43LucNeo3G43 (Neo) were aligned with a mouse DNA reference sequence (ref) using CLUSTAL Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/)

SEQUENCE ALIGNMENT OF THE 3' HA FOR p5G43pLucGFP3G43 AND p5G43pLucNeo3G43

In the 3' GAP-43 HA two mutations were observed. At position 180 there was a deletion of a T and an addition of an AT evident in GFP and Neo respectively. Furthermore, a single base mutation was apparent in GFP at position 600, where an A replaces a G.

```
GFP TCTAACATGCGGTGACGTGGAGGAGAATCCCGGCCCAATGCTGTGCTGTATGAGAAGAAC 60
Ref ------------------------------------ATGCTGTGCTGTATGAGAAGAAC 60
Neo TCTAACATGCGGTGACGTGGAGGAGAATCCCGGCCCAATGCTGTGCTGTATGAGAAGAAC 60
.                                        **********************

GFP CAAACAGGTAGAGCTAAAGATTTCTTTTTTACTTCTTTGCTGTTGTGAAATTATCAGAGT 120
Ref CAAACAGGTAGAGCTAAAGATTTCTTTTTTACTTCTTTGCTGTTGTGAAATTATCAGAGT 120
Neo CAAACAGGTAGAGCTAAAGATTTCTTTTTTACTTCTTTGCTGTTGTGAAATTATCAGAGT 120
.   ************************************************************

GFP ACAGGGTTTCCTCGTGAAAGGCAGAAAAAAAAT--TTTTTTTTTTAAAAAAAAACTGCTT 180
Ref ACAGGGTTTCCTCGTGAAAGGCAGAAAAAAAAT-TTTTTTTTTTAAAAAAAAACTGCTT 180
Neo ACAGGGTTTCCTCGTGAAAGGCAGAAAAAAAAATTTTTTTTTTTAAAAAAAAACTGCTT 180
.   ******************************   ************************

GFP CTGCCCTGGCATGATGCTCTGGCTTCCTTAGCATACGGTAACTGATGCTTGCATCCCGGC 240
Ref CTGCCCTGGCATGATGCTCTGGCTTCCTTAGCATACGGTAACTGATGCTTGCATCCCGGC 240
Neo CTGCCCTGGCATGATGCTCTGGCTTCCTTAGCATACGGTAACTGATGCTTGCATCCCGGC 240
.   ************************************************************

GFP GTTATTCTTTTCTGCCTTTCATGATCTGGTTTTTGGAATGCTGCTACTAATTAGGGTAAG 300
Ref GTTATTCTTTTCTGCCTTTCATGATCTGGTTTTTGGAATGCTGCTACTAATTAGGGTAAG 300
Neo GTTATTCTTTTCTGCCTTTCATGATCTGGTTTTTGGAATGCTGCTACTAATTAGGGTAAG 300
.   ************************************************************

GFP GGGAGAGAAATATGCCGGCTTGGCTAGAAATATGATTCCCCTCGCCTATTAGTAAGTGCT 360
Ref GGGAGAGAAATATGCCGGCTTGGCTAGAAATATGATTCCCCTCGCCTATTAGTAAGTGCT 360
Neo GGGAGAGAAATATGCCGGCTTGGCTAGAAATATGATTCCCCTCGCCTATTAGTAAGTGCT 360
.   ************************************************************

GFP CAGCCGCTAGGTCTCTGGTTTTGAGGGTGTGGATGCAGAAAGGGGTGTGGGGACGATGTG 420
Ref CAGCCGCTAGGTCTCTGGTTTTGAGGGTGTGGATGCAGAAAGGGGTGTGGGGACGATGTG 420
Neo CAGCCGCTAGGTCTCTGGTTTTGAGGGTGTGGATGCAGAAAGGGGTGTGGGGACGATGTG 420
.   ************************************************************

GFP GGCTCTATCTACGAGATCAAAAAAGCTAATCTTGATATTATTTGTGGAAAATTAGGTCTG 480
Ref GGCTCTATCTACGAGATCAAAAAAGCTAATCTTGATATTATTTGTGGAAAATTAGGTCTG 480
Neo GGCTCTATCTACGAGATCAAAAAAGCTAATCTTGATATTATTTGTGGAAAATTAGGTCTG 480
.   ************************************************************

GFP GGGGAATTATAGTCACATTTCAACATTGCCTGTTCCGTGATTCAAATTTTCTCACATGTG 540
Ref GGGGAATTATAGTCACATTTCAACATTGCCTGTTCCGTGATTCAAATTTTCTCACATGTG 540
Neo GGGGAATTATAGTCACATTTCAACATTGCCTGTTCCGTGATTCAAATTTTCTCACATGTG 540
.   ************************************************************

GFP CCACGGAAGATACCTGATTTCGTGCTCGTACTGGATCCTCTAGAGTCGACCTGCAGGCAT 600
Ref CCGCGGAAGATACCTGATTTCGTGCTCG------------------------------- 600
Neo CCGCGGAAGATACCTGATTTCGTGCTCGTACTGGATCCTCTAGAGTCGACCTGCAGGCAT 600
.   ** ************************
```

Figure 11. Sequencing results of the amplified 3' forward GAP-43 HA from the reporter donor plasmids (3' reverse was the same). The 3' HA sequence obtained from the p5G43LucGFP3G43 (GFP) and p5G43LucNeo3G43 (Neo) were aligned with a mouse DNA reference sequence (Ref) using CLUSTAL Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/)

Appendix 2: Sequence Maps of the Reporter Donor Plasmids

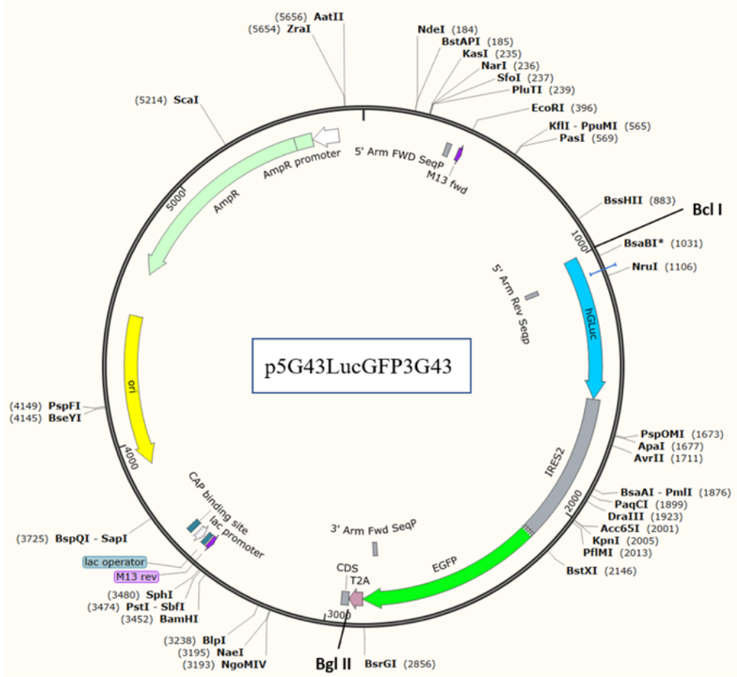

Figure 12. Map of the p5G43LucGFP3G43 reporter donor plasmid with the BclI and the BglII sites indicated.

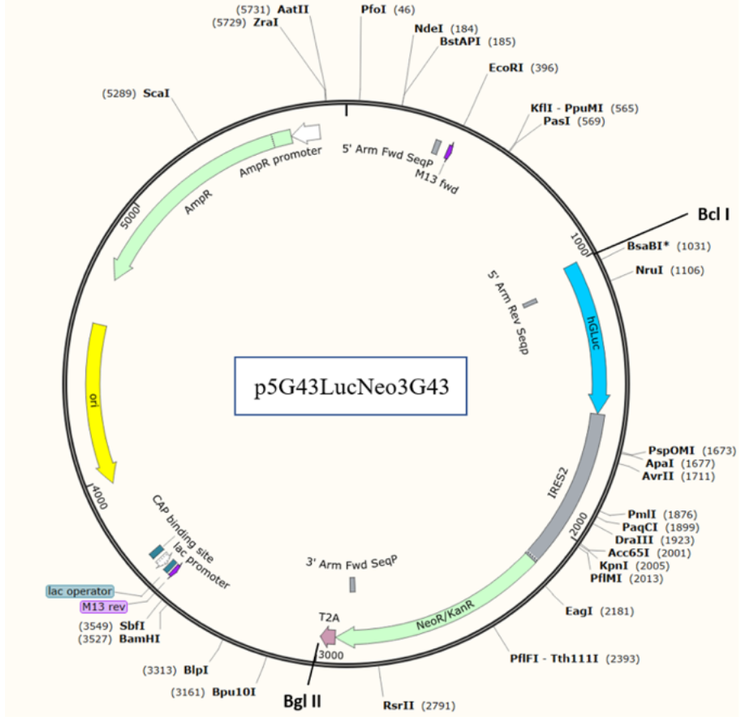

Figure 13. Map of the p5G43LucNeo3G43 reporter donor plasmid with the BclI and BglII sites indicated.

Efficacy of the Informed Consent Process for Surgical Procedures: A Review of the Literature

JULIE BESHARA

Abstract

BACKGROUND: Informed consent is a communication process between a patient and their healthcare team to acquire a patient's approval to undergo a medical intervention. It is essential to the delivery of legal, safe, and patient-centred health care. Despite this, it is often inadequately implemented in clinical practice which frequently contributes to patients having little understanding and can lead to unfavourable outcomes. Furthermore, interventions to improve the consent process are not well recognized. Ultimately, the evaluation of these factors in this review will be of relevance in improving patient-centred care.

OBJECTIVES: Explore degree of understanding and retention of information amongst surgical patients during the informed consent process, identify outcomes of obtaining inadequate informed consent, and evaluate interventions that improve comprehension of surgical treatment.

METHODS: The first electronic search was conducted through EBSCOhost to identify relevant literature on MEDLINE, Academic Search Complete, and CINAHL Plus. A second search was completed through PubMed. Exclusion and inclusion filters were applied, and duplicates removed, which yielded 200 articles. Title/Abstract screening yielded 15 articles which have then undergone full text review to assess for eligibility. This generated the 10 articles used in this review.

RESULTS: Surgical patients have poor comprehension with regards to the benefits, risks, and alternatives of their procedure. Although most patients receive some information about their procedure, this was not suited to their personal goals and needs. Surgical patients also greatly benefited from interventions that were assessed to increase patient understanding and improve the informed consent process.

CONCLUSION: Informed consent is poorly delivered based on the analysis of patient understanding and outcomes. Further research on interventions to improve these elements are recommended as previous studies show notable improvement.

Introduction

Informed consent is a fundamental element of adequate patient communication (1). It is an ethical and legal requirement before proceeding with any form of surgical care (2) as well as emphasizing the concept of autonomy, giving patients the right to be informed about their well-being and to make decisions about their healthcare (1). Informed consent is said to be defined based on three main measures: sufficient delivery of knowledge regarding relevant risks and benefits of the procedure and alternatives; ensuring patient understanding; and obtaining patient's approval for treatment (1,3). Evidence shows that the communication quality correlates with patient comfort and knowledge with respect to their proposed treatment plan (3). Satisfactory patient knowledge will bring about an effective discussion with their physician to create a treatment plan that meets the patient's medical, social, emotional, and economical demands (3). However, patient understanding relating to surgical informed consent is often poor and effectiveness of interventions to improve this remains unknown (2). The literature calls attention to health professionals training needs when facilitating informed consent within clinical consultations (2). This proposes the requirement to identify the level of patient understanding with regards to acquiring informed consent to determine the appropriate approach to best meet the patient's needs (4). Failure to adequately address informed consent violates the principles of biomedical ethics: beneficence, autonomy ,justice and nonmaleficence and exposes the doctor to professional and legal sanction (5). Hence, the focus of this paper is to search for and assess literature with regards to patient understanding, outcomes and communication interventions related to informed consent from surgical patients, to assess the efficacy of this process.

OBJECTIVES:

1. Explore the degree of understanding and recall of information amongst surgical patients during the process of informed consent
2. Identify the outcomes of obtaining inadequate informed consent from surgical patients
3. Evaluate the interventions put in place to aid patients in comprehending surgical treatment

Methodology

SEARCH STRATEGY: Two electronic data bases were organized on EBSCOhost and PubMed to gather relevant literature with regards to the aim and objectives of this review. The following keywords were used on EBSCOhost using Boolean operators:

"patient" AND "informed consent" AND "surgical"

The following databases yielded the maximal results through EBSCOhost:

1. MEDLINE
2. Academic Search Complete
3. CINAHL Plus with Full Text

PubMed was searched separately (using the same keywords as above) and yielded relevant publications.

PROCESS OF SELECTION

Figure 1 below shows a summary of the selection process. The searches on EBSCOhost and PubMed with the keywords "patient" and "informed consent" and "surgical" were conducted. This yielded the maximum results in MEDLINE, Academic Search Complete and CINAHL Plus with Full Text databases through EBSCOhost. PubMed also yielded results, giving a total of 3912 results combined, 1,201 results from MEDLINE, 555 results from Academic Search Complete, 466 results from CINAHL and 1690 results from PubMed. Initial inclusion criteria were selected (see table 1), and these filters were applied: articles that were in available in full text, available in English, published in academic journals between 2000 and 2021, and only qualitative and quantitative studies. This yielded 620 results in total, 138 results from MEDLINE, 203 results from Academic Search Complete, 95 results from CINAHL Plus with Full Text, and 184 results from PubMed. Next, a filter criterion of only adults who are 19+ years was selected leaving 220 articles, excluding articles with only specific age ranges such as just 45–50-year-olds. Duplicates were removed using Zotero reference manager and 200 articles remained. Exclusion/inclusion criteria (see table 1) were applied while abstract screening and this yielded a total of 15 articles. Full text review (see table 3) produced 10 articles meeting criteria for inclusion in this study.

SELECTION CRITIERIA

Table 1: Exclusion and inclusion criteria

Inclusion Criteria	Exclusion Criteria
Available in the English Language	Unavailable in the English Language
Available in full text	No full text available
Available through University College Cork Library	Not available through University College Cork Library or required subscription
Quantitative and qualitative studies reporting original research only	Systematic reviews, literature reviews, protocols, meta-analysis
Published in academic journals and peer reviewed between 2000 and 2021	Non-peer reviewed literature (ex: magazine articles) and published literature before 2000
Population included all adults (19+) who do not required assisted decision making	Patients under 19, specific adult ranges only, and patients who required assistance in decision making
Surgical patients in a hospital setting	Patients who are receiving non-surgical interventions or not receiving surgical treatment at a hospital (ex: in a clinic or ambulatory care center)
Studies investigating the interventions that aid in comprehending surgical treatment	Studies investigating interventions that aid in comprehending post operation treatment

Table 2: Reasons for exclusion of articles during full text review

Reason for Exclusion	Number of Articles
Guideline protocol	2
Not exclusively consent for surgical treatment	3
Total excluded	5
Remaining articles	10

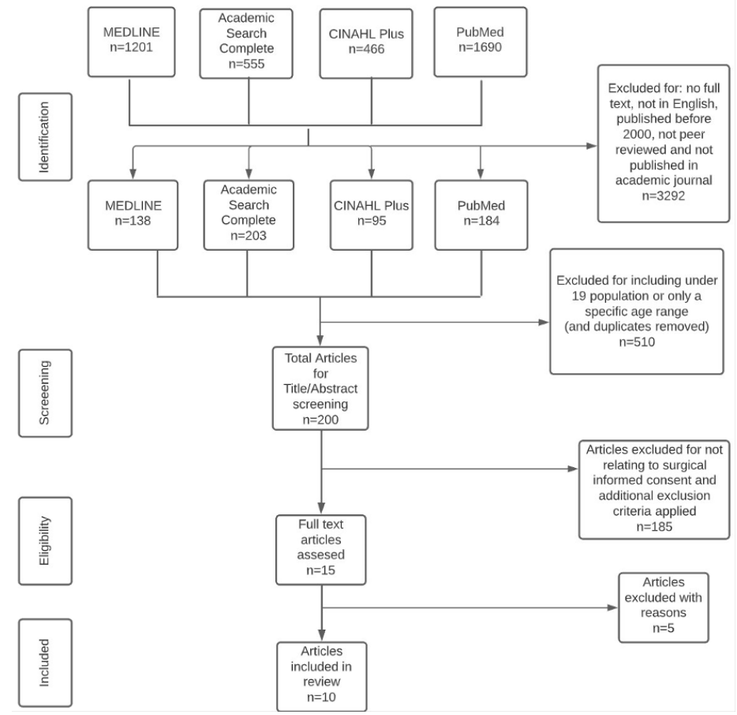

Figure 1: Summary of study selection process

Results

During the selection process, ten articles meeting study criteria were included. Of the ten, eight were quantitative (6–13) and two were qualitative (14,15). Specific study designs included four cross sectional studies (6,7,10,13), two narrative studies (14,15), two randomized control studies (8,12), and two prospective cohort studies (9,11). Many methods were used to collect data, such as structured questionnaires (6,7,11,13), semi-structured questionnaires (9), and interviews (14,15). The location of these studies varied with one study taking place in Croatia (6), one in Uganda (7), one in New Zealand (14), one in Australia (8), two in Britain (9,10), two in the USA (11,13), one in India (12) and one in Israel (15). The sample sizes ranged from 12 (15) to 371 (7) participants. The summary of each of the ten articles is included in Table 4.

ASSESSMENT OF STUDY QUALITY

The EBL appraisal tool was used for the eight quantitative studies (6–13). See appendix A for the EBL checklist. The CASP tool was used for the two remaining qualitative studies (14,15) selected for this literature review. The validity scores of the quantitative studies using the EBL critical appraisal tool checklist (16) are summarized in table 5 below along with the summary of the CASP checklist (17) for the qualitative studies in Table 6.

Table 3: Summary of selected articles

Author (Year), Title, Location	Aim(s), Design, Sample Size, Population	Study Methodology	Key Findings	Study Strengths and Limitations	Future Directions
Vucemilo *et al* (2013) Are physician-patient communication practices slowly changing in Croatia? – a cross-sectional questionnaire study (6) Zagreb, Croatia	Examine practices of doctor-patient communication while acquiring informed consent in hospitals Cross-sectional questionnaire study N= 250 Internal medicine and surgical patients	Two-part questionnaire given to 250 patients from five tertiary level hospitals Questionnaires were anonymous and focused on communication and informed consent Part one of questionnaire: 32 questions (20 on patient doctor communication and informed consent, 6 on doctor-patient relationship, and 6 on patient rights) Part two of questionnaire: 12 questions on socio-demographic aspects	Fifty seven percent of patients rated the comprehension level of benefits, risks, and alternatives as high, 37% as average and 5% as low Patients were informed about risks regarding a rejection of a surgical procedure in 69% of cases Of the total, 76% of patients received information on risks of proposed treatment Of the total, 46% of patients received information on other methods of treatment	*Strengths:* Large sample size Population had both sexes and range of age groups (18-86 years) Large response rate *Limitations:* Actual conversations between physicians and patients were not recorded so study relied on patients recall of information No qualitative analysis was done which can be of benefit	Further quantitative and qualitative data should be gathered Further assessment of communication practices in health care, particularly in Croatia Increase the importance of informed consent processes within hospitals in Croatia Have communication skills training for medical health professionals including medical students
Ochieng *et al* (2015) Informed consent in clinical practice: patients' experiences and perspectives following surgery (7) Uganda	Identify patients' events and viewpoints with regards to surgical informed consent Cross-sectional questionnaire study N=371 Post-operative patients from different surgical disciplines	Within two weeks after surgery, participants were given a semi structured questionnaire to complete Questionnaire composed of questions related to surgical details and the informed consent discussion with their medical professional	Eighty percent of participants have received explanations of their surgery and 56.1% had their concerns addressed before the surgery Of the total,17% of patients were not aware of the kind of surgery they had Seventeen percent of patients did not recall giving consent for surgery Of the total, 23.7% of participants can name the medical professional that received consent from them	*Strengths:* Participants portrayed a range of adult age groups Participants in the study had different education and social class Large sample size *Limitations:* Study was only conducted at major referral hospitals which have the most senior surgeons, findings may not be applicable for other hospitals Inclusion and exclusion criteria of participants not outlined	Improve patients' participation during decision making by providing education programs for doctors
Agnew *et al* (2012) Informed Consent: A Study of the OR Consenting Process in New Zealand (14) New Zealand	To address if the patient's comprehension of the procedure and the information given appropriate for the needs of each patient Qualitative study N=18 Surgical patients from 3 specialty areas: orthopaedics, general surgery, and urology	Telephone contact was made with patients ten to fourteen days after surgery that lasted 30 minutes Interviews were recorded and patients were asked to explain how they gave consent for surgery, to share how much information they received about their surgery and to describe things that they found to be positive and negative about the consenting process	One hundred percent of patients concluded that they were provided with some information with regards to their surgery One hundred percent of patients stated that the information was not given in an appropriate way and did not meet their personal goals and needs	*Strengths:* Qualitative study design is appropriate for the aims of the study One interviewer reduces chance of variability between observers Compares surgical patients from different speciality areas *Limitations:* Small number of participants General nature of information is hard to gather	Future research can focus on more specific populations such as gaining consent within different ethnic groups Perioperative nurses should receive specific training for the consenting process

Table 3: Summary of selected articles (continued)

Author (Year), Title, Location	Aim(s), Design, Sample Size, Population	Study Methodology	Key Findings	Study Strengths and Limitations	Future Directions
Fraval *et al* (2015) Internet based patient education improves informed consent for elective orthopaedic surgery: a randomized control trial (8) Australia	To determine if providing patients to educational websites as part of the consent process will improve the efficacy of the informed consent process Randomized control study N= 211 Surgical patients from orthopaedic outpatient clinic and were booked for orthopaedic procedures	Patients received standard informed consent discussion with their surgeon then randomized to either the control arm or intervention arm The experiment arm was asked to read the appropriate part of the website and then both groups were directed to complete the same survey	Clinically notable increase in patient knowledge for the intervention arm relative to the control arm ($p< 0.01$) Satisfaction of patient was improved in the intervention arm compared to control arm ($p=0.043$)	*Strengths:* Used validated survey to test comprehension First randomized control trial to examine effectiveness of a online education resource designed for hospital patients *Limitations:* No longitudinal follow up to assess if the enhancement in knowledge is still present	Future research focus on the efficacy of exposing patients to online resources before and after consent, particularly in elective orthopaedic procedures
Zarnegar *et al* (2015) Patient perceptions and recall of consent for regional anaesthesia compared with consent for surgery (9) Britain	Evaluate the efficacy of consent for interscalene block and compare this with consent for surgery Prospective observational Study N=46 Adult patients who had shoulder replacement procedures under general anaesthesia (interscalene brachial plexus block)	Participants were interviewed using a structured questionnaire comprised of 18 questions relating to interscalene brachial plexus block and shoulder arthroplasty consent	All patients stated that they agreed to undergo both interscalene block and the arthroplasty of their shoulder Of the total, 60% believed that once a consent form has been signed, they cannot change their mind Of the total, 60% of patients did not read the consent form for their surgery prior to signing Twenty four of the forty-six patients viewed the consent form as a way by which hospitals protect themselves against litigation Of the total, 33% of patients did not think the preoperative discussion concerning the general anaesthesia was as important as the consent form for surgery	*Strengths:* One of few studies that evaluate consent for anaesthetic procedures Compares patient experience and recall of consent of an anaesthetic procedure with a surgical procedure *Limitations:* Small sample size Examined patients who had a specific intervention in one speciality	Emphasize the importance of developing efficient strategies to increase patient comprehension of consent for anaesthetic procedures
Howlader *et al* (2004) Patients' views of the consent process for adult cardiac surgery: questionnaire survey (10) Britain	Examine patient perception and recollection of the surgical consent process Cross-sectional questionnaire study N=100 Patients who underwent cardiac surgery from January to February 2003 in the same London teaching hospital	Patients completed questionnaires after their surgery, and a day before they are discharged from the hospital Questionnaire was on information they were given during consent process	Eighty- nine percent of patients stated that information given at consent has been adequate Of the total, 38 % of patients stated that use of booklets alone in comparison with verbal explanations is less discouraging Thirty one percent of patients could recall the risk of surgery at time of discharge Themes emerged: most patients will not comprehend or recall risks that have been communicated to them verbally	*Strengths:* Cardiac surgery is suitable for assessing consent No other studies examining consent process for post-Bristol area *Limitations:* Recall bias as questionnaires were given prior to discharge	Use of booklets, audiotapes, and videotapes for consent process Communicate risks and probabilities to patients Research on optimum timing of consent

Table 3: Summary of selected articles (continued)

Author (Year), Title, Location	Aim(s), Design, Sample Size, Population	Study Methodology	Key Findings	Study Strengths and Limitations	Future Directions
Feiner *et al* (2016) Preoperative Surgical Discussion and Information Retention by Patients (11) USA	Assess the degree of information communicated to patients is comprehended and recalled after pre-operative discussion of upper limb procedures Prospective cohort study N=20 Patients who were scheduled to undergo elective upper extremity surgery	Patients given same 20 item questionnaire to complete twice, once after the first preoperative discussion (several weeks before surgery) and the second time after preoperative discussion two (one week before surgery)	0 out of the 20 patients did not retain 100% of information in any of the two visits There was less comprehension of the preoperative discussion during the second visit (patients retained 73% of information in first visit and 61% during the second visit) Of the total, 50% of patients stated that they comprehended 100% of the discussion but this value dropped to 10% after preoperative discussion two	*Strengths:* Easily reproducible Evaluates patient comprehension over time which can minimize recency effect *Limitations:* Small sample size Patient population only represents only one surgeons practice Data is quantitative and does not tell complete story of each patient	Investing time in educating patients about their operations is vital Continue further research that improves patient care
Karan *et al* (2014) The effect of multimedia interventions on the informed consent process for cataract surgery in rural South India (12) India	Test effect multimedia resources on the comprehension of cataract surgery if it is added to the informed consent process Randomized control study N=97 Patients at a private surgical hospital scheduled to undergo a cataract surgical procedure	Patients allocated randomly in intervention group and control group Intervention group was given a verbal informed consent with a educational pamphlet and a 3-D model of the eye Control group was still given informed consent but only verbally The two groups were tested using a quiz before informed consent, after informed consent and one day before surgery The quiz had a True/False/I don't know setup	Both groups showed enhancement in between scores of pre and post informed consent quizzes (P value on the order of 10^{-6}) more improvement in the intervention group (P value on the order of 10^{-16}) No notable differences observed in change of scores between post-informed consent and post-operative quizzes Multimedia aid is effective in improving patient comprehension even in a patient population with limited knowledge	*Strengths:* Appropriate representation of population due to non bias selection criteria *Limitations:* Absence of true randomization into control and intervention groups Small sample size Challenge to make sure there is complete standardization of informed consent experiences	Further research on usefulness of multimedia interventions in diverse patients Identify optimal multimedia images and models that target different patient populations

OBJECTIVE 1: DEGREE OF UNDERSTANDING AND RETENTION AMONGST SURGICAL PATIENTS DURING THE INFORMED CONSENT PROCESS

Seven of the studies evaluated patient comprehension of the informed consent process prior to surgical treatment (7–11,13,15). Feiner et al concluded that 0 of the 20 patients fully retained the surgical detail provided to them at preoperative discussions and 50% of patients expressed that they comprehended 100% of the discussion during the first preoperative discussion, however this figure dropped to 10% at their second preoperative discussion, one week prior to surgery (11). Another group of physicians in Britain focused on recall of information at time of discharge and found that 31 of the 100 patients were able to recall the risk of their completed surgery (10). On the other hand, Zarnegar et al assessed the consent process of anaesthetic procedures in comparison with consent for surgery and findings showed that 24 of the 46 patients only regarded consent forms as a method of protection from litigation for hospitals (9). A further study centred on patient's experiences of the informed consent process, following surgery (7). Seventeen percent of participants were unaware of the name of their surgical procedure (7).

OBJECTIVE 2: OUTCOMES OF ACQUIRING INADEQUATE INFORMED CONSENT FROM SURGICAL PATIENTS

The efficacy of the consent process was assessed in six studies, and several diverse outcomes were identified (6,7,9,10,14,15). The two qualitative studies found that patients were not given information concerning their surgical procedure that was suitable for their personalized needs (14,15). However, Howlader et al indicated that 89% of patients were satisfied with information

Table 3: Summary of selected articles (continued)

Author (Year), Title, Location	Aim(s), Design, Sample Size, Population	Study Methodology	Key Findings	Study Strengths and Limitations	Future Directions
Lorenzen et al (2008) Using Principles of Health Literacy to Enhance the Informed Consent Process (13) USA	Assess the use of health literacy in improving patient knowledge of medical interventions Cross-sectional questionnaire study N=41 Patients aged 26-80 years who underwent varying surgical procedures	Patients were given two different consent forms (original and new reader friendly) with access to surgery nursing staff for assistance Data collection was made through nurses filling out survey based on patient performance and comprehension	Common language used in surgical consent forms often surpass average reading level of US patients Of the total, 75% of patients do not read the consent documents Addition of reader friendly language makes it more likely patient will read consent documents (52% increase in reading of documents) Adding teach back methods performed by nurses increases patient understanding	*Strengths:* Study contributed to body of evidence-based practice Study evaluated the development of better practices in health literacy aspect of medical practice *Limitations:* Some patients were scheduled to undergo eye surgery and may have experienced vision problems while attempting to read consent documents which can have effect on results Methodology was not clearly stated enough for replication	Increase staff member awareness of health literacy concepts
Gabay et al (2019) What do patients want? Surgical informed-consent and patient-centered care - An augmented model of information disclosure (15) Israel	Identify the major concerns and preferences of patients concerning disclosure information prior to surgery Narrative study N=12 Patients who underwent major surgeries in public hospitals with varying ages and socio-demographic traits	Two interviews were carried out with each participant at their homes Interviews were from 90 minutes to 2 hours; the first interview was two days after discharge and the second interview was three weeks after Participants were asked to comment on the reason they came to the hospital and their experience	Participants expected that the information they were given would be tailored to their constraints and aims Participants wanted to be aware of the risks of the surgery to feel in-control Themes from narrative analysis: objectification of patients, intimidating scenarios and lack of information for patients	*Strengths:* Study supported the view of patient-centeredness Narrative study led to the creation of an augmented model of information disclosure *Limitations:* Restricted awareness of time constraints Controversy over what aspect of the organizational culture at the hospital had suboptimal surgical informed consent	Further research using narrative methods to fully acknowledge patients' experiences of surgical consent Future studies to determine challenges that surgeons face with application of the augmented model of surgical informed consent

Table 5: Validity scores of quantitative studies based on the EBL Quantitative Checklist

Study	Population validity (%)	Data Collection validity (%)	Study Design validity (%)	Results validity (%)	Overall validity (%)
Vucemilo et al. (2013)	100	87.5	100	100	95.8
Ochieng et al. (2015)	66.6	100	80	100	87.5
Fraval et al. (2015)	100	100	80	50	84
Zarnegar et al. (2015)	83.3	83.3	100	83.3	86.9
Howlader et al. (2004)	100	66.6	80	100	86.9
Feiner et al. (2016)	66.7	66.6	80	83.3	73.9
Karan et al. (2014)	75	66.6	80	83.3	76
Lorenzen et al. (2008)	66.6	50	80	83.3	69.5

provided (10). A range of 57-100% expressed that they received information regarding their surgery (7,10) but in another study, 60% of the total thought there was no withdrawal of consent after signing forms (9) and 57% of patients in the Vucemilo et al study stated they have highly obtained the benefits, risks, and alternatives of their recommended procedure (6). Another study in Uganda found that 17% of the 371 participants did not remember providing consent for surgery (7).

Table 6: Summary of qualitative studies' quality based on CASP assessment

Study	Was there a clear statement of the aims of the study?	Is qualitative methodology appropriate?	Was the research design appropriate to address the aims of the research?	Was the recruitment strategy appropriate to the aims of the research?	Was the data collected in a way that addressed the research issue?	Has the relationship between researcher and participants been adequately considered?	Have ethical issues been taken into consideration?	Was the data analysis sufficiently rigorous?	Is there a clear statement of the findings?	Are the research findings valuable?
Agnew et al. (2012)	Y	Y	Y	Y	Y	C	Y	Y	Y	Y
Gabay et al. (2019)	Y	Y	Y	Y	Y	C	Y	Y	Y	Y

Key: Y=Yes, N=No, C=Can't tell

OBJECTIVE 3: COMMUNICATION INTERVENTIONS PUT IN PLACE TO AID PATIENTS IN COMPREHENDING SURGICAL TREATMENT

Different types of interventions to help patients' comprehension of various aspects of their surgical procedure were assessed in three of the studies (8,12,13). Participants often showed improvements in understanding of surgical procedure and its elements of risks, benefits, and alternatives (8,13). Lorenzen et al first reported that seventy five percent of the participants signed the consent form without reading (13). But with the introduction of newly developed surgical consent forms with patient-friendly language and use of teach back methods while communicating with patients increased reading of surgical consent forms by 52% and patient understanding of their procedure by 12%, respectively (13). In another study, use of multimedia resources such as a pamphlet and 3-dimensional model of the eye was used to assess effect on patient comprehension of cataract surgery (12). The use of this intervention increased scores in the post-informed consent quiz by a notable amount compared to control (12).

Discussion

DEGREE OF UNDERSTANDING AND RECALL AMONGST SURGICAL PATIENTS THROUGHOUT THE INFORMED CONSENT PROCESS

The degree of understanding and level of retention among surgical patients was suboptimal according to the selected literature (7–11,13,15). Studies varied in time of patient approach, some focused on preoperative recall and comprehension (7,8,11,13) while others examined these two points postoperatively (9,10,15). Despite the time of recall, patients still exhibited poor recall diminishing the possibility of the results being due to recall bias. The analysis of comprehension and recall is a constructive tool for examining the efficacy of the informed consent process, which in turn reflects the collaboration between the patient and their doctor (7). This concept is supported by other literature, Shah et al conclude that by declaring informed consent was obtained, it is presumed the physician assessed patient understanding (18). These findings are pertinent since informed consent is said to be carried out, but its purpose is often not attained. It is expected to be a practice that enables patients to have sufficient information to make competent decisions, yet its implementation is hindered (7).

OUTCOMES OF OBTAINING INADEQUATE INFORMED CONSENT FROM SURGICAL PATIENTS AND ASSISTANCE INTERVENTIONS

The signed consent form does not inevitably constitute informed consent (19). This seems to be the case in modern medicine, nevertheless, adequate acquisition of informed consent is of increasing importance and physicians are required to maintain this to meet legal and ethical expectations. Several themes emerged as the review progressed. Participants did not feel like they received sufficient detail about their procedure and the discussion was not suitable for their intentions, affecting quality of co-decision making (6,7,10,14,15). This highlights the necessity to determine factors that contribute to these results. Previous studies show considering a patient's level of education, time constraints, use of confusing language and medical jargon, and patients who may not speak English as a first language are factors that play a role in poor comprehension and therefore lead to inadequate patient consent (2,4). The use of websites and models were associated with improvements in patient comprehension during pre-operative consultations (12,13). These findings signify the need for interventions that will improve not just patient understanding but the delivery of information by physicians. Previous studies show that templates provided to surgeons may facilitate general discussion and can remind surgeons of key details, but discussion must still be individualized for each patient (19). Further research can be conducted to identify ways in which consent discussions can be modified to meet each patient's ideas, concerns and expectations while still providing generic information.

STRENGTHS AND LIMITATIONS OF STUDIES

Although only ten articles were selected, varying strengths of each study provided relevant contributions to the aims of this review. In one study, there was a range in age groups and educational levels of participants and results were similar between them (7). This takes in consideration the poor efficacy of the informed consent process while eliminating confounding factors. Another study was one of the first to run a randomized control trial to test effectiveness of online educational tools (8). This is contributory to modern medical practice as technology plays a big role in the delivery of healthcare.

Certain limitations were present in the studies, and they were highlighted by using EBL and CASP critical appraisal tools. Six of the ten selected studies had a sample size under 100. This can affect the validity of results. Future research can aim to conduct studies with more appropriate sample sizes. Another limitation was failure to account for confounding variables in four of the studies (8,9,11,12). This provided a low score for the results section in the EBL checklist. For example, patient factors such as vision or hearing difficulties may contribute to inaccurate completion of questionnaires. For the qualitative studies, it was not clear whether the researcher-participant relationship was considered, which is essential for minimizing bias in qualitative research (14,15).

STRENGTHS AND LIMITATIONS OF REVIEW

This review included both qualitative and quantitative studies. This is advantageous for analysing aspects of informed consent since it is tailored on generalized human rights but also patient specific goals. The qualitative studies provided patient own experiences by using narrative methods while the quantitative studies were key in identifying prevailing issues using numerical data. This review also provides perspective from eight different countries in five different continents. This addresses the efficacy of the process of informed consent in various hospital healthcare systems. Therefore, similar findings listed above can be beneficial internationally and not confined to specific locations.

This review also had some limitations. Firstly, studies selected participants from different surgical departments. This may influence the results since surgical procedures are simpler to comprehend than others. Although patient comprehension is a key measure of the efficacy of informed consent, it is difficult to achieve a standardized assessment of patient comprehension when the surgeries are of different complexities. Therefore, further reviews could focus on comparing the efficacy of the consent process within similar surgical departments to determine consistency of results. This may propose research in areas of developing specific interventions that will be effective with helping patients undergoing different surgeries understand the aspects of informed consent. Secondly, only ten articles were chosen, and they were in English and chosen if available from the University College Cork library which could have modified results. Finally, this review was done by one researcher leading to a reduction in quality and limited interpretative viewpoint.

CONCLUSION

Existing literature claims that some aspects of collaborative decision making during the informed consent-obtaining process are present, but patient-doctor communication appears to be suboptimal according to the level of understanding in patients and poor outcomes of the informed consent process. However, interventions established to support this process are proven to be of high effectiveness. This literature review highlighted the necessity for improvements in surgical consultations to facilitate informed consent. More research on the quality of informed consent within other medical specialties and doctor and patient factors that affect efficacy of this process is recommended. The paramount goal of research in this area is enhancing patient-centred care in practice.

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Appendix

Appendix A: EBL Critical Appraisal Checklist

EBL Critical Appraisal Checklist		Vucemilo et al. (2013)	Ochieng et al. (2015)	Fraval et al. (2015)	Zarnegar et al. (2015)
Section A: Population	Is the study population representative of all users, actual and eligible, who might be included in the study?	Y	Y	Y	Y
	Are inclusion and exclusion criteria definitively outlined?	Y	N	Y	Y
	Is the sample size large enough for sufficiently precise estimates?	Y	Y	Y	N
	Is the response rate large enough for sufficiently precise estimates?	Y	Y	Y	Y
	Is the choice of population bias-free?	Y	N	Y	Y
	If a comparative study: Were participants randomized into groups? Were the groups comparable at baseline? If groups were not comparable at baseline, was incomparability addressed by the authors in the analysis?	N/A	N/A	 Y Y N/A	N/A
	Was informed consent obtained?	Y	Y	Y	Y
Section B: Data Collection	Are data collection methods clearly described?	Y	Y	Y	Y
	If a face-to-face survey, were inter-observer and intra-observer bias reduced?	N/A	N/A	N/A	N/A
	Is the data collection instrument validated?	Y	Y	Y	U
	If based on regularly collected statistics, are the statistics free from subjectivity?	U	Y	N/A	N/A
	Does the study measure the outcome at a time appropriate for capturing the intervention's effect?	Y	Y	Y	Y
	Is the instrument included in the publication?	Y	Y	Y	Y
	Are questions posed clearly enough to be able to elicit precise answers?	Y	Y	Y	Y
	Were those involved in data collection not involved in delivering a service to the target population?	Y	Y	Y	Y
Section C: Study Design	Is the study type / methodology utilized appropriate?	Y	Y	Y	Y
	Is there face validity?	Y	U	U	Y
	Is the research methodology clearly stated at a level of detail that would allow its replication?	Y	Y	Y	Y
	Was ethics approval obtained?	Y	Y	Y	Y
	Are the outcomes clearly stated and discussed in relation to the data collection?	Y	Y	Y	Y
Section D: Results	Are all the results clearly outlined?	Y	Y	Y	Y
	Are confounding variables accounted for?	Y	Y	N	U
	Do the conclusions accurately reflect the analysis?	Y	Y	Y	Y
	Is subset analysis a minor, rather than a major, focus of the article?	Y	Y	U	Y
	Are suggestions provided for further areas to research?	Y	Y	Y	Y
	Is there external validity?	Y	Y	U	Y

Key: Y= Yes, N=No, U=Unclear, N/A=Not applicable

Appendix

Appendix B: EBL Critical Appraisal Checklist

EBL Critical Appraisal Checklist		Howlader et al. (2004)	Feiner et al. (2016)	Karan et al. (2014)	Lorenzen et al. (2008)
Section A: Population	Is the study population representative of all users, actual and eligible, who might be included in the study?	Y	Y	Y	Y
	Are inclusion and exclusion criteria definitively outlined?	Y	N	Y	N
	Is the sample size large enough for sufficiently precise estimates?	Y	N	N	N
	Is the response rate large enough for sufficiently precise estimates?	Y	Y	Y	Y
	Is the choice of population bias-free?	Y	Y	Y	Y
	If a comparative study: Were participants randomized into groups? Were the groups comparable at baseline? If groups were not comparable at baseline, was incomparability addressed by the authors in the analysis?	N/A	N/A	 N Y N/A	N/A
	Was informed consent obtained?	Y	Y	Y	Y
Section B: Data Collection	Are data collection methods clearly described?	Y	Y	Y	N
	If a face-to-face survey, were inter-observer and intra-observer bias reduced?	N/A	N/A	N/A	N/A
	Is the data collection instrument validated?	N	U	U	N
	If based on regularly collected statistics, are the statistics free from subjectivity?	N/A	N/A	N/A	N/A
	Does the study measure the outcome at a time appropriate for capturing the intervention's effect?	Y	Y	Y	Y
	Is the instrument included in the publication?	N	U	U	U
	Are questions posed clearly enough to be able to elicit precise answers?	Y	Y	Y	Y
	Were those involved in data collection not involved in delivering a service to the target population?	Y	Y	Y	Y
Section C: Study Design	Is the study type / methodology utilized appropriate?	Y	Y	Y	Y
	Is there face validity?	U	U	U	Y
	Is the research methodology clearly stated at a level of detail that would allow its replication?	Y	Y	Y	N
	Was ethics approval obtained?	Y	Y	Y	Y
	Are the outcomes clearly stated and discussed in relation to the data collection?	Y	Y	Y	Y
Section D: Results	Are all the results clearly outlined?	Y	Y	Y	Y
	Are confounding variables accounted for?	Y	N	N	Y
	Do the conclusions accurately reflect the analysis?	Y	Y	Y	Y
	Is subset analysis a minor, rather than a major, focus of the article?	Y	Y	Y	U
	Are suggestions provided for further areas to research?	Y	Y	Y	Y
	Is there external validity?	Y	Y	Y	Y

Key: Y= Yes, N=No, U=Unclear, N/A=Not applicable

Reviewing the effect of creative arts therapy on the development of cognitive and social skills in children and adolescents with autism spectrum disorder

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Abstract

Creative art therapy has been utilized for many years as a treatment regimen for certain mental disorders. Overall, results of studies on creative art therapy show a positive trend, with general improvements in mental health and reduction of neurological symptoms in the majority of cases. The results regarding the effectiveness of creative art therapies in treating autism spectrum disorder are unclear and varied, due to the range of symptoms involved in autism spectrum disorder, the effect of age on therapy, and the variety of creative art therapies available for use. Of all the creative art therapies chosen to review, music was the most investigated. The studies performed on music therapy showed positive results, however, a large-scale randomized controlled trial refuted the positive benefits, leading to an inconclusive overall result on the effectiveness of music therapy. Research on the benefits of creative art therapies for children with autism spectrum disorder is inconclusive. Studies generally demonstrate a positive effect on many different types of creative art therapies. However, these studies have major limitations in generalizability due to small sample sizes and their descriptive nature. Future research should focus on developing randomized controlled trial protocols that can be used on a larger scale to generate more definitive results on the benefits, or lack thereof, of creative art therapies. Research should also be performed on the effect of adolescence on creative art therapies, and whether targeting it at younger children with autism spectrum disorder would be more or less beneficial than targeting it towards adolescents.

Introduction

Creative art therapy (CAT) has been utilized for many years as a treatment regimen for certain neurological and mental health disorders, becoming more of an attractive choice in recent years in order to avoid the unwanted adverse effects of pharmacological agents (1). CAT also comes in many forms, as it can be based around a multitude of art styles, including visual arts, dance, theatre, music, or, more recently, filmography (1). Various types of studies of the effects of CAT have been conducted on numerous conditions, including bipolar disorder, schizophrenia, depression, and post-traumatic stress disorder (PTSD), focusing mainly on disease-specific symptoms, as well as improving mental health, the satisfaction of care, and overall wellbeing of the patients (1,2,3). However, the effects of CAT on autism spectrum disorder (ASD) require more attention, and its effects are particularly important as there is a very limited pharmacological treatment for the condition and a rate of occurrence of approximately 1 in 100 children (4). The pathophysiology of ASD is yet to be fully understood, regardless of its high prevalence, due to the complex neurophysiology involved and the lack of suitable animal models, however current research suggests that both genetic and environmental factors play a role in the occurrence of the condition (4). The signs and symptoms of ASD vary amongst age groups, however, the symptoms that usually begin in childhood, include delayed and stunted development of social communication and interaction skills, therefore improvement of these skills through CAT may be beneficial for the symptomatic treatment of ASD (4).

Overall, results for the majority of cases tend towards a positive effect of CAT, with improvements in general mental health and a reduction in disease-specific symptoms in a multitude of conditions (1). The mechanisms with which CAT improves these symptoms are unclear. However, suggested hypotheses include the influence of an environment that fosters connection and communication between individuals and encourages creativity and the development of talents. Ultimately, these environmental conditions lead to increased self-esteem, cognitive development, mood, and social functioning (5). CAT has also been shown to foster trust between the individual and the healthcare team in the in-patient setting, providing a better environment for patient care (1). This category of therapy would have the potential to be useful for treating ASD, which typically presents in patients with social interaction difficulties, as well as the tendency to restrict their behaviours and difficulty in dealing with emotional stressors (4).

The results regarding the effectiveness of CAT on numerous disorders are contradictory and diverse (6), which may be due to the range of variability present in psychiatric disorders, along with the scope of options available for the types of art used in therapy (7). Furthermore, the age of the individual undertaking CAT may have an effect on its treatment capabilities, as it may be more critical to develop the connections and enhance the brain plasticity, seen in the body and mind approach, at a younger age (8). A study performed in 2019 demonstrated that adolescents with mental health disorders undergoing drama therapy influenced the adaptation of the individuals to peer groups and enhanced the formation of normal behavioural patterns (9). Overall, although the effects of CAT have been widely studied, the effects on the paediatric population with ASD are less elucidated, and in particular, it has not been determined which types of CAT provide the greatest benefit to this population.

The aim of this literature review is to investigate the effect of different types of CAT on the social and cognitive outcomes of paediatric individuals with ASD. The objectives of this review include examining previously published research studies in order to

1. Investigate the effects of music therapy on the cognitive and social outcomes of children with ASD.
2. Investigate the effects of visual art therapy on the cognitive and social outcomes of children with ASD.
3. Investigate the effects of novel creative art therapy mechanisms (dance therapy, theatre therapy, and film therapy) on the cognitive and social outcomes of children with ASD.

Methodology

SEARCH STRATEGY

An electronic search was performed using two databases, Academic Search Complete and MEDLINE through EBSCOhost to identify literature relevant to the aims and objectives of this review.

The following search terms were used for Academic Search Complete and MEDLINE:

art therapy [All Text] OR art psychotherapy [All Text] OR creative arts therapies [All Text]
AND
children [All Text] OR adolescents [All Text] OR youth [All Text] OR child [All Text] OR teenager [Text]
AND
autism [All Text] OR asd [All Text] OR autism spectrum disorder [All Text]

The following filters were applied to Academic Search Complete and MEDLINE:

Table 1: List of filters applied to Academic Search Complete and MEDLINE

No.	Filter	Specifications
1	Limiters	Scholarly (Peer-Reviewed) Journals
2	Date of Publication	2011-2021
3	Source Types	Academic Journals
4	Language	English

INCLUSION AND EXCLUSION CRITERIA

Articles selected had to be available in full-text in order to properly select and critically appraise appropriate articles, and the articles had to be either written or translated in English for personal understanding of the contents of the article. Location was not limited to Ireland as the searches from these two databases yielded no articles of a relevant nature from Ireland. Articles that were published before 2011 were excluded in order to ensure the most relevant research was included. Studies performed on animals were excluded, and human subjects included in the study must be between the ages of 3 and 18 years old in order to exclude studies investigating the effects of CAT on neonates, infants, and adults. The remaining inclusion and exclusion criteria were selected in order to focus the review on the specific topic of the effects of CAT on the cognitive, verbal and social development of children with ASD. All types of primary research, both observational and interventional studies, were utilized in order to visualize all ranges of responses to CAT and reduce bias.

Table 2: Inclusion and exclusion criteria for studies selected for analysis in this review

Inclusion Criteria	Exclusion Criteria
Articles available with full text online or are available through UCC online library loans	Articles without full-text availability
Articles available in English	Studies auditing specific CAT programs
Articles published between 2011 and 2021	Studies looking at non-cognitive and non-social developmental milestones of children with ASD utilizing CAT
Studies performed on humans	Studies that examine children with multiple diagnosed mental and cognitive conditions
Studies performed on children aged between 3 and 18 years old	Studies that are review articles or meta-analyses
Studies that determine the cognitive outcomes of CAT on children with ASD	Studies that investigate the outcomes of CAT on the parent's care of the child
Studies that determine the social outcomes of CAT on children with ASD	Studies that investigate the outcomes of CAT on the artist
Studies that determine the verbal outcomes of CAT on children with ASD	Studies that investigate the outcomes of CAT on the healthcare team
Studies that are performing primary research	Studies that aim to propose a standard type of CAT for the treatment of ASD

SELECTION CRITERIA

The initial search on Academic Search Complete yielded 1,107 results, and was reduced to 669 results after the filters were applied. On MEDLINE, the primary search yielded 85 results, and was then decreased to 75 results after the application of filters. The initial search was left with a large number of results, as after initial screening, many entries were not relevant to the objectives of this review. After the removal of the duplicate entries, 611 articles remained. Of the 611 abstracts screened, 572 did not fit the inclusion criteria. The remaining 39 articles were then read in full to determine their eligibility within the exclusion and inclusion criteria. 10 final articles were selected for the review.

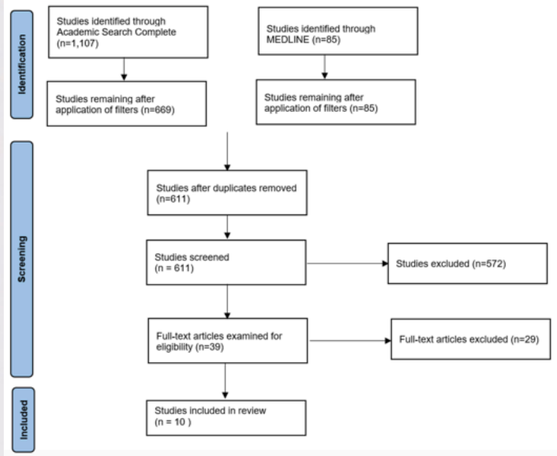

Figure 1: Search strategy for selecting relevant articles for inclusion in the literature review

CRITICAL APPRAISAL

Due to the variety of study designs examined, 3 different checklists were utilized in order to critically evaluate the studies included in the review. The EBL checklist was utilized for quantitative studies, the CASP checklist was used for qualitative studies, and the mixed methods appraisal tool (MMAT) was used for mixed-methods studies. The results from these checklists are summarized in Table 4 (EBL), Table 5 (CASP), and Table 6 (MMAT). More detailed results are included in Appendix A (EBL).

Table 3: Reasons for exclusion of research articles throughout the screening process

Reason for exclusion	Number
Articles without full-text availability	5
Studies auditing specific CAT programs	89
Studies looking at non-cognitive and non-social developmental milestones of children with ASD utilizing CAT	201
Studies that examine children with multiple diagnosed mental and cognitive conditions	52
Studies that are review articles or meta-analyses	137
Studies that investigate the outcomes of CAT on the parent's care of the child	4
Studies that investigate the outcomes of CAT on the healthcare team	16
Studies including individuals below 3 years of age and above 18 years of age	23
Studies performed on animals	73
Studies that aim to propose a specific type of CAT for the treatment of ASD	1
Total	601

Results

OBJECTIVE 1: INVESTIGATE THE EFFECTS OF MUSIC THERAPY ON THE COGNITIVE AND SOCIAL OUTCOMES OF CHILDREN WITH ASD

Of the 10 studies that fit the criteria for this review, five were focused on music therapy (MT) (10, 12, 13, 15, 19). Music therapy encompasses more than just listening to music, and involves the creation of music, singing, discussing the lyrics and playing an instrument (10).

Overall the majority of studies that focused on MT found that it led to positive outcomes for children with ASD (10, 12, 15, 19). A case series of 4 children demonstrated that MT increased the quantity of overall vocal communication in all participants as therapy progressed, however, this growth was not in a linear pattern and did fluctuate throughout sessions (12). In further detail, vocal communication became focused on creating interpersonal relationships, and vocalization of other topics that were not aimed at connecting with others decreased over time (12). A more recent case series performed on 10 children observed that MT aided with

Table 4: Summary of articles

Author, year, location, title	Objectives	Study design, population, sample size	Study methodology	Key findings	Strengths and limitations
Rabeyron T *et al* (2020) France 'cars" (10)	To determine if music therapy (MT) is more effective than music listening (ML) on the outcomes of children with ASD	Single-blind randomized controlled trial (RCT) Children with ASD, ages 4-7 from Nantes, recruited from 5 psychiatric facilities, 31 boys, 5 girls n=36 (17 in ML, 19 in MT)	25 sessions of designated intervention over a period of 12 months Patients were randomly assigned to one of the two groups Outcome measures were Clinical Global Impression (CGI), Childhood Autism Rating Scale (CARS) and the Aberrant Behaviour Checklist (ABC)	1. Childhood Autism Rating Scale (CARS) scores decreased for both MT (39.3 to 35.9) and ML (36.4 to 33.8) groups with no significant difference between the two (p = 0.92) 2. Clinical Global Impression (CGI) scores decreased more for participants in MT (5.1 to 2.8) than in ML (4.6 to 3.4) [Odds Ratio (OR)=0.44, 95% Confidence Interval (CI) = 0.20-0.93] 3. Increased improvement of symptoms of ASD based on Aberrant Behaviour Checklist (ABC) subscales for MT group (58.7 to 48.6) than for ML group (53.1 to 49.8) [ABC scores were higher for MT group than ML group to begin with] (Mean difference (MD) 12.48, CI [0.35 -24.68], and a difference was no longer observed post treatment (MD 6.83, 95% CI [-5.81-19.86])	**Strengths:** Detailed analysis, use of control group of ML for comparison from baseline, controlled for all foreseeable variables **Limitations:** Did not utilize the gold standard instrument for assessing ASD, no follow-up past the endpoint of treatment, bias of therapists involved in the ML group, not fully replicable due to the nature of MT, wide confidence intervals for ABC subscales
Parvathi G (2020) India "Arts based therapeutic intervention on an adolescent living in autism spectrum" (11)	Explore the value of CAT on the development and growth of a boy with autism Discuss the importance of arts-based therapy approaches and techniques as a therapeutic intervention Discuss how children diagnosed with autism will benefit from arts-based therapy	Case Report A 17-year-old male with ASD n=1	10 months worth of art therapy sessions, ranging from body movement, drawing, clay, and theatre	Arts-based therapy caused a gradual progression in the boy's behaviour, where he was able to develop object constancy, build relationships with others, and develop language skills.	**Strengths:** Detailed description of the art therapy sessions, and what was being taught in the session A detailed description of the boy's outcomes **Limitations**: Due to the nature of a case report, it is not able to be deciphered whether these observations are an anomaly, or how the development would compare to a child with autism undergoing typical therapy and treatment
Salomon-Gimmon M and Elefant C. (2019) Israel "Development of vocal communication in children with autism spectrum disorder during improvisational music therapy" (12)	Examine the development of vocal communication in children with ASD during music therapy Investigate trends and patterns of vocal development during music therapy	Case Series n=4 4 children (aged 4-5, diagnosed with ASD) were chosen from a previously run RCT (2 children had music therapy twice a week, 2 children had music therapy 3 times a week 3 children out of the 4 were non-verbal	Analysis of videos from children in a prior RCT who received MT sessions, 30 minutes each over a course of 5 months One session per month was analyzed, therefore a total of 20 sessions Vocal communication was encoded into categories from the VQR scale (vocal pre-speech quality of relationship) and frequency of speech in these categories was noted.	1. Vocal communication of children with ASD developed in a non-linear pattern in music therapy 2. A downward trend was observed in the development of vocal communication if it was not directed at creating contact with others, and an upward trend in vocal communication when creating interpersonal relationships 3. An upward trend in the quantity of communication overall	**Strengths**: Therapy only performed by two therapists reduced variability, in-depth analysis of particular issues **Limitations:** Small sample size, videos were only analyzed by one researcher therefore the analysis could be biased
Bieleninik L *et al.* (2017) Australia, Austria, Brazil, Israel, Italy, Korea, Norway, United Kingdom, United States "Effects of improvisational music therapy vs enhanced standard care on symptom severity among children with autism spectrum disorder: the TIME-A randomized clinical trial" (13)	Evaluating the effects of improvisational music therapy on the development of social skills and communication of children with ASD	Assessor-blinded, RCT n=364, (182 in enhanced standard care (ESC), 92 in ESC plus low-intensity improvisational music therapy (IMT), 90 in ESC plus high-intensity IMT) Children aged 4-7 with ASD from 9 countries (Australia, Austria, Brazil, Israel, Italy, Korea, Norway, United Kingdom, United States)	Children were recruited from 2011-2015, follow up between 2012 and 2016. Autism severity was measured using the Autism Diagnostic Observation Schedule (ADOS) and Social Responsiveness Scale (SRS). Secondary outcomes were related to parents' visualization of changes in social skills of the children Outcomes were assessed at 2 months of therapy, at the end of therapy intervention (5 months) and 7 months post-therapy	1. Both groups of children undergoing IMT and the ESC group had reductions in the ADOS score, however, the difference in reduction was non-significant (MD 0.06, 95% CI[-0.70 to 0.81) p=0.88). 2. Of the 20 secondary outcomes in the SRS assessed by parents, 17 showed no significant differences between treatment groups (high-intensity IMT was associated with greater improvements in social motivation and autistic mannerisms, low-intensity IMT was associated with a greater improvement in social awareness)	**Strengths:** Large scale study in numbers and duration, a detailed protocol, narrow confidence intervals within results **Limitations**: Potential variable with cohort (83% boys, 17% girls), only 19 IMT sessions in addition to ESC, and more therapy in total for IMT group vs ESC group, the study was terminated early due to lack of funding, length of intervention may have been too small to see significant effects, arguments exist about whether symptom severity is less important than the well-being of the individual
Schweizer C *et al.* (2020) The Netherlands "Evaluation of 'Images of Self', an art therapy program for children diagnosed with autism spectrum disorders (ASD)" (14)	To evaluate the outcome of art therapy on children with ASD, including the sense of self, emotional regulation, flexibility, and social behaviour	Mixed-Methods n=12 Children aged 6-12 with ASD undergoing 15 weekly, individual art therapy sessions of 45 min duration	The improvement of the children was measured using the Behaviour Rating Inventory of Executive Functioning (BRIEF) and Children's Social Behaviour Questionnaire (CSBQ) instruments for measuring executive functioning and social behaviour respectively Measurements occurred before therapy, during the 3rd, 8th and 15th sessions, and 15 weeks post-treatment. Parents filled out qualitative surveys Therapists recorded three sessions and evaluated the recordings with parents and the researcher	1. 7 children strongly improved on the BRIEF and CSBQ measurements post-treatment, indicating improved social behaviour 2. All 12 children remained in the stable zone of the BRIEF and CSBQ measurements, indicating that none were negatively affected by the therapy	**Strengths:** Multiple perspectives are gathered using the mixed-methods approach, took baseline measurements before beginning treatment **Limitations:** Small sample size limits the ability to generalize the results, only utilizing children that do not show resistance to art therapy may bias the results of the study

Table 4: Summary of articles (continued)

Author, year, location, title	Objectives	Study design, population, sample size	Study methodology	Key findings	Strengths and limitations
Rickson D (2021) New Zealand "Family members and other experts' perceptions of music therapy with children on the autism spectrum in New Zealand: Findings from multiple case studies" (15)	To observe the perceived outcomes of MT on the emotion, cognition, and sensory regulation, as well as communication and relationships of children with ASD	Case Series n=10 Children with ASD aged 5 - 10 (7 boys and 3 girls) with no previous MT receiving up to 40 sessions	2-4 Family members and other experts with close connections to the child involved examined clinical materials from therapy and gave written feedback Six other experts who did not know the child also reviewed the material	1. MT allowed children to express emotions appropriately, involving increased expression of positive emotions, and increased regulation of negative responses, leading to decreased behavioural disturbances 2. MT supported the learning of new methods of communication in the cohort and the development of interpersonal interactions 3. MT aided in developing trusting and secure relationships between children with ASD and other individuals	**Strengths:** Variety of perspectives provided by both people who know and are strangers to the child, wide range of people involved throughout New Zealand **Limitations:** No perspectives from the children on their own experiences, limited detail in the manuscript
Saladino V *et al.* (2020) Italy "Filmmaking and video as therapeutic tools: case studies on autism spectrum disorder" (16)	To evaluate the behavioural and psychosocial outcomes of adolescents with ASD undergoing film therapy (FT)	Case Series n=4 Italian adolescents with ASD, aged 10-13 years old, 2 females and 2 males	Interview conducted prior to the start of the study, one with the parents and one with the child Six sessions of FT followed, where the four individuals worked in pairs to create short films Two sessions of cinematherapy, where the individuals watched and discussed the film with their peers and family Two follow-up sessions, one month later and three months later, with interviews conducted in the same fashion as prior to the start of the study	Participants improved their social skills throughout the study Mutual interaction, social-emotional reciprocity and play and behaviour regulation increased post-study Relationships amongst adolescents with ASD and between adolescents with ASD and neurotypical individuals (in this case, the researchers) increased	**Strengths:** Detailed description of results of each individual **Limitations**: Cannot generalize results due to the nature of the study, no overarching summary of the results post-study
Corbett BA *et al.* (2016) United States "Improvement in social competence using a randomized trial of a theatre intervention for children with autism spectrum disorder" (17)	To evaluate the impact of a theatre-based art therapy intervention on the social outcomes of children with ASD	RCT n=30 Children with ASD aged 8-14, randomly assigned to treatment (n=17) or wait-list control group (n=13)	Theatre intervention delivered over 10 4-h sessions. Theatre sessions included working towards creating a theatre performance, as well as peer-modelling practices and video training sessions Social functioning was measured using the SRS and Adaptive Behaviour Assessment System (ABAS), social interaction was measured using the Peer Interaction Paradigm (PIP), and social cognition was measured with Memories of Faces Delayed (MFD) and Theory of Mind (TOM) tools. Electroencephalograms (EEGs) were also performed.	Improvements were seen post-treatment in social ability (d=0.77) and communication symptoms, (d=0.86) and communication symptoms continued to improve after 2 months of follow-up (d=0.82). Theatre interventions result in improvement in social cognition compared to wait-list control groups (p=0.02). The Theatre intervention group had a greater time effect on changes in social cognition, brain amplitude differences, and social functioning (ABAS, p=0.04, TOM, p=0.02, EEG readings brain amplitude differences, p=0.016).	**Strengths**: control group for comparison was included, randomization between the groups reduced bias, groups were comparable at baseline, multiple measures were used, and effect sizes were large **Limitations:** Parents were aware of which treatment group their child was in, therefore leading to potential bias in parent-led measurements, the follow-up did not go farther than 2 months, coordinating schedules of all participating parties may make this treatment method difficult to employ
Vaisvaser S. (2019) Israel "Moving along and beyond the spectrum: creative group therapy for children with autism" (18)	To investigate the effect of creative group therapy on children with ASD	Case Report n=3 Boys with ASD, aged 4.5-6 years old	Group met in 45 min sessions, once weekly for 30 weeks Four phases: 1: opening song, 2: drawing on paper, 3: free movement with stretch bands, 4: closing song. Groups were co-led by an occupational therapist and a dance therapist	Members of the group were able to develop social engagement through creative play, and discovered motivation to connect and be seen by their peers	**Strengths:** Very detailed observation of the group process and development of social cognition **Limitations**: Cannot generalize results due to the nature of the study, no clear statement of aims or objective
Sharda *et al.* (2018) Canada "Music improves social communication and auditory-motor connectivity in children with autism" (19)	Evaluate the effect of a MT intervention on social communication in school-age children with ASD	Assessor-blinded, RCT n=51 Children aged 6-12 years with autism, randomized into two groups (n=26 in intervention, n=25 in non-music intervention)	8-12 weeks of 45-minute music intervention or non-music intervention Music intervention involved use of improvisation in song and rhythm in order to target communication between peers Non-music intervention was structurally matched therapy but did not include musical elements Assessment of social communication and the functional connections between frontal and temporal lobes was performed pre and post intervention Assessment was performed through the SRS, the Children's communication checklist (CCC) and resting-state functional magnetic resonance imaging (rs fMRIs) for functional connectivity	1. Communication scores were higher in the music group than in the non-music group after intervention [CCC MD: 4.84 95%CI(0.76-8.92), p=0.01] 2. Activity between the auditory and subcortical frontal regions in a resting state was higher post-music intervention than post-non-music intervention, along with lower brain connectivity between the auditory and visual regions for the music intervention compared to the non-music intervention 3. Covariate analysis showed that the change in communication scores was related to the increased activity between the auditory and subcortical frontal regions on rs fMRI ($z=3.57$, $p < 0.0001$), and lower brain connectivity between the auditory and visual regions ($z=3.64$, $p < 0.001$)	**Strengths:** Detailed study, the groups matched at baseline, studied functional effects of music on the brain, expressed calculations performed in order to ensure a proper sample size **Limitations:** Excluded older children from the study, could potentially have different effects in adolescents, particularly in the rsfMRI outcomes

emotional regulation in the children, as well as improved communication skills and development of interpersonal relationships (15). Overall, all studies agreed upon an improvement in the development of the children's communication skills post-MT (12, 15, 19).

Functional magnetic resonance imaging (fMRI) images performed on children (n=26) undergoing music therapy showed increased connectivity between the auditory and frontal subcortical centres compared to a non-intervention group (n=25) (19), as well as increased communication in the Children's Communication Checklist (CCC) (19). Current theories about the impaired development of communication skills in children with ASD point towards alterations and miscommunications between the sensorimotor and cognitive functions in the brain itself, therefore growth in the connections between these two centres may lead credence to this theory in the pathophysiology of communication skills in children with ASD (19). A more recent randomized-controlled trial (RCT), using the Clinical Global Impression (CGI) and the Aberrant Behaviour Checklists (ABC), showed increased communication, decreased behavioural upsets, and decreased severity of symptoms in children with ASD who were in the MT groups (n=19) compared to children undergoing simple music listening (n=17) (10).

However, one large RCT (n=364) found that MT in extension to enhanced standard care (ESC), did not provide any additional benefit to the children compared to those receiving ESC alone, as children in both groups received a reduction in their Autism Diagnostic Observation Scale (ADOS) and Social Responsiveness Scale (SRS) scores (13). However, there was a significantly higher result observed in the MT group compared to the ESC group in the subcategories of social motivation, social awareness and autistic mannerisms based on SRS scores (13).

OBJECTIVE 2: INVESTIGATE THE EFFECTS OF VISUAL ART THERAPY ON THE COGNITIVE AND SOCIAL OUTCOMES OF CHILDREN WITH ASD.

Of the 10 studies that fit the criteria for this review, three studies focused on the outcomes of visual art therapy (VAT) on children with ASD (11, 14, 18). Prior studies have shown that visual art therapy tends to increase the connectivity between the amygdala and the prefrontal cortex, which works to decrease anxiety, whereas music therapy stimulates the excess release of dopamine, which may also work to decrease anxious emotions, as well as depression, therefore there may be differences in the effects of both types of therapy (20).

A case study on a boy undergoing VAT reported that it was able to help him develop object constancy, build relationships with others, and improve language skills (11). A case report on three boys also reported an increase in social engagement between the boys after VAT, as well as expanded motivation to seek out connections with their peers (18). Another mixed-methods study on 12 children reported that the majority of children that underwent VAT improved on both the Behaviour Rating Inventory of Executive Functioning (BRIEF) and Children's Social Behaviour Questionnaire (CSBQ) measurement systems, and none of the children was negatively affected by the treatment (14).

OBJECTIVE 3: INVESTIGATE THE EFFECTS OF NOVEL CREATIVE ART THERAPY MECHANISMS (DANCE THERAPY, THEATRE THERAPY AND FILM THERAPY) ON THE COGNITIVE AND SOCIAL OUTCOMES OF CHILDREN WITH ASD.

Of the 10 studies that fit the criteria for review, one focused on theatre therapy (TT) (17), and another focused on film therapy (FT) (16). Dance therapy was incorporated into two case studies focusing on art CAT (11, 18), the results of which are described above in the section referring to Theme 2.

The RCT showed that compared to the control group (n=13), a theatre intervention (n=17) observed an increase in social ability (d=0.07), social cognition (p=0.02) and improvement in communication symptoms (d=0.86) as measured by the SRS, Adaptive Behaviour Assessment System (ABAS), Peer Interaction Paradigm (PIP), Memories of Faces Delayed (MFD), and Theory of Mind (TOM) tools, as well as electroencephalogram (EEG) measurements (17). The social cognition further continued to improve at 2 months follow-up (d=0.82) (17).

A case series was performed in 2020 to analyse the effect of FT on children with ASD, and after completion of the sessions it was found that the social skills of the children had improved, not just amongst other children with ASD, but also with neurotypical adults (16). It was also reported by the parents and researchers during follow-up with the children, that mutual interaction, social-emotional reciprocity and play and behaviour regulation of the children had continued to improve post study (16).

Discussion

The aim of this review was to investigate the effect of different types of CAT on the social and cognitive outcomes of paediatric individuals with ASD.

INVESTIGATE THE EFFECTS OF MUSIC THERAPY ON THE COGNITIVE AND SOCIAL OUTCOMES OF CHILDREN WITH ASD.

The majority of the research performed on CAT for children with ASD has focused on MT, with five out of the 10 studies relevant to this review specifically addressing its effects. Furthermore, four out of the five studies provided evidence that MT did supply a benefit to the social and cognitive outcomes of children with ASD (10, 12, 15, 19). However, one study had a different finding and showed that although MT was not negatively affecting the children, it didn't provide any additional benefit when combined with enhanced standard therapy which is already recommended for children with ASD (13). In particular, there were only minor differences in social motivation and awareness, as well as autistic mannerisms being reported post-MT completion (13).

The study refuting the benefits of music therapy does have the most strength, due to the number of participants, and the variety of countries that participated in the study, reducing the bias that may occur in geographic specific methods of how music therapy is performed (13). The other studies, although beneficial for the purpose they served and still critically of value, were much smaller scale, and therefore less generalizable to the population as a whole (10, 12, 15, 19). The RCT performed by Rabeyron et al., in particular, also did not receive a favourable "Population" score with the EBL checklist, due to the lack of comparison at the baseline of the two groups and a deficiency of clarity around the selection methods used for the participants (10).

Therefore, based on the findings, it may not be beneficial to implement MT as a therapy, due to the evidence of a potential lack of therapeutic benefit. Furthermore, studies show an increased economic burden on the healthcare system towards therapies for ASD, therefore it may not be financially beneficial to implement widespread MT, as studies show that behavioural therapies tend to cost significantly more than pharmacological therapies for the average ASD patient (21, 22).

INVESTIGATE THE EFFECTS OF VISUAL ART THERAPY ON THE COGNITIVE AND SOCIAL OUTCOMES OF CHILDREN WITH ASD.

VAT has been less researched than MT, with only 3 studies total discussing the topic (11, 14, 18). In addition, the two case studies (11, 18), were also confounded by including dance therapy in the treatment regimens being investigated. Therefore, it is difficult to investigate whether the benefits observed were due to the VAT, dance therapy, or the combination. However, the mixed-methods study (14) which did not include dance therapy concluded that there was a benefit for the use of VAT in the treatment of ASD. Although, the study is limited in its generalizability due to small sample size and selection bias towards children who did not show resistance to VAT (14).

Therefore, further investigations need to be performed to understand the benefits of VAT, ideally with larger sample sizes and more RCT's rather than observational studies.

INVESTIGATE THE EFFECTS OF NOVEL CREATIVE ART THERAPY MECHANISMS (DANCE THERAPY, THEATRE THERAPY AND FILM THERAPY) ON THE COGNITIVE AND SOCIAL OUTCOMES OF CHILDREN WITH ASD.

TT and FT are relatively new methods of CAT, and therefore only one study per therapy fits the criteria for review (23).

Both the studies on TT and FT showed improvements on the cognitive and social skills in children with ASD (16,17), however both studies have limitations. The study by Corbett et al. (17) on TT had several strengths in its protocol including a large sample size, randomization of subjects, and diversity of statistical analyses. However, it was noted that some parents during this study managed to discover which cohort their child was allocated. As the parents were responsible for the measurements, this knowledge may have introduced the potential for bias. Furthermore, the therapeutic potential of this therapy may be limited by its feasibility to be organized and scheduled according to all participants' schedules and needs. Similarly, although the Saladino et al. (16) study on FT shows promising results for participants, the sample size is too small to generalize. Therefore, there is a need for additional investigations in this field with larger sample

There are several limitations to this review. Firstly, the word count restrictions limited the amount of detail that could be included in each investigation. Similarly, only studies written or translated into English could be included, limiting the findings that could be found in other countries. There were also no investigations into the impact of age on the study findings, which is particularly important due to the knowledge that the transition from childhood into adolescence impacts the effects of therapy due to the increased challenges faced by individuals in this period (24). Furthermore, all studies used a variety of different measurement tools, leading to difficulties comparing results directly between the studies. Finally, selection bias may have been introduced as a single researcher completed this review.

Conclusion

In conclusion, evidence of the benefits of CAT for children with ASD is inconclusive, however even the small benefits may help children with ASD to avoid a multi-drug pharmacotherapy plan, particularly during a vulnerable period of brain development as children age. Studies generally demonstrate a positive effect for many different types of CAT, including MT, VAT, TT, and FT, however, these studies have major limitations in generalizability due to small sample sizes and their descriptive nature. Negative effects were not demonstrated, however, null effects were seen in some studies. Future research should focus on developing RCT protocols that can be used on a larger scale to generate more definitive results on the benefits, or lack thereof, of CAT. Research should also be performed to determine if CAT is more beneficial when used for younger children with ASD compared to adolescents. Furthermore, ensuing research should delve into other variables of creative arts therapy, such as the environmental setting, whether the therapy occurs virtually or in-person, and the number of instructors present, as these factors may play a role in the efficacy of creative arts therapy in the symptomatic treatment of autism. Depending on future results, it may still be worth suggesting such therapies as a treatment method for children with ASD in order to ensure safe, enjoyable and accessible therapies for the developing youth.

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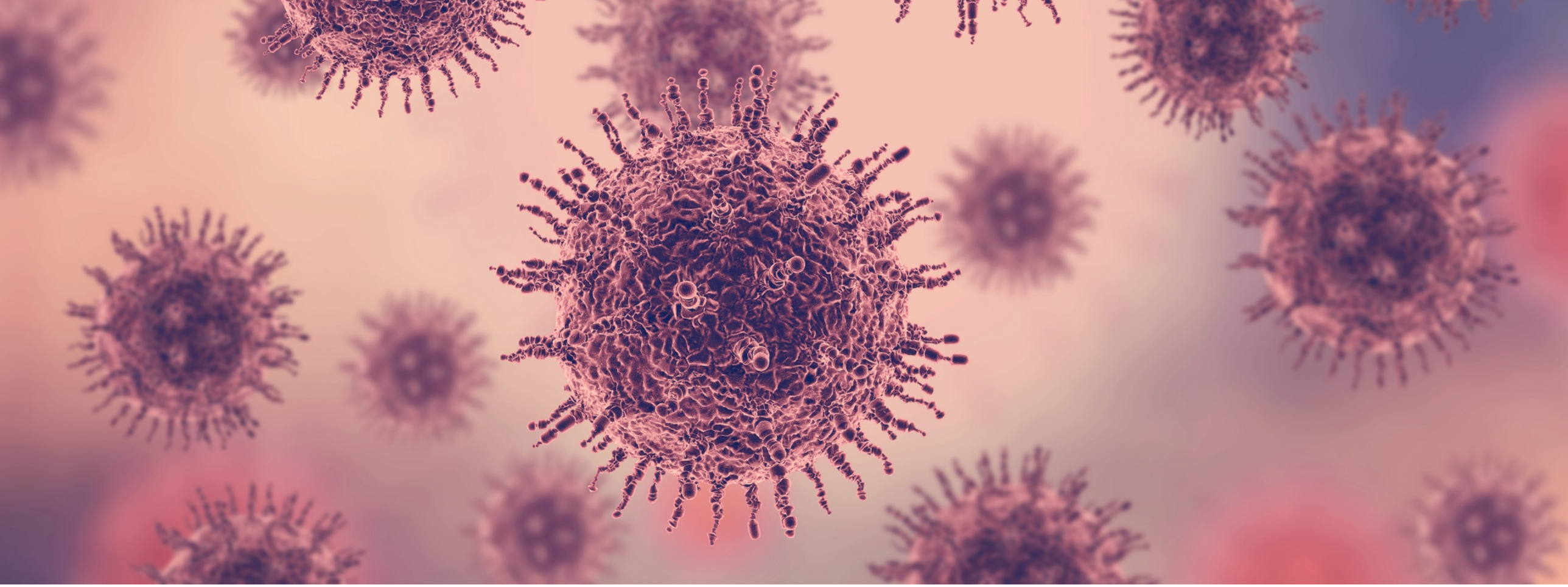

Immunogenicity and safety of Cervarix HPV-16/18 AS04-adjuvant vaccine against HPV induced cervical cancer in women (15+ years)

ADINA ELENA ZAGONEANU

Abstract

BACKGROUND: The incidence of cervical cancer has decreased by over 50% from the mid-1970s to the mid-2000s. This is due in part to an increase in screening, which can identify cervical changes before they turn cancerous. Decreasing incidence rates in young women may be due to the use of the HPV vaccine [1]. There are currently three types of HPV vaccines on the market: bivalent, quadrivalent and nine-valent HPV vaccines. These are administered in three-doses to both sexes from age 15 upwards to prevent HPV infection. Many medical systems support the vaccination of teenage girls and boys with HPV vaccines during their secondary school years in order to reduce the incidence of HPV-related cervical cancer in women. Despite the discovery of the link between HPV and cervical cancer in 1985, the beginning of vaccine production in 2002, and promotion of screening procedures since the 1960's, cervical cancer is still the fourth most common cancer in women worldwide today [2].

OBJECTIVE: This review aims to analyse the immunogenicity and safety of Cervarix®, also known as HPV-16/18 AS04-adjuvant vaccine, in women (15+ years) against cervical cancer.

METHODS: The literature review analysing the effectiveness and immunogenicity of HPV-16/18 AS04-ADJUVANTED Vaccine against cervical cancer, was conducted by using two main databases: EbescoHost (Medline and Academic Search) and PubMed. Advanced searches were conducted using specific key words, various filters and Boolean operators 'OR' and 'AND' to find the most suitable literature. This process resulted in over 100 results. From this cohort, clinical trials were selected in accordance with the specific inclusion and exclusion criteria determined for the review. After duplicates from both databases were removed, the studies were assessed in order or title, population and objective relevance, and the top 10 most relevant articles to the title of the literature review, were selected for the literature review.

RESULTS: The 10 clinical trials selected all obtained a minimum of 10 out of the 11 CASP[3] requirements indicating good study quality. All 10 studies were successful in proving the immunogenicity and safety of the HPV-16/18 AS04-adjuvant vaccine in protecting women 15+ (15-55 years) from developing HPV-induced cervical cancer [15, 16, 17, 18, 19, 20, 21, 22, 23, 24]. This confers that vaccination against HPV-16/18 virus proves to be effective in preventing HPV-induced CIN and cervical cancer and is a safe to administer vaccine.

Introduction

Cervical cancer is the fourth most common cancer affecting women globally [2]. It is diagnosed in more than 500,000 women every year and leads to 250,000 deaths [4]. Statistics show, a woman dies from cervical cancer every two minutes [4]. 99% of cervical cancer are due to human papillomavirus [HPV] infection [6], most commonly HPV-16 and HPV-18 [5].

Given the knowledge that most cervical cancers are caused by HPV, for which there are already vaccination programmes in place, the above figures are startling.

Anti-HPV vaccinations began in the early 2000's leading to a marked decrease of 50% in yearly cervical cancer rates.

Currently we have three HPV vaccines on the market, namely the "9-valent HPV vaccine (Gardasil® 9, 9vHPV), quadrivalent HPV vaccine (Gardasil®, 4vHPV), and bivalent HPV vaccine (Cervarix®, 2vHPV)" [12] that protect against HPV-16 & -18.

However, despite the FDA approval and medical recommendation that all teenage girls and women should avail of HPV vaccines general acceptance of HPV vaccines are low in many countries [12] due to lack of public health education, public health funding or drive for this campaign or vaccine availability, which is reflected by the high number of infections and subsequent cervical cell abnormalities.

The vaccine is developed based on a virus-like particle (VLP) of the major papillomavirus capsid protein L1" [14] that doesn't contain the active virus and is thus non-infectious and safe. "Cervarix comprises HPV16 and 18 VLPs, monophosphoryl lipid A (MPL), and aluminium hydroxide (together called adjuvant system 04, AS04) as an adjuvant" [14].

In my literature review I aim to assess the immunogenicity and safety of Cervarix® HPV Vaccine against cervical cancer in women, aged 15 and over. This aims to see if "AS04-adjuvanted HPV 16/18 vaccine administered in a three-dose schedule over 6 months elicits a high immunogenic response and is highly protective against cervical intraepithelial neoplasia and infection causally related to high-risk oncogenic HPV types" [13].

OBJECTIVES

- Review the literature for evidence of the immunogenicity of Cervarix® vaccine in protecting women against cervical cancer.
- Review the literature regarding the safety of Cervarix® vaccine.

METHODOLOGY

i) Search Database

Two Databases were used to conduct this literature review, namely:

EBSCOhost (Academic Search Complete & Medline) & PubMed.

ii) Key Words Identified

"HPV Vaccine 16/18", "bivalent HPV Vaccine", "AS04-adjuvant HPV vaccine", "AS04-adjuvant vaccine" , "Cervarix", "efficacy", "protection", "effectiveness", "immunogenicity", "immunity", "Cervical Cancer", "Cervical Carcinoma" and "CIN".

iii) Search Criteria

a] The following search criteria was implemented into EbescoHost [Academic Search & Medline] and PubMed:

EbescoHost had 276 results and Pubmed had 196 results for the initial search.

Table 1: Search Criteria Used for EbescoHost & PubMed

Term 1	"Efficacy" **OR** "Effectiveness" **OR** "Efficiency"
Command	**AND**
Term 2	"Immunogenicity" **OR** "Immunity" **OR** "Immune Response"
Command	**AND**
Term 3	"Cervarix" **OR** ""Bivalent HPV 16/18 Vaccine" **OR** "AS04-adjuvant vaccine" **OR** "16/18 AS04 Vaccine" **OR** "AS04 HPV Vaccine"
Command	**AND**
Term 4	"Cervical Cancer" **OR** "Cervical Carcinoma" **OR** "CIN"

iv) Filters Used

The following filters were applied to ensure the studies found were relevant and suitable. After filter application, EbescoHost had 84 results and PubMed had 21 results, for a total of 105 research papers.

Table 2: Advanced Search Filters for EbescoHost & PubMed

EbescoHost [Academic Search Complete & Medline]	PubMed
• Full Text • Peer Reviewed • Language: English • Published: 2000 – 2021 **Special Limiters for Academic Search Complete** • PDF Full Text **Special Limiters for Medline** • Human • Abstract Available • Sex: Female • Age: Adolescent 13-18, Adult 19+ Years • Publication Type: Clinical Study	• Full Text • Peer Reviewed • Language: English • Results by Year: 2000 – 2021 • Clinical Trials • Randomised Control Trial **Additional Filters** • Species: Human • Sex: Female • Age: Adolescent 13-18, Adult 19+ Years

v) Inclusion & Exclusion Criteria

Mendeley was used to save the 105 results and remove duplicates resulting in 67 papers to be assessed following inclusion and exclusion criteria below.

vi) Screening Exclusion Process

Out of the 58 research papers left to be examined, 6 papers were removed due to their titles not being fully relevant to the study, leaving 52 studies to be assessed for their eligibility. 42 studies were excluded for various reasons listed in the table below leaving 10 articles [12,13,14,15,16,17,18,19,20,21] as the most relevant to use in the literature review.

Table 3: Inclusion & Exclusion Criteria for Papers

Inclusion Criteria	Exclusion Criteria
• Original Research • Pilot study • Free PDFs • Full Free Text • Research Published between 2000 - 2021 • Research Conducted on Humans • Women aged 15 years and over • Cervarix® vaccine clinical trials • Different ethnicities • Peer Reviewed Research	• Case Reports • Review Articles • Meta Analysis • Research Published before 2000 • Research conducted on animals • Not available in English • Not scholarly reviewed articles • Protocols • Studies on male participants • Studies on females under 15.

Table 4: Study Exclusion Criteria

Research Paper Exclusion Reasons	# of Papers
Title didn't include all 4 search items together	6
Study didn't have full free text/PDF online	7
Case Study/Review	4
Study analysed financial efficacy of vaccine as opposed to prophylactic efficacy	2
Title didn't include 'AS04-adjuvant vaccine'	5
Study involved teenage girls & boys	4
Study involved men	2
Study involved women under 15 years of age	6
Study involved vaccine administration as protection against other diseases, not cervical cancer	3
Study didn't have results published	2
Study involved vaccination in patients already suffering from HPV induced cervical cancer	4
Total Excluded with Reason	**42**
Remaining Studies	**10**

vii) Data Extraction & Management

The data obtained from the studies included author name, publication year, study title, population, intervention, control, duration, design, methodology, objectives, key findings and strengths and limitations. Mendeley was the reference manager of choice for this literature review.

Results

In this literature review, 10 clinical trials were included [15,16,17,18,19,20,21,22,23,24] 8 of which were randomized and double-blinded except for two studies; one which was partially randomized and partially blinded [23] and one which was non-randomized and open label [16]. All data obtained and analysed by the clinical trials was quantitative. The findings resulted from multicentre trials and single centre studies which ranged from India [12], Japan [14], Brazil [15], China [16,19], Malaysia [21], South Africa [20] and Korea [24]. All study participants were female and study populations ranged from 120 [20] to 6051 [22]. The age range of the participants was 15-55+ years and no participants studies on women under 15 years were assessed, as per inclusion & exclusion criteria (Table 4). Controls were used in all bar one study [16] which assessed the immunogenicity and safety of the HPV-16/18 AS04-adjuvant vaccine in age stratified cohorts and discussed the differences in these groups. 9 out of the 10 studies used parallel placebo groups to help determine the immunogenicity of the HPV 16/18 AS04 Adjuvant vaccine. Placebo vaccines did not pose any harm to the wellbeing of the population. Study duration varied from 7 months [15,17,19,24] – 9.4 years [18]. All 10 studies used in this review had the same objectives analysing the immunogenicity and safety of HPV 16/18 AS04 vaccine against CIN1+, irrespective of HPV infection. All 10 studies had the same method of vaccine administration – 3 vaccine doses at months 0, 1 and 6 [15,16,17,18,19,20,21,22,23,24]. All 10 studies had the same safety recording measures to record AEs, SAEs, NOCDs and other MSCs [15,16,17,18,19,20,21,22,23,24]. All 10 papers performed the same methods of data analysis, namely ELISA and PBNA to determine GMTs [15,16,17,18,19,20,21,22,23,24].

STUDY VALIDITY & QUALITY

The study population, objectives, methodology and data analysis were all appropriate and indicative of a good quality study, according to the CASP Checklist [3]. Confounding variables were assessed in all 10 papers by PIs. The validity of these studies was analysed in accordance to CASP Clinical Trials Review [3], in Table 7 included in the appendix. All the articles assessed for the review were of a very high standard, having a minimum of 10 out of the 11 requirements as stated on CASP [3] form.

Discussion

IMMUNOGENICITY

All participants in all the 10 studies selected were seropositive for anti-HPV 16 & anti-HPV-18 antibodies post vaccination, meaning that the vaccine induced an antibody producing immune response to prevent HPV infection [15,16,17,18,19,20,21,22,23,24]. This serostatus can be detected at one month post vaccination dose 1, 2 or 3. All data provided shows how the vaccine elicits a similar GMT response in all participants, spiking at month 7 of study, exactly one month after vaccination with dose 3 of the HPV-16/18 AS04 adjuvant [15,16,17,18,19,20,21,22,23,24]. The vaccine has this effect on women irrespective of their age, serostatus at

Table 4: Summary of results

Author [Year], Title	Objective	Study Type, Population, Sample Size	Study Methodology	Key Findings	Strengths and Limitations
Neerja Bhatla et al. [2015] "Immunogenicity and safety of human papillomavirus-16/18 AS04-adjuvanted cervical cancer vaccine in healthy Indian women"[15]	Assess the **immunogenicity and safety** of HPV-16/18 AS04-adjuvanted vaccine against cervical cancer	Study type: Double-blind, randomized [1:1], controlled and multicentre trial with two parallel groups. P= women [18-35 years] N = 330	Participants were randomly divided into two groups. Group 1 = HPV vaccine Group 2 = Placebo [aluminium hydroxide] Women were vaccinated at month 0, 1 and 6. Immunogenicity: -Serum samples collected at pre-vaccination, month 0, and at month 7 -Analysed using ELISA method & PBNA Safety: -Safety log diaries were given to participants on the day of vaccination to record solicited and unsolicited [local/general] symptoms during the 7-day and 30-day post-vaccination follow-up period, respectively. -SAEs NOCDs and MSCs were recorded throughout. - Data was analysed accordingly	Immunogenicity: - All participants seropositive for HPV-16 & HPV-18 antibodies post vaccination. - Anti HPV-16 GMT: 10226.5 EL.U/ml [95% confidence interval: 8847.1–11821.0] -Anti HPV-18 GMT: 3953.0 EL.U/ml [95% confidence interval: 3421.8–4566.8], Safety: - 6 SAEs detected, non-vaccine related - Equal no. of reported vaccine related local AEs in both groups >97% Compliance to vaccination course . The AS04-adjuvanted HPV-16/18 cervical cancer vaccine resulted in being highly immunogenic and easily tolerable.	Limitation: None mentioned in the papers which is a limitation in itself. Limitation: Short study [7 months] Limitation: Local AE numbers not reported.
Tino F Schwarz et al. [2008] "Immunogenicity and tolerability of an HPV-16/18 AS04-adjuvanted prophylactic cervical cancer vaccine in women aged 15-55 years"[16]	Assess the efficacy, **immunogenicity and safety** of an HPV-16/18 AS04-adjuvanted vaccine in women.	Study Type: Multicentre, Phase III, non-randomized, open-label, age-stratified study. P=Women [15-55 years] N= 676	Women were divided into three groups by age Group 1: [15-25], Group 2: [26-45], Group 3: [46-55]. All women got three doses of the Cervarix Vaccine at month 0,1 and 6. Immunogenicity: -Phase 1: Follow up visits at month 0,1,2,6,7,12. -Serum samples collected and assessed at months [0,2,7,12] using ELISA method. -Phase 2: Follow up visits at months 18 and 24. -Serum samples collected at month 18 & 24 -Data analysed using ELISA method & PBNA -GMTs calculated Safety: -Safety log diaries were given to participants on the day of vaccination to record solicited and unsolicited [local/general] symptoms during the 7-day and 30-day post-vaccination follow-up period, respectively. -SAEs NOCDs and MSCs were recorded throughout. - Data was analysed accordingly	Immunogenicity: -Study findings reported high immunogenicity and tolerability of HPV-16/18 AS04 vaccine in study population. -Vaccine induced a high immune response against both HPV-16 & -18 for 24 months, in women >26 years, after first vaccination [month 0]. -Seropositivity = 100% from months [0-24] -Peak antibody titres at months 7 for all participants. -Antibody levels in women >26 years reached a plateau 24 months after first vaccination [month 0] -Site-specific immunity [at cervix] induced by vaccine supported by high antibody levels present in secretions at month 24. Safety: - Compliance to vaccination course was 98.7 to 99.3% in the respective phases. -Approximately 11% of participants experienced medically significant adverse effects [bronchitis, depression, hypertension] -Approximately 5% of participants [14 women] experienced serious adverse effects, out of which only 1 was a side-effect of the vaccine.	Limitation: Ethnic demographic of women not specified. Limitation: No identified serological correlate of protection,

Table 4: Summary of results (continued)

Author [Year], Title	Objective	Study Type, Population, Sample Size	Study Methodology	Key Findings	Strengths and Limitations
Ryo Konno et al. [2014] "Efficacy of the human papillomavirus [HPV]-16/18 AS04-adjuvanted vaccine against cervical intraepithelial neoplasia and cervical infection in young Japanese women"[17]	Assess HPV 16/18 AS04-adjuvanted vaccine efficacy, **immunogenicity and safety** up to 4 years after first vaccination in Japanese women aged 20-25 years.	Study Type: multicentre phase II double blinded, parallel group RCT. P = Women [20-25 years] N = 752	Women were split in [1:1] manner into two groups. Group 1 = HPV-16/18 L1 VLP AS04 vaccine Group 2 = Placebo [Aluminum hydroxide] Vaccine administrations at months 0, 1, and 6. Immunogenicity: -Cervical liquid-based cytology samples were collected at the yearly follow-up visits[months 12, 24, 36, 48] -Analysed using ELISA method & PBNA -GMTs calculated. Safety: -Safety log diaries were given to participants on the day of vaccination to record solicited and unsolicited [local/general] symptoms during the 7-day and 30-day post-vaccination follow-up period, respectively. -SAEs NOCDs and MSCs were recorded throughout. - Data was analysed accordingly	A Immunogenicity: -All participants seropositive for HPV-16 & -18 antibodies at 48 months. -GMTs HPV-16 = 1283.2 EL.U/mL [95% CI: 1150.1–1431.7] -GMTs HPV-18 = 473.0 EL.U/mL [95% CI: 416.8–536.8] -GMTs for antibodies peaked at month seven, declined until month 18 and then plateaued. Safety: -The number of SAEs/AEs were similar between the two groups.	Strength: Vaccine efficiency also calculated
Paulo S. Naud et al. [2014] "Sustained efficacy, **immunogenicity, and safety** of the HPV-16/18 AS04-adjuvanted vaccine"[18]	Assess the efficacy, **immunogenicity and safety** of an HPV-16/18 AS04-adjuvanted vaccine in women from 5 Brazilian centres during 9.4 years.	Study Type: Double-blind, randomized [1:1], placebo-controlled study. P= Women [25-55 years] N=437	Women were randomized [1:1] into two groups. Group 1 received 3 doses of HPV-16/18 vaccine. Group 2 received 3 doses of placebo [Al[OH]3] Vaccine administrations occurred at months 0, 1, and 6. Immunogenicity: -Cervical swabs & cytology specimen collections were performed every 6 months and 12 months respectively for HPV testing, for 113 months. -Anti HPV 16/18 antibodies were measured via ELISA and PBNA. -VE was determined based of HPV, cytological lesions and histopathological abnormalities detected at months 6 and 12 throughout the 113 month study. Safety: -AEs were reported by participants up to 7 days post vaccination. -Reports of SAEs, pregnancies, etc. were recorded up to 30 days post vaccination. -All other SAEs, AEs etc. were recorded during 113 month study.	Immunogenicity: - All women seropositive for HPV-16/18 at month 113. - GMTs HPV 16 =180·1 ED50 [95% CI: [153·3 to 211·4]] - GMTs HPV 18 = 137·3 ED50 [95% CI: [112·2 to 168·0]] - Antibody titres [HPV vaccine induced] x10 above natural infection levels. Safety: -Safety profile is acceptable. -All reported SAEs, AEs and pregnancy outcomes in this study were not associated to the vaccine.	Strength: Longest follow-up reported for a licensed HPV vaccine.

Table 4: Summary of results (continued)

Author [Year], Title	Objective	Study Type, Population, Sample Size	Study Methodology	Key Findings	Strengths and Limitations
Hextan Y S Ngan et. al [2010] "Human papillomavirus-16/18 AS04-adjuvanted cervical cancer vaccine: **immunogenicity and safety** in healthy Chinese women from Hong Kong"[19]	To assess the **immunogenicity and safety** of human papillomavirus-16/18 AS04-adjuvanted cervical cancer vaccine in Chinese women aged 18 to 35 years enrolled from Hong Kong.	Study Type: Double-blind, randomized controlled trial with vaccine and placebo groups. P = Women [18-35 years] N = 300	Women were randomized [1:1] into two groups. Group 1 received 3 doses of HPV-16/18 vaccine. Group 2 received 3 doses of placebo [Al[OH]3] Vaccine administrations occurred at months 0, 1, and 6. Immunogenicity: HPV-16/18 antibodies, seroconversion rates and geometric mean titres were measured at month 7. -Data analysed using ELISA method & PBNA Safety: -Safety log diaries were given to participants on the day of vaccination to record solicited and unsolicited [local/general] symptoms during the 7-day and 30-day post-vaccination follow-up period, respectively. -SAEs NOCDs and MSCs were recorded throughout. - Data was analysed accordingly	Immunogenicity: Group 1 seropositive for HPV-16/18 antibodies by month 7. Anti HPV-16/18 GMT mean titres = 10 422 [95% confidence interval, 8730-12 442] EL.U/mL for HPV 16and 4649 [3975-5437] EL.U/mL, for HPV18. Safety: -High vaccination compliance among both groups [99%] and well tolerated vaccine. -Post-vaccination local injection reactions at site were higher in the vaccine than placebo group; Most common symptom: pain. - Other symptoms = fatigue and myalgia, frequent in both groups. SAEs = 5/300: 4 in Group 1, 1 in group 2. All unrelated to HPV vaccine or placebo.	Limitation: Not a very lengthy study considering peak titres occur at month 7 post initial vaccination at month 0. Limitation: Single Site study including only 300 participants from the same population – not representative.
Cosette M Wheeler et. Al [2016] "Efficacy, **safety, and immunogenicity** of the human papillomavirus 16/18 AS04-adjuvanted vaccine in women older than 25 years: 7-year follow-up of the phase 3, double-blind, randomised controlled VIVIANE study"[20]	To assess the efficacy, **safety, and immunogenicity** of the human papillomavirus 16/18 AS04-adjuvanted vaccine in women,	Study Type: 7-year follow-up of the phase 3, double-blind, randomised controlled VIVIANE study. P = Women [25-49 years] N = 5747	Participants were split in [1:1] manner into two groups. Group 1 received the HPV-16/18 L1 VLP AS04 vaccine and group 2 received the Hepatitis A vaccine [control]. Vaccine administrations occurred at months 0, 1, and 6. Immunogenicity: Cytology testing performed for HPV DNA testing every 6 months and Pap cytology testing every 12 months via PBNA. Antibody responses assessed by ELISA19 every 6-months, for the 2 year study duration. [24 months]. Safety: -Safety log diaries were given to participants on the day of vaccination to record solicited and unsolicited [local/general] symptoms during the 7-day and 30-day post-vaccination follow-up period, respectively. -SAEs NOCDs and MSCs were recorded throughout. - Data was analysed accordingly	Immunogenicity: -Geometric peaked at approximately month 7 and plateaued thereafter. ->95% participants seropositive for HPV-16 /18 at month 84. -GMTs similar in all age groups. Safety: Serious adverse events related to vaccination occurred in 5 [0·2%] participants in Group 1 and 8 [0·3%] participants in Group 2 [Control].	Strength: Multi-Site, Ethnically diverse study with a large subject co-hort. Limitation: The ratio of women with a history of HPV infection or disease and women with no history of disease used in VIVIANE study might not be representative general population.

Table 4: Summary of results (continued)

Author [Year], Title	Objective	Study Type, Population, Sample Size	Study Methodology	Key Findings	Strengths and Limitations
B K Lim et. al [2014] **"Immunogenicity and Safety** of the AS04-adjuvanted Human Papillomavirus-16/18 Cervical Cancer Vaccine in Malaysian Women Aged 18–35 years: A Randomized Controlled Trial"[21]	To evaluate the vaccine **immunogenicity against HPV-16 and HPV-18 as well as its safety** and reactogenicity in Malaysian women.	Study type: Phase IIIb, double-blind, randomized [1:1] and placebo controlled trial P = Women [18-35 years] N = 271	Participants were randomized [1:1], divided into two groups, to receive three doses of HPV-16/18 vaccine or Al[OH]$_3$ [control] at M 0, 1, 6. Group 1 – HPV vaccine, Group 2 – Control. Immunogenicity: -Antibody titters were measured at month 7 by [ELISA] method & PBNA. -Gynaecological examination and cervical swab performed at month 7. Safety: -Any local or general, solicited symptoms were recorded in diary cards for 7 days after each vaccination. -Unsolicited symptoms were recorded for 30 days after each vaccination. -SAEs were reported throughout the study.	Vaccine was immunogenic and well tolerated in Malaysian women aged 18-35 years. Immunogenicity: - 100% seroconversion, in initially seronegative participants, one month post-dose-3 for anti HPV-16 and anti-HPV-18 antibodies. - GMTs HPV 16 = 11107.5 [95% CI: 9727.3-12683.4] EL.U/mL - GMTs HPV 18 = 4273.5 [95% CI: 3771.8-4841.9] EL.U/mL Safety: -Vaccine compliance >96% -AEs such as local injection pain and post vaccine general symptoms [fatigue etc.] present equally among both groups. - 8 SAEs reported [5 in group 1, 3 in group 2], not connected to HPV vaccine.	Limitation: Vaccine efficiency not assessed. Limitation: Relatively small sample size Limitation: Short Study [7 months].
Feng-Cai Zhu et al. [2014] "Efficacy, **immunogenicity and safety** of the HPV-16/18 AS04-adjuvanted vaccine in healthy Chinese women aged 18-25 years: results from a randomized controlled trial"[22]	Efficacy, **immunogenicity and safety** of the HPV-16/18 AS04-adjuvanted vaccine in healthy Chinese women aged 18-25 years: results from a randomized controlled trial	Study Type: randomized, double-blind, controlled trial P = Women [18-25 years] N = 6051	Participants were randomized [1:1], divided into two groups, to receive three doses of HPV-16/18 vaccine or Al[OH]$_3$ [control] at M 0, 1, 6. Group 1 – HPV vaccine, Group 2 – Control. Immunogenicity: -Blood samples at months 0, 7, 12, 24, 36 and 48. HPV-16 and HPV-18 antibodies were measured [ELISA] & PBNA. -Gynaecological examination with cervical samples collected at day 0 and at 6-month intervals until month 48. -VE assessed Safety: -Any local or general, solicited symptoms were recorded in diary cards for 7 days after each vaccination. -Unsolicited symptoms were recorded for 30 days after each vaccination. -SAEs were reported throughout the study. -Data was analysed accordingly.	Immunogenicity: -Seroconversion was 100% for anti-HPV-16 and 99.7% for anti-HPV-18, in initially seronegative participants. -GMTs[95% CI] were 6,996 [6,212 to 7,880] against HPV 16 and 3,309 [2,942 to3,723] EU/mL, against HPV 18. -All initially seropositive women in the vaccine group remained seropositive for anti-HPV-16 and anti-HPV-18 antibodies at month 7 and GMTs were 5,698 [4,703 and 6,904] and 3,242 [2,736 and 3,842]EU/mL, respectively. Safety: -Similar AEs were reported among both groups, - Group 1 reported more local injection site symptoms. -1 SAE, GIT infection associated with HPV vaccine. -1 fatal SAE, suicide, reported but unrelated to HPV vaccine.	Limitation: SAE & AE incidences not reported numerically.

Table 4: Summary of results (continued)

Author [Year], Title	Objective	Study Type, Population, Sample Size	Study Methodology	Key Findings	Strengths and Limitations
Denny L et. al [2013] **"Safety and immunogenicity** of the HPV-16/18 AS04-adjuvanted vaccine in HIV-positive women in South Africa: A partially-blind randomised placebo-controlled study"[23]	Evaluated the **safety and immunogenicity** of the HPV-16/18 AS04-adjuvanted vaccine in women in South Africa.	Study type: Phase I/II, partially-blind, partially-randomised, placebo-controlled trial at a single centre in Khayelitsha, Cape Town, Republic of South Africa. P = Women [18-25 years] N = 120	Participants were divided into HIV positive and HIV negative groups. Women in the HIV positive group were randomised [1:1] to receive the HPV-16/18 AS04-adjuvanted vaccine [Group 1] or control [Group 2]. Women in the HIV negative group were given the HPV vaccine also [Group 3]. Immunogenicity: -Blood samples for assessment of HPV-16/18 antibody and CD4+ T-cell responses were collected at months 0, 2, 7, and 12 and analysed. -Data analysed using ELISA method & PBNA -GMTs calculated Safety: -Solicited adverse events were recorded by a trained field worker daily for 7 days after each vaccination. Severity of solicited adverse events was graded on a scale of 0 [absent] to 3 [preventing normal activities]. -Data was analysed accordingly	Immunogenicity: All participants seropositive for HPV-16 and HPV-18 after their vaccinations and at month 12. -GMTs peaked in both group 1 and 3 at month 7. Group 1 HPV-16 :3558.2 [95% CI: 2723.6; 4648.6] EL.U/ml Group 1 HPV-18: 1945.8 [95% CI: 1451.4; 2608.6] Group 3 HPV-16: 8168.8 [95% CI: 6341.0; 10,523.5]EL.U/ml Group 3 HPV-18 : 3703.0 [95% CI: 2502.5; 5479.4] EL.U/ml -GMTs at Month 12: Group 1 HPV-16: 748.1 [95% CI: 520.0; 1076.3] EL.U/ml Group 1 HPV-18: 343.1 [95% CI: 236.2; 498.2] Group 3 HPV-16: 2793.6 [95% CI: 2087.8; 3738.0] EL.U/ml Group 3 HPV-18: 210.3 [95% CI: 627.4; 1662.6] EL.U/ml. In placebo group – no change was seen in GMT levels. Safety: Administration of the HPV-16/18 vaccine did not influence HIV disease progression. The HPV-16/18 vaccine had a clinically acceptable safety and reactogenicity profile. AEs: 30 days post vaccine 86.9% [group 1], 78.0% [group 2], 86.7% [group 3] including headache, local injection pain. SAEs: **1 SAE, rhinitis associated with HPV vaccine in group 1.**	Limitation: immunogenicity was assessed in young adult women, many of whom were already seropositive for HPV-16 and/or HPV-18 prior to vaccination, Limitation: Small study population. Small number of participants [approx. 40] in each group.
Seung Cheol Kim et. al [2011] "Human papillomavirus 16/18 AS04-adjuvanted cervical cancer vaccine: **Immunogenicity and safety** in 15-25 years old healthy Korean women"[24]	The study assessed the **immunogenicity and safety** of human papillomavirus [HPV]-16/18 AS04-adjuvanted cervical cancer vaccine in healthy Korean women.	Phase IIIB, double-blind, randomised [2:1], multi-centre clinical trial. P: Women [15-25 years] N = 225	Women were divided into two groups HPV vaccine [N=149] and placebo [N=76]. Vaccine administrated at month 0, 1, and 6 months. Patient assessment happened monthly until month 7. Immunogenicity: -Serum samples were collected pre-vaccination and one month post-dose 3. - Blood samples [5 mL] were collected before vaccination and one month post-dose 3 to evaluate the antibody response against HPV-16 and HPV-18 using ELISA & PBNA. Safety: -Safety log diaries were given to participants on the day of vaccination to record solicited and unsolicited [local/general] symptoms during the 7-day and 30-day post-vaccination follow-up period, respectively. -SAEs NOCDs and MSCs were recorded throughout. - Data was analysed accordingly	Immunogenicity: At month 7, all initially seronegative women had seroconverted for HPV-16 and HPV-18 antibodies with anti-HPV-16 and anti-HPV-18 GMTs of 9,351.4 El.U/mL [95% CI, 8,145.5 to 10,735.8] and 4204.1 El.U/mL [95% CI, 3,626.5 to 4,873.6], respectively. Safety: Vaccination compliance 95.3% in HPV and 89.5% in placebo group. AEs: Symptoms of local pain, headache, fatigue were reported in both groups in equal proportion. SAEs: 3 were reported [2 in HPV group; 1 in placebo group]. AEs were tabulated with exact 95% confidence intervals [CIs] for all vaccine doses and overall. All SAES unrelated to vaccination.	Limitation: Relatively short study duration – 7 months. Limitation: No VE calculated.

month 0 or underlying health conditions such as HIV [15,16,17,18,19,20,21,22,23,24].

Thereafter follows the decline of the serum GMT levels until month 18 post initial vaccination, where antibody levels reach a plateau [15,16,17,18,19,20,21,22,23,24]. However, this result has been challenged in one of the 10 studies in use, which stated women vaccinated at age 26+ had their GMT levels reach a plateau at month 24, approximately 6 months later than the 15+ age cohort [16]. The 10 studies selected were based in different countries and included different ethnicities. This diverse population cohort allowed for an inclusive and globally representative analysis to be conducted [15,16,17,18,19,20,21,22,23,24]. There was no significant difference in the GMT levels induced by HPV vaccine among populations with no underlying health conditions [15,16,17,18,19,20,21,22,24].

HIV-positive individuals have an impaired or reduced immune response to routine vaccines [2], however, the adjuvant in Cervarix is an important contributor towards the immunogenicity induced by the vaccine. Factors affecting immunogenicity in the case of the review, are age [16] and autoimmune diseases i.e. HIV [23]. These factors either affect the plateau period of serum GMT levels [16] or quantity of serum GMTs at any given time [23], respectively.

Although these factors contribute to overall immunogenicity, they don't suppress the effectiveness of the vaccine. Therefore according to the mentioned papers, women vaccinated at 26+ years or women that are HIV positive can acquire immunity against HPV 16 and HPV 18 on vaccination with HPV-16/18 AS04-adjuvant [16]. On the other hand, in both studies mentioned, which included parameters affecting the immunogenicity of the study (in this age, older age and HIV positive status), these may invite scepticism as their population cohorts were relatively small and they were not fully double blinded and randomized, which can lead to biased results [16][23].

SAFETY

The HPV-16/18 AS04-adjuvant vaccine was approved for use by the FDA in 2009, indicating it's a safe and efficacious vaccine

[25]. Among the 10 studies listed, each study carefully analysed any adverse effects of the vaccine up to day 7 post vaccination and then up to day 30 post vaccination, by allowing participants to record their details in a diary [15,16,17,18,19,20,21,22,24]. Only 1 study involves personnel gathering this information from the participants themselves [23], which although may be a more scientifically reliable method, can be a confounding variable given possible 'white coat syndrome' amongst participants, as this may influence participants to give biased answers regarding side effects of the vaccine.

Current literature promotes the HPV-16/18 AS04 adjuvant vaccine as safe and tolerable among patients, as we've seen with a vaccine compliance rate with an average of 97% among the 10 studies selected [15,16,17,18,19,20,21,22,23,24]. The majority of adverse effects reported were related to the injection itself and certain symptoms such as headache and fatigue [15,16,17,18,19,20,21,22,23,24] are usual Cervarix side effects [26]. The accuracy of self-reported symptoms is something that can be questioned regarding AEs reported, as some participants may over-report/under-report their symptoms as self-reporting bias is a concerning aspect in research [27]. The time of year of symptom reporting would also be interesting to determine and see if there were any confounding variables present which may have coincided with any presenting symptoms e.g. head cold caught during winter at the same time of vaccine, which may influence reported side effects of the vaccine. Only 3 out of the 10 papers showed SAEs as a result of the HPV vaccine. These were all easily treatable and the participants recuperated without sequalae.

STRENGTHS & LIMITATIONS OF REVIEW

Among the 10 studies selected, many were found set in Asia: China, Japan, Malaysia, India, one in Europe – France, one in America, South America – Brazil, and one in Africa [15,16,17,18,19,20,21,22,23,24].

Studies on Caucasian women or Asian [Indian, Chinese, Japanese and Malaysian] women were the most abundant, which although is a great discovery among women of these populations, is limited to only representing specified groups. Finding studies that included more than one ethnic group and analysing the immunogenic effect of the vaccine and its safety for the patients was difficult. Equally only one study investigating the immunogenicity of the vaccine in black women[23] was found on both databases used [EbescoHost & PubMed]. This one paper then went to analyse the immunogenicity and safety of the vaccine in a small population of equal numbers of HIV positive & negative participants in South Africa – which is not an accurately representative of the South African demographic [23] due to the small sample size.

Another limitation of this research topic is that it was difficult to find 10 papers featuring populations of the same broad age bracket (15 – 55 years). Although a review was possible to conduct given the scope specified (15+ years) it would have been preferable to have 10 articles including the exact same age cohort, preferably age stratified.

GAPS IN LITERATURE

Based on the overall limitations mentioned it would be great to see a clinical trial analysing the immunogenicity and safety of HPV-16/18 AS04-Adjuvant vaccine on women of different ethnicities and see how their results compare. There should be more studies performed on women of colour, especially black women as they're grossly under-represented in the current literature. For increased statistical accuracy and bias elimination all studies should be double-blinded and randomised. It would also be very interesting to assess the immunogenicity and safety of the Cervarix vaccine against another HPV vaccine brand i.e. bi-valent but without AS04 adjuvant, Gardasil, quadrivalent or nine valent. This would be an interesting analysis and would help determine which vaccine induces more immunogenicity, as the stronger the immune response against the HPV induced virus the less likely it is for dyskaryosis, CIN and Cervical cancer to progress. As we can observe from the literature, HPV vaccination is an effective prophylactic treatment against cervical cancer with its immunogenicity present up to 9.4 years[18], and perhaps beyond. Therefore, the question of why cervical cancer is the 4th most common cancer among women[2] is not answered by the lack of vaccine protection & safety, but rather by the lack of vaccination, regular screening and awareness.

Conclusion

The administration of Cervarix, HPV-16/18 AS04-adjuvant vaccine is highly immunogenic, safe and tolerable among women aged 15+ and protects them against HPV induced cervical cancer, which counts for approximately 90% of all cases [15,16,17,18,19,20,21,22,23,24].

All women, regardless of initial HPV-16/18 serostatus prior to vaccination, tested seropositive for both HPV-16/18 antibodies post vaccination [15,16,17,18,19,20,21,22,23,24]. GMTs level reach their peak at month 7, exactly 1 month post dose 3 vaccination, after which they decrease to hit a plateau at month 18 [15,16,17,18,19,20,21,22,23,24]. The antibody induced response is still present post month 18 and the longest study to confirm presence of GMT levels is 9.4 years [18]. Despite local injection site AEs, the Cervarix vaccine is highly tolerable among women and has a high administration compliance. The majority of SAEs detected at any time post vaccination are rarely vaccine associated [15,16,17,18,19,20,21,22,23,24]. However, even those that are vaccine associated are generally easily treatable. Therefore, along with proven immunogenicity, the Cervarix HPV-16/18 AS04-adjuvant vaccine is also safe and an appropriate prophylactic treatment in women aged 15+ to prevent the development of HPV induced cervical cancer [15,16,17,18,19,20,21,22,23,24].

Appendix A: Abbreviations

AE[s]	Adverse Events/Effects
SAE[s]	Serious Adverse Events/Effects
CIN1+	Cervical Intraepithelial Neoplasia [grade one or grater]
GMT[s]	Geometric Mean Titre[s]
NOCD[s]	New Onset Chronic Disease
MSC[s]	Medically Significant C
IM	Immunogenicity
VE	Vaccine Efficiency
ELISA	Enzyme-linked immunoassay
PBNA	Pseudovirion-based Neutralization Assay
CASP	Critical Appraisal Skills Program [3]

Appendix B: CASP Checklist for Clinical Trials [3]

CASP Checklist: Clinical Trial Based Checklist	Neerja Bhatla et al. [2015]	Tino F Schwarz et al. [2008]	Ryo Konno et al. [2014]	Paulo S. Naud et al. [2014]	Hextan Y S Ngan et. al [2010]	Cosette M Wheeler et. Al [2016]	B K Lim et. al [2014]	Feng-Cai Zhu et al. [2014]	Denny L et. al [2013]	Seung Cheol Kim et. al [2011]
1. Did the study address a clearly focused research question?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2. Was the assignment of participants to interventions randomised?	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
3. Were all participants who entered the study accounted for at its conclusion?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4a. Were the participants 'blind' to intervention they were given?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4b. Were the investigators 'blind' to the intervention they were giving to participants?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4c. Were the people assessing/analyzing outcome/s 'blinded'?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5. Were the study groups similar at the start of the randomised controlled trial?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6.Apart from the experimental intervention, did each study group receive the same level of care [that is, were they treated equally]?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7. Were the effects of intervention reported comprehensively?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8. Was the precision of the estimate of the intervention or treatment effect reported?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9. Do the benefits of the experimental intervention outweigh the harms and costs?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10. Can the results be applied to your local population/in your context?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11. Would the experimental intervention provide greater value to the people in your care than any of the existing interventions?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Total Score:	**11/11**	**10/11**	**11/11**	**11/11**	**11/11**	**11/11**	**11/11**	**11/11**	**10/11**	**11/11**

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Focused gluteal region dissection on the human sciatic nerve

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Abstract

Dissection provides an understanding of human anatomy that is difficult to achieve solely with the use of textbooks. The knowledge obtained from anatomical dissection has allowed medical practitioners the ability to understand the anatomy that forms that basis of a particular clinical presentation, and more effectively treat the condition. These conditions include piriformis syndrome and sciatica. Clinical conditions may also arise due to anatomical variations which are rare presentations, absences, or arrangements of structures. The ultimate goal of the dissection in this report was to discern the pathway of the sciatic nerve and its divisions. In this report, the dissection of the gluteal region of a left leg from a female cadaver is detailed. The findings reveal two anatomical variations, a high division of the sciatic nerve and a muscular slip lateral to the long head of the biceps femoris. Additional research is needed to determine the implications of these anatomical variations in the clinical context. This prosection will be an important educational resource for future students, and could not have been possible without the generous donors.

Table 1: Acronym and abbreviations

Acronym/ Abbreviations	Definition
Adductor Magnus	AMag
Biceps Femoris	BF
Biceps Femoris Slip	BFS
Common Peroneal Nerve	CPN
Gluteus Maximus	GMax
Gluteus Medius	GMed
Gluteal Region Muscles	GRM
Gracilis	Grc
Inferior Gluteal Vessels	IGV
Long Head of Biceps Femoris	LHBF
Piriformis	PRF
Posterior Femoral Cutaneous Nerve	PFCN
Semimembranosus	SMem
Semitendinosus	STen
Short Head of Biceps Femoris	SHBF
Superior Gluteal Vessels	SGV
Tibial Nerve	TN

Introduction

The sciatic nerve is the largest peripheral nerve in the human body (2) and care is required to avoid injury during intramuscular (IM) injections. The dorsogluteal injection site is the gluteus maximus (GMax) in the superior lateral aspect of the gluteal region when it is divided into four equal parts (Fig. 1A) (3). The ventrogluteal injection site is located in the gluteus medius (GMed): more superior, lateral, and anterior to the dorsogluteal site (Fig. 1B) (3). As there is less risk to the sciatic nerve if the injection is given to the gluteus medius, the ventrogluteal site is preferred for IM injections (3).

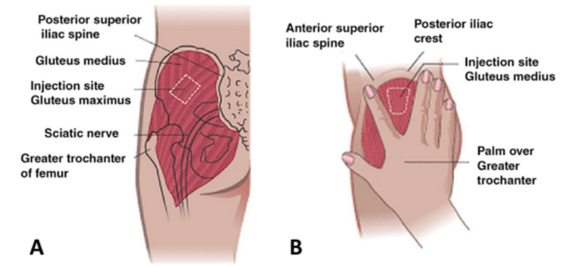

Figure 1: Sites for Intramuscular Injections. Adopted from springer.com (1) (A – Dorsogluteal site, B – Ventrogluteal site)

Another clinical consideration with regards to the sciatic nerve is a radiculopathy commonly known as sciatica. Sciatica is a condition that affects approximately 40% of adults at some point in their lives (4). Patients who suffer from sciatica often experience a sharp and aching back and unilateral leg pain that radiates from the gluteal region to the lower leg and often to the toes (5). Associated symptoms might also include tingling, numbness, or loss of muscle

strength in the affected leg (5). The most common cause of sciatica is a prolapsed intervertebral disc in the lumbar spine between L4-L5 or S1-S2 causing nerve entrapment and inflammation (4). Diagnosis is usually based on clinical presentation, but imaging may also be performed when there is suspicion of a malignancy or infection (6).

For females, sciatica can also be caused by endometriosis and pregnancy (7). Lower back pain and sciatica is a common occurrence in pregnancy due to the pressure of the fetus in the uterus on the surrounding anatomical structures (8). A rare cause of sciatica is piriformis (PRF) syndrome, a neurological disorder that causes the PRF to spasm and compress the sciatic nerve as it exits the greater sciatic foramen (9). The treatment for sciatica consists of physiotherapy, exercise, pain medications or surgery (6). In the case of PRF syndrome, botulinum toxin injections might be recommended (7).

Anatomical knowledge is a core component of every medical student's education. The clinical presentation of many conditions can be better understood with a high level of anatomical knowledge. The process of dissecting cadavers creates a deeper understanding of the anatomical layers, orientation, and position. Reflection and documentation are important aspects of this educational experience. This report contains information detailing a 7-day dissection of the posterior thigh with a focus on the gluteal region of a left leg from a female cadaver. The structures studied included the muscles of the gluteal region, posterior thigh muscles, superior and inferior gluteal vessels, posterior femoral cutaneous nerve, and sciatic nerve branches. The age and cause of death of the cadaver were not known.

Methods

On day 1, the initial incision into the gluteal skin was made using a size 20 scalpel blade on a size 4 handle (referred to as scalpel blade from this point forward throughout the text). The superficial incision was made transversely just below the location of the inferior border of the GMax, visualized on the medial aspect of the left leg cadaver. Next, a deeper dissection was performed using the scalpel blade while ensuring the underlying muscle was not accidentally cut. Blunt dissection with fingers was used on the medial side of the cadaver to pull apart the subcutaneous tissue from the gluteal muscles. Next, the scalpel blade was used to reflect the layer of tissue covering the GMax using feathering motions with light pressure. After completion of the lateral reflection, there was subcutaneous tissue remaining that needed to be cleaned to visualize the GMax.

On day 2, the scalpel blade continued to be used until the layer covering the GMax was thin enough to see the color of the muscle. At this stage, the fascia protecting the GMax was lifted using forceps and cut with a pair of small scissors until the muscle was entirely exposed. These two surgical instruments were the choice for cleaning of all anatomical structures for the remainder of the project.

On day 3, tissue was cleaned from the GMed using the same techniques described for exposing the GMax. Afterwards, large portions of fat were removed from the lateral aspects of the GMax by cutting with small scissors. Finally, two additional superficial incisions were made using a scalpel blade in the lower region of the posterior thigh. One incision was made transversely in the posterior thigh region, and another was made in the frontal plane on the medial aspect of the cadaver. Both incisions intersected with each other, and the frontal incision also intersected with the original incision made below the inferior border of the GMax. Thus, a window was created that was reflected laterally to allow for exposure of the thigh muscles.

On day 4, the posterior muscles of the thigh were cleaned of fat. Next, a lateral incision was made along the GMax with a long, sharp blade. Medial resection of the GMax began with blunt dissection, using fingers to pry the muscle away from the underlying anatomical structures. To continue the resection, a scalpel blade was used. The resection was completed by the beginning of day 5. The remainder of day 5 was used to clean the structures underlying the anterior surface of the GMax.

Day 6 was spent cleaning the biceps femoris (BF), semitendinosus (STen), semimembranosus (SMem), adductor magnus, and gracilis. On the final day, cleaning was completed for the tibial nerve (TN), common peroneal nerve (CPN), posterior femoral cutaneous nerve (PFCN), and anterior surface of the GMax. By the end of the final day of the dissection, the gluteal and posterior thigh regions of the cadaver were exposed to show several anatomical structures in detail.

Results

The cadaver was received after a transverse and median cut was made through the pelvis. Thus, the subcutaneous tissue and muscle layers of the gluteal region could be identified on the medial aspect of the cadaver (Fig. 2).

By the end of day 2, the GMax had been completely exposed but the GMed remained covered. It was noted that the GMax was smaller than expected. Figure 3 depicts the exposed and cleaned GMax and GMed muscles.

Several vessels were cut to ensure full resection of the muscle was possible. During the resection, a large vessel was grazed by the scalpel blade as it was difficult to distinguish the vessel from the surrounding tissue. To avoid severing the vessel, as it was hypothesized to be the sciatic nerve based on the large diameter, the resection was continued but followed a path much closer to the GMax.

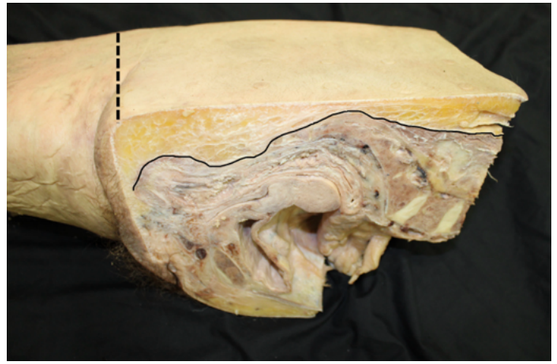

Figure 2: **Presentation of Cadaver Prior to Dissection of Gluteal Region.** The initial superficial incision was made transversely along the dotted line. Blunt dissection with fingers was used along the solid line to divide the subcutaneous tissue from the underlying anatomical structures.

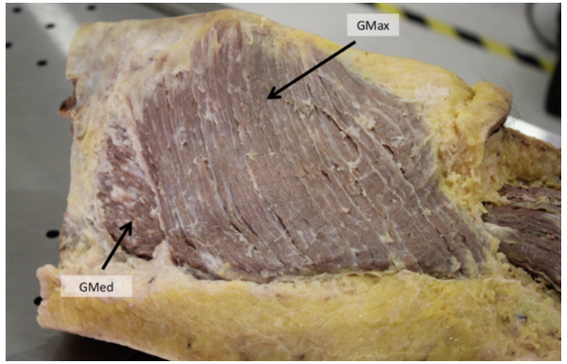

Figure 3: **Gluteus Maximus and Gluteus Medius of a Gluteal Region in the Left Leg of a Female Cadaver.** The subcutaneous tissue has been removed with forceps and a pair of small scissors to expose both muscles. (GMax – Gluteus maximus, GMed – Gluteus medius)

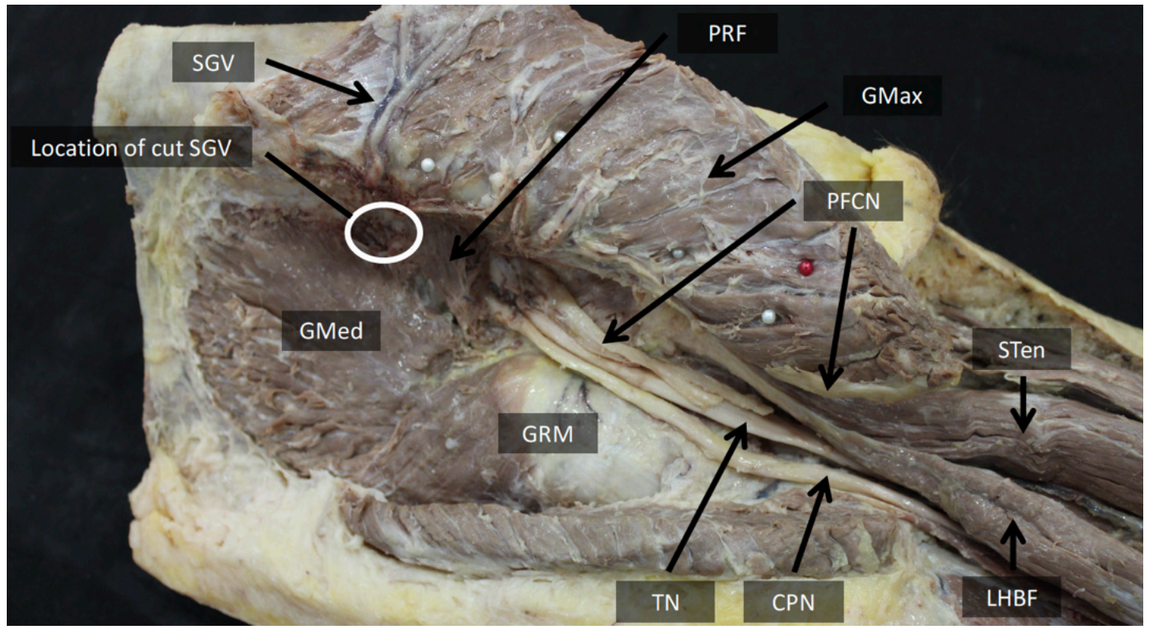

Figure 4: **Gluteal Region in the Left Leg of a Female Cadaver.** Resection of the gluteus maximus and removal of subcutaneous tissue was performed. (SGV – Superior gluteal vessels, PRF – piriformis, GMed – Gluteus medius, GMax – Gluteus maximus, GRM – Gluteal region muscles, PFCN – Posterior femoral cutaneous nerve, TN – Tibial nerve, CPN – Common peroneal nerve, STen – Semitendinosus, LHBF – Long head of biceps femoris)

During the process of cleaning the anterior portion of the GMax, it was observed that the superior gluteal vessels (SGV) including the artery, vein and nerve were perfectly preserved in the fascia located in the superior region and on the anterior side of the GMax (Fig. 4). A piece of a large vessel was also observed attached to the inferior region of fascia on the anterior aspect of the GMax. The identity of this vessel, as a part of the posterior femoral cutaneous nerve (PFCN), was not discovered until after the contents of the gluteal region were cleaned.

The first structure in the gluteal region clearly identified was the PRF muscle based on the triangular shape and the superior medial location (Fig. 5). The pathways of the superior and inferior gluteal vessels (IGV) were clearly seen above and below the muscle (Fig. 5). It was noted that the PRF was very atrophied.

After cleaning around the PRF, it was observed that three additional vessels emerged from the inferior region. The most medial vessel was cut and seemed to fit with the piece of a vessel remaining on the GMax. Thus, this vessel was identified as the PFCN (Fig. 4 and Fig. 6). The lateral two vessels followed paths consistent with the sciatic nerve (10). Therefore, the most lateral

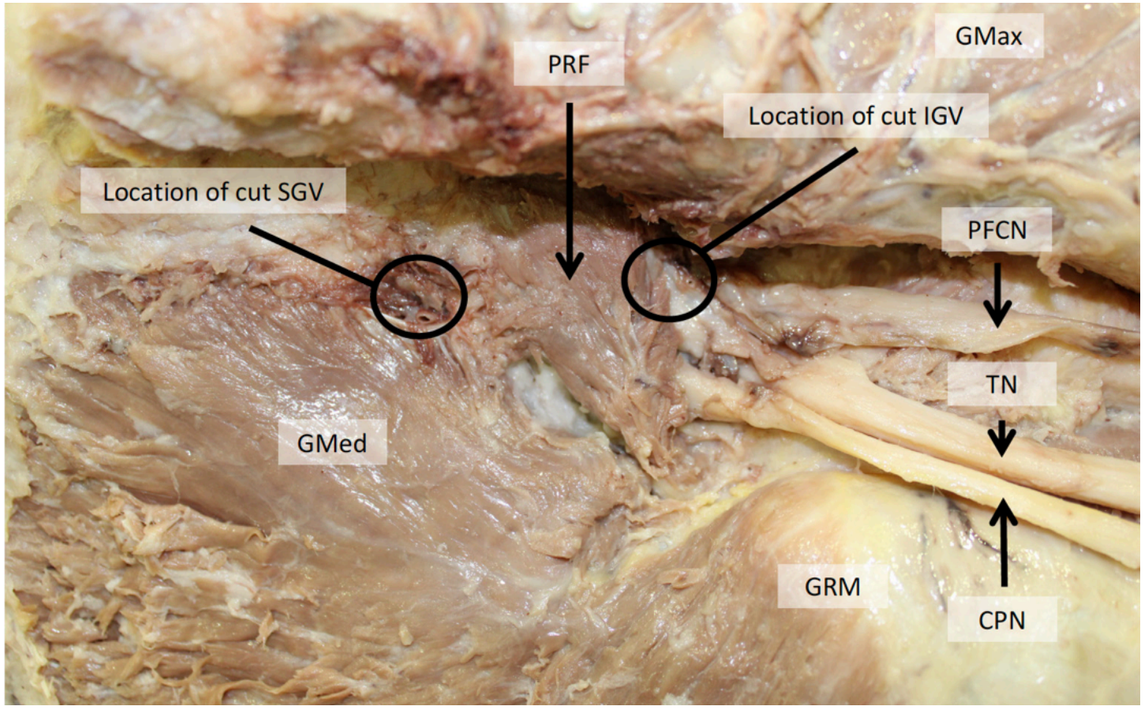

Figure 5: **Piriformis as a Landmark in the Gluteal Region.** Piriformis muscle was used to identify the superior gluteal vessels, inferior gluteal vessels, posterior femoral cutaneous nerve, and divisions of the sciatic nerve. (SGV – Superior gluteal vessels, IGV – Inferior gluteal vessels, PRF – piriformis, GMed – Gluteus medius, GMax – Gluteus maximus, GRM – Gluteal region muscles, PFCN – Posterior femoral cutaneous nerve, TN – Tibial nerve, CPN – Common peroneal nerve)

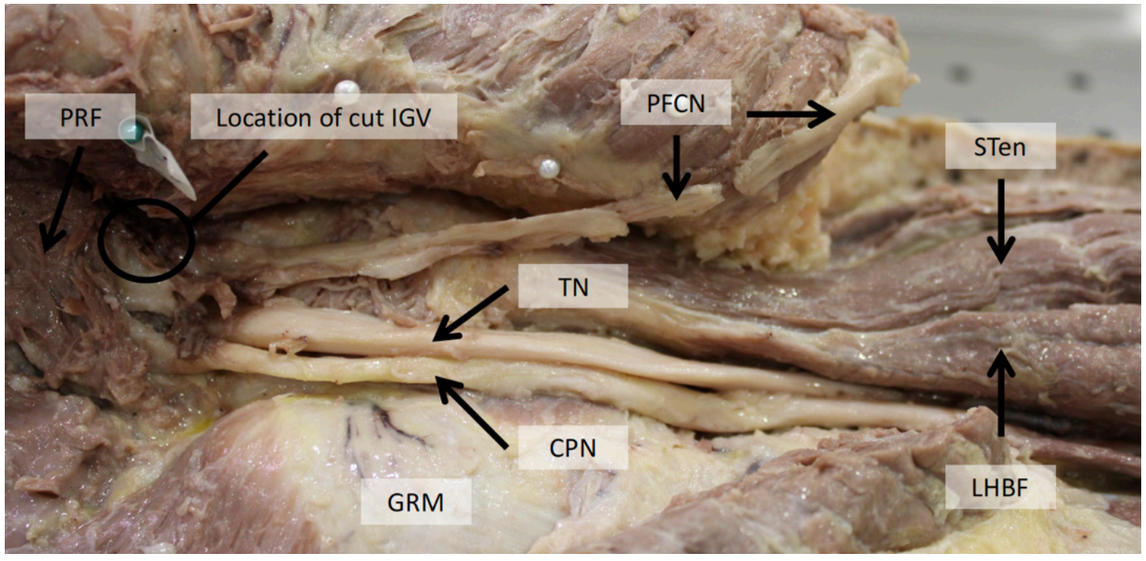

Figure 6: **Pathway Followed by the Divisions of the Sciatic Nerve in the Gluteal Region.** In this prosection, the divisions of the sciatic nerve, the tibial and common peroneal nerves, pass over the gluteal region muscles including the obturator internus, superior gemellus, inferior gemellus, and quadratus femoris before entering the posterior region of the thigh. The posterior femoral cutaneous nerve was cut during the dissection, but a piece of the vessel remained on the gluteus maximus. The remaining portions of the posterior femoral cutaneous vessel could not be demonstrated in this dissection. (IGV – Inferior gluteal vessels, PRF – piriformis, GMax – Gluteus maximus, GRM – Gluteal region muscles, PFCN – Posterior femoral cutaneous nerve, TN – Tibial nerve, CPN – Common peroneal nerve, STen – Semitendiosus, LHBF – Long head of biceps femoris)

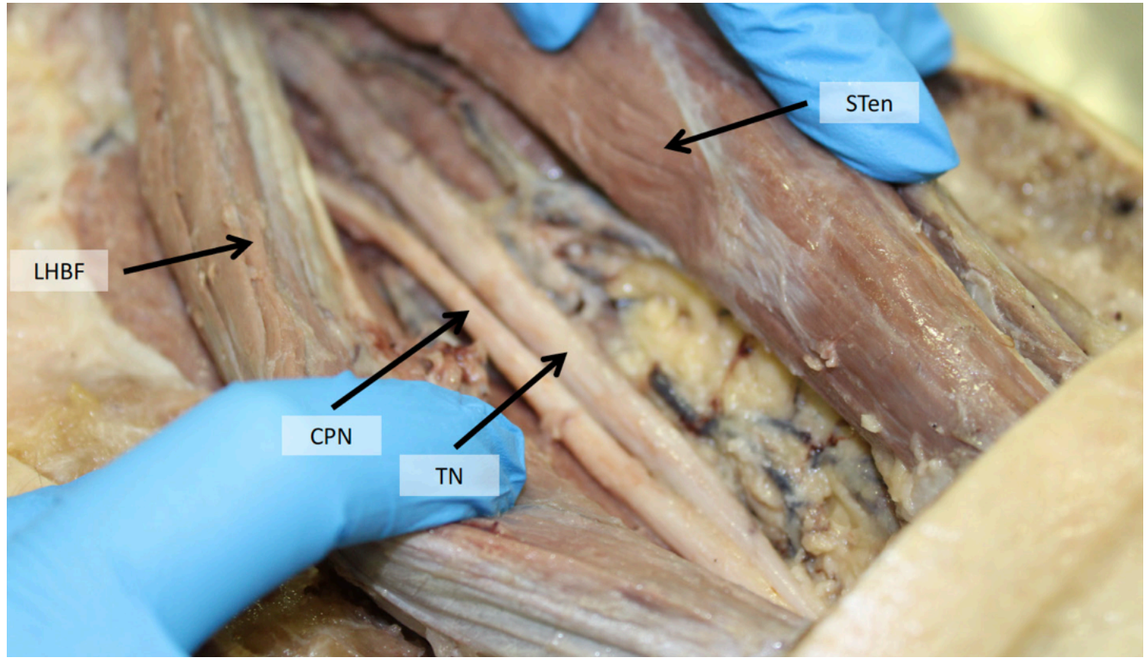

Figure 7: **Pathway Followed by the Divisions of the Sciatic Nerve in the Posterior Thigh Region.** In this prosection, the divisions of the sciatic nerve, the tibial and common peroneal nerves, travel between the semitendinosus and long head of the biceps femoris in the posterior region of the thigh. (TN – Tibial nerve, CPN – Common peroneal nerve, STen – Semitendiosus, LHBF – Long head of biceps femoris)

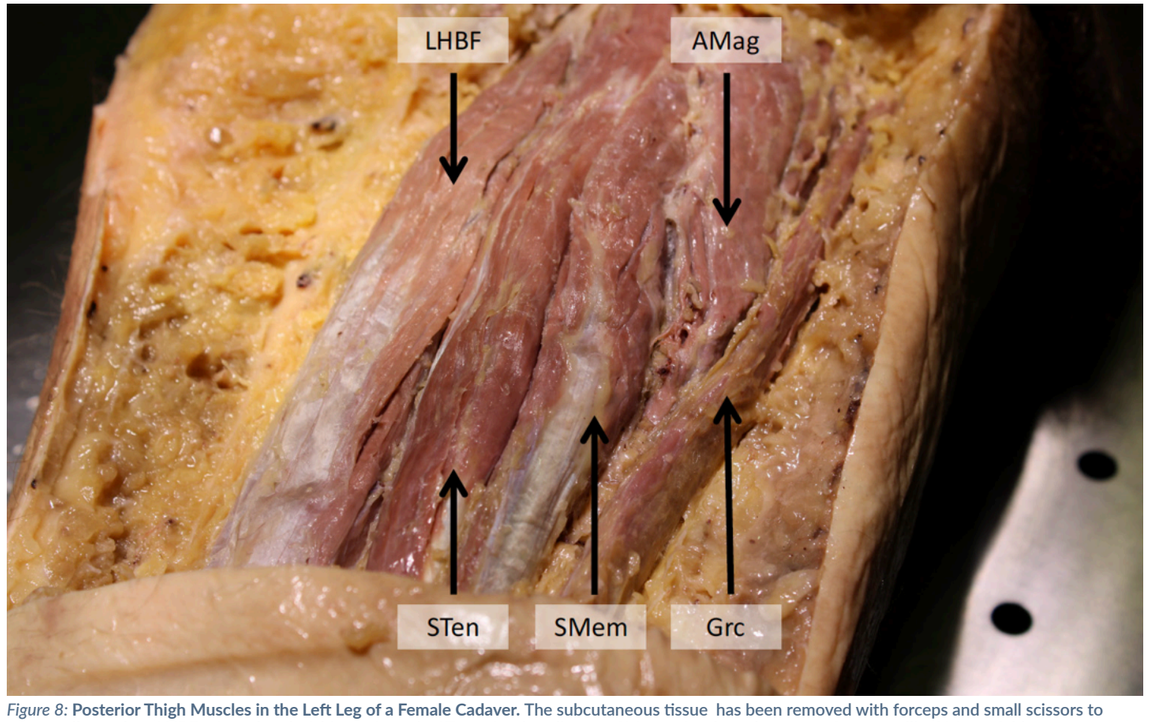

Figure 8: **Posterior Thigh Muscles in the Left Leg of a Female Cadaver.** The subcutaneous tissue has been removed with forceps and small scissors to expose all muscles. (LHBF – Long head of biceps femoris, STen – Semitendinosus, SMem – Semimembranosus, Grc – gracilis, AMag – Adductor magnus)

vessel was identified as the common peroneal nerve (CPN) and the vessel medial to this one was identified as the tibial nerve (TN), two branches of the sciatic nerve.

Both branches of the sciatic nerve passed over the obturator internus, gemelli muscles, and the quadratus femoris before traveling between the long head of the biceps femoris (LHBF) and

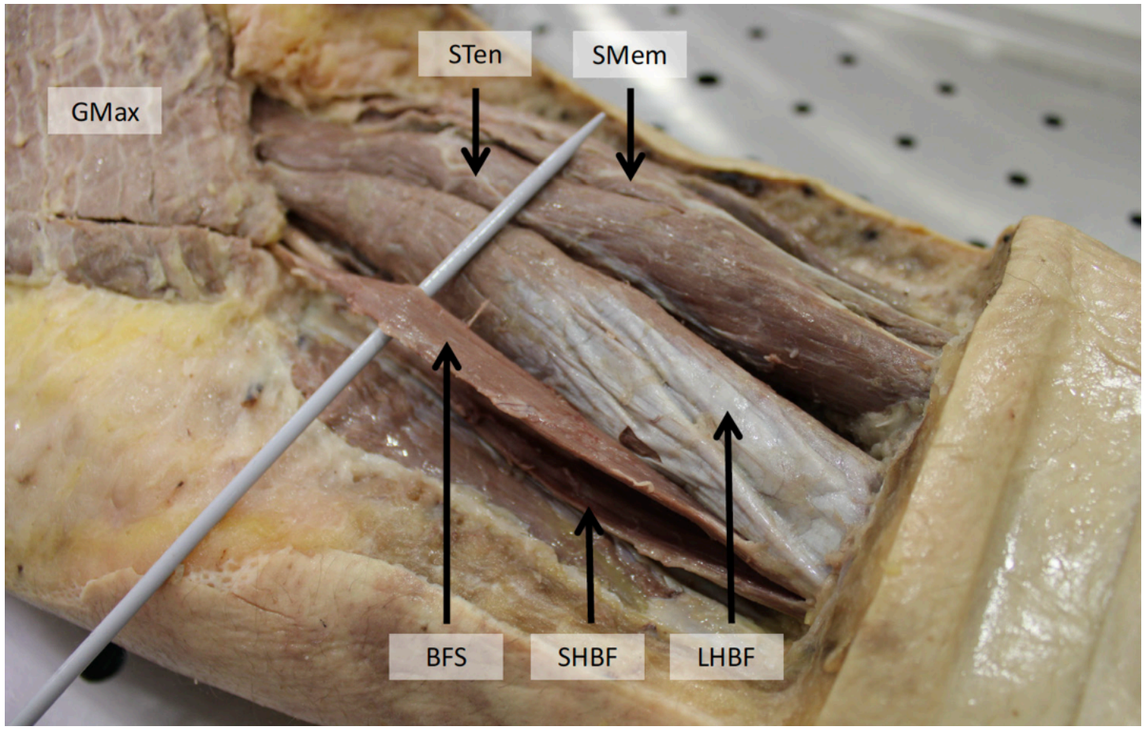

Figure 9: **Anatomical Variation of the Long Head of the Biceps Femoris.** A slip of muscle was identified lateral to the long head of the biceps femoris and medial to the short head of the biceps femoris. (GMax – Gluteus maximus, STen – Semitendiosus, SMem – Semimembranosus, LHBF – Long head of biceps femoris, BFS – Biceps femoris slip, SHBF – Short head of biceps femoris)

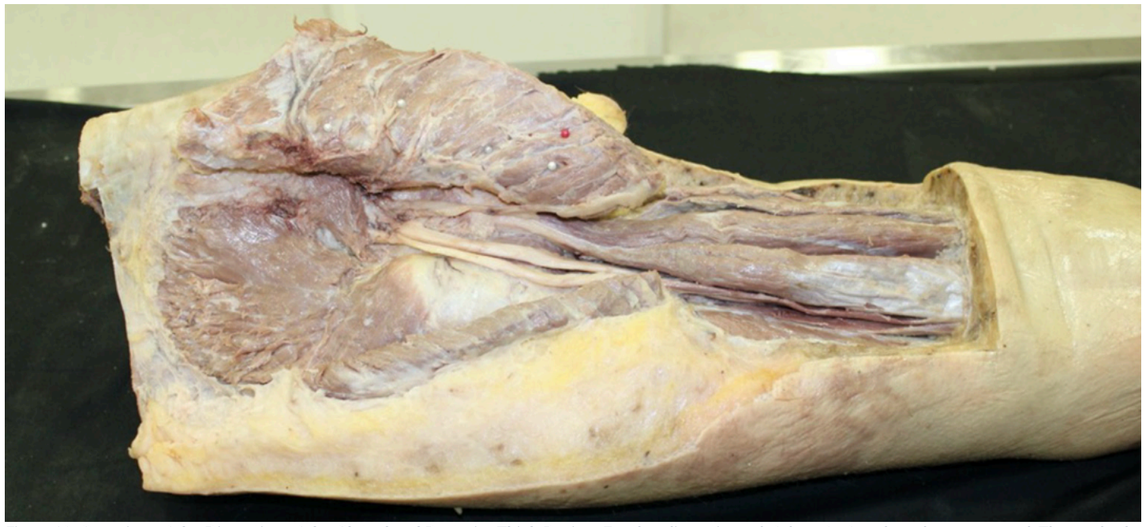

Figure 10. **Overview of the Dissection of the Gluteal and Posterior Thigh Region.** For the dissection, a left leg of a female cadaver was used. The methods and techniques used for the dissection are detailed in this report.

the semitendinosus (STen) muscle (Fig. 6 and Fig. 7).

The muscles of the posterior thigh are shown in Figure 8. The muscles were orientated anatomically as expected. The muscles in this compartment listed from most lateral to medial are LHBF, STen, SMem, adductor magnus (AMag) and gracilis (Grc). These muscles were embedded under a thinner layer of subcutaneous tissue compared to the GMax in the gluteal region.

Exploration of the posterior thigh led to discovery of a muscular slip lateral to the LHBF. The slip appeared to originate from the fascia surrounding the femur head, anterior to the GMax and medial to the insertion of the GMax on the gluteal tuberosity. It was thin, delicate, highly mobile and continued alongside the LHBF into the lower leg (Fig. 9).

Discussion

The divisions of the sciatic nerve were revealed in this dissection after medial resection of GMax. Medial resection is the dominant surgical method used when attempting to expose and treat lesions of the proximal sciatic nerve (11). The sciatic nerve innervates all posterior thigh muscles, all foot and ankle muscles, and provides sensation to skin in the lower limb (10). More specifically, the TN supplies the muscles of the posterior compartment of the leg (12) and the CPN supplies the lateral and anterior compartment (13). In four previous studies, it was observed that the sciatic nerve could divide into its divisions either before or after entering the pelvis (14-17). Unfortunately, it is not possible to determine where the sciatic nerve divided in the current project because the PRF was blocking visualization of the nerve's exit through the greater sciatic foreman. If the PRF had not been atrophied, a window or resection of the PRF might have been considered. Atrophy of muscles is experienced by patients who experience extended bed rest (18). It was decided not to dissect the PRF as not to reduce the educational value of the prosection. The sciatic nerve can also divide at various locations after entering the pelvis. The most common location is at the upper angle of the popliteal fossa (14). In one study, 8% of cadavers acquired from India, contained a sciatic nerve characterized by high division in the posterior thigh (14). Rare instances of the sciatic nerve entering the gluteal region from above the PRF have also been observed (19). In the current prosection, the sciatic nerve exhibited a rare occurrence of division before emerging from below the PRF.

An important and rare anatomical variation was discovered in the posterior thigh. There was a BF slip found lateral to the LHBF. BF slips have been previously reported in the literature (20). Additional anatomical variations of the BF have been reported including absence of the short head and presence of additional heads (21). Further research is required to understand the impact of these anatomical variations on the clinical presentation of a patient and the physical challenges that might be encountered.

There were a few limitations to the project. After the gluteal region was cleaned, it was determined that the GMed would be too small and frail to transect. In addition, the obturator internus, gemelli muscles and the quadratus femoris were difficult to distinguish from each other unlike in anatomical textbooks. There was a tough, white layer of fascia covering the lateral regions of these muscles that could not be cut with a scalpel. Thus, it was decided not to expose the insertion sites on the head of the femur. Despite these limitations, the main objective of the project to observe the path of the sciatic nerve and its divisions through the gluteal region and posterior thigh of the left leg of a female cadaver was achieved.

Conclusion

The ultimate goal of this dissection was to discern the path of the sciatic nerve and its divisions. Most of the steps in the dissection plan were completed, although in a slightly different order. The steps that could not be completed included transection of the gluteus medius, exposure of the insertion points on the head of the femur and cutting the PRF to observe the greater sciatic foramen. Performing these steps was reconsidered after encountering a few unexpected issues including severe muscle atrophy and a layer of fascia that could not be cut with the tools available. However, upon completion of the project, the path of the sciatic nerve divisions was visible.

The inspiration behind the project was the sciatic nerve. However, during the dissection, decisions were made to preserve anatomical structures that were not mentioned in the original dissection plan. For example, the PFCN and posterior gluteal muscles. The dissection also revealed an anatomical variation, a muscular slip lateral to the LHBF. This cadaver will serve well for educational purposes as it demonstrates that not all human bodies have the same anatomy, a difficult concept to grasp in first year anatomy classes.

In conclusion, this project provided a greater understanding of human anatomy that could not be achieved solely with the use of a textbook. The cadaver will serve as a useful educational resource for future students to understand the clinical relevance of various medical conditions and clinical procedures related to the gluteal region. In addition, the format of the prosection provides a unique experience as it unfolds like a book and allows the demonstrator to explain to the students the various layers that conceal the gluteal region. Telling a story while teaching the students could help with information retention and make the learning process more fun.

Acknowledgments

I would like to thank Dr. André Toulouse and Mr. Michael Cronin for the opportunity to learn human anatomy through dissection. Thank you for coordinating the class and providing excellent advice on dissection techniques and anatomical structure identification. I would also like to thank the teaching assistants Audrey, Patricia, and Roisin for their constant and unwavering encouragement, without which I wouldn't have made a single cut. Finally, I would like to thank the donors and their families for providing this educational experience not only for myself, but for the future students who will benefit from this project. Every care was taken to respect the cadaver and in doing this, respect the donor's life and their family. I hope that this project will be as educational for future students as it was for myself.

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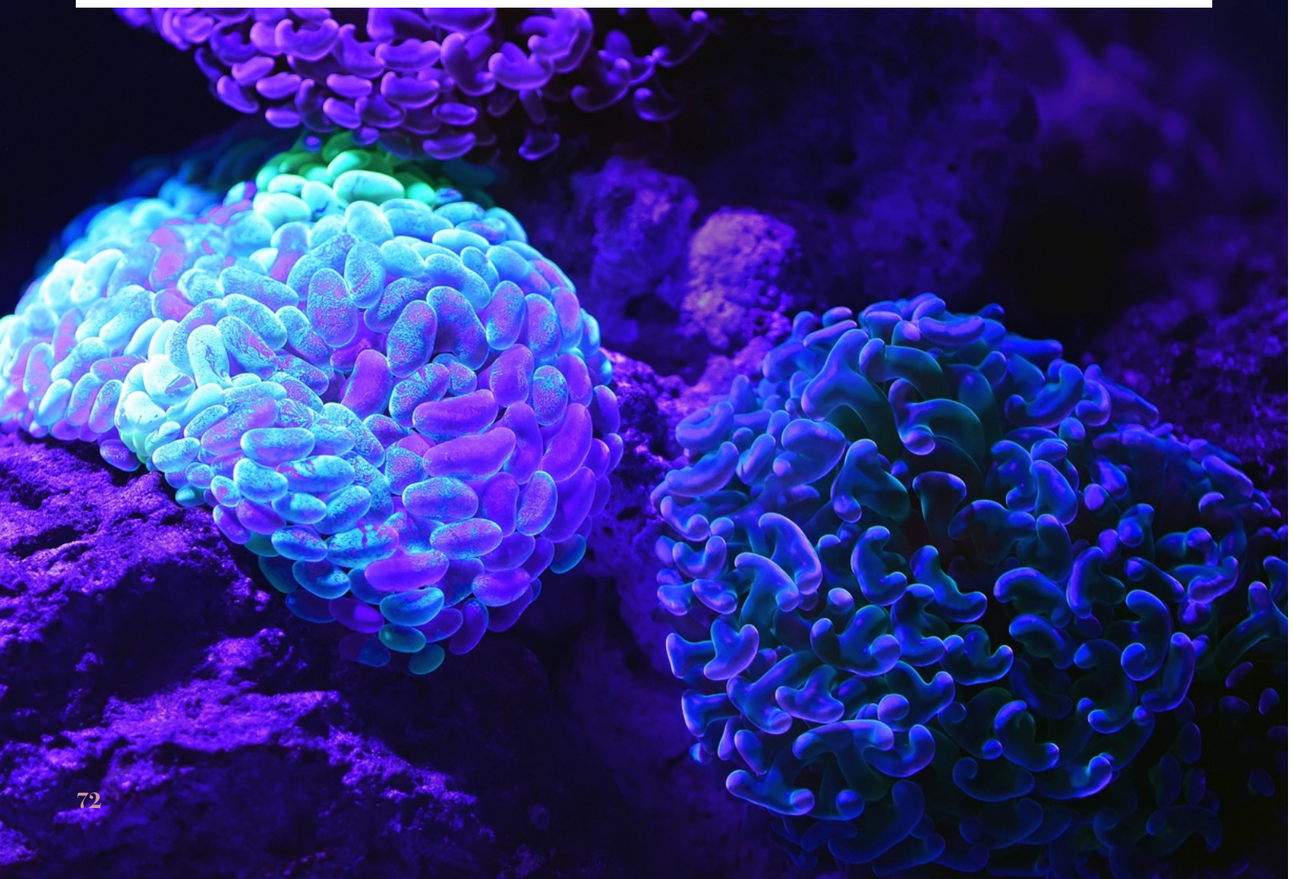
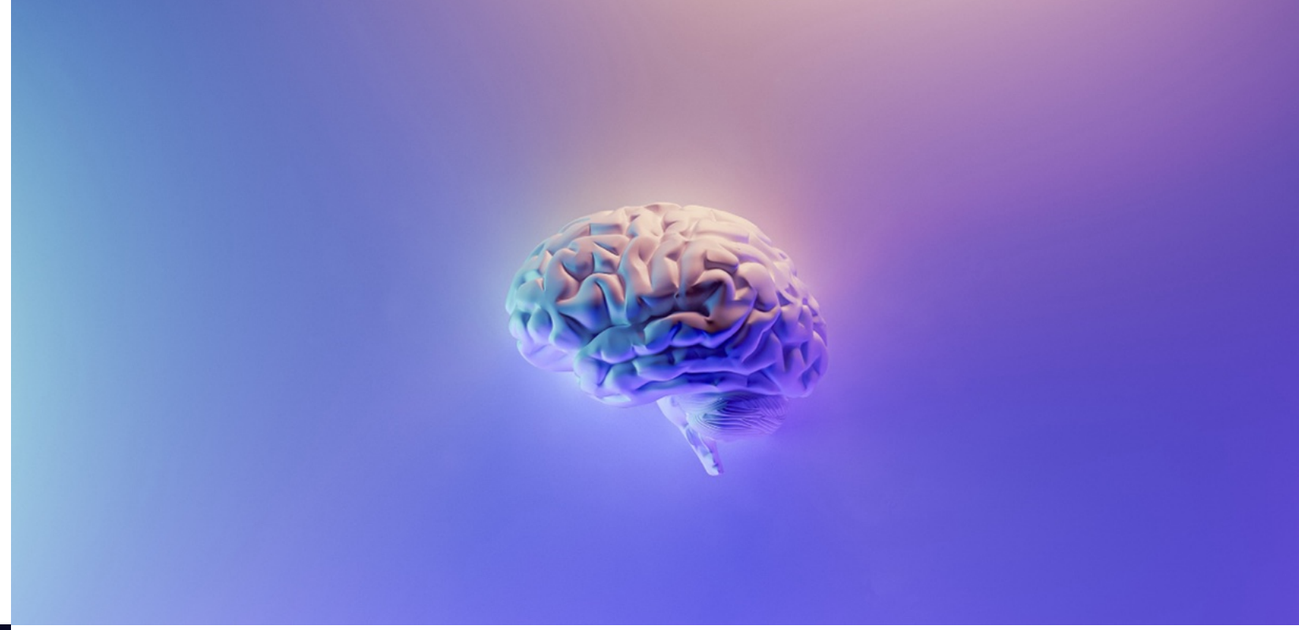
Behavioral Addictions and Psychosis

KASHAF AAMER

Abstract

M.K* is a 27-year-old, single, unemployed male that was driven to the Emergency Department by his foster father, four weeks ago with first episode of psychiatric symptoms of tactile hallucinations, auditory hallucinations of male and female voices making inappropriate sexual comments about him, persecutory delusions and delusions of control for the past five days. M.K is a daily cannabis user of 0.5-1g use for the past 2 years and has 5.5 pack year smoking history. M.K also reports having a pornography and masturbation addiction for 2 years with marked intrusive thoughts of sexual nature the past week. M.K has no medical or psychiatric history. Recent stressor was moving out of foster father's house 8 days ago. The symptoms led to significant impact at work which he has quit since then. On presentation, M. K showed partial insight into his third person running commentary hallucinations but believed adamantly that he was being watched, followed and controlled. He reported hearing his female neighbour stating that he has sexual interest in animals and later heard his manager making pedophilic allegations on him. M.K was so distressed, he quit work and isolated himself in his room. He reported poor concentration and lack of appetite for the past week. M.K describes his baseline mood to have "always been not the greatest" and denied anhedonia, fatigue, or sleep changes. He denied thoughts of self-harm, harm to others or suicidal ideation. Four weeks following inpatient, referral to HBCT and cannabis abstinence has shown improvement in psychiatric symptoms with absence of hallucinations, however, there has been increased symptoms of anxiety with "panic attacks". M.K's biological mother is reported to have history of substance misuse and sister with depressive symptoms. He continues to withdraw from society with increased time spent indoors gaming and shows emotional dependence on foster father.





Discussion

Substance addictions, particularly cannabis, has been shown to be associated with psychosis. When cannabis-induced psychosis is differentiated against schizophrenia, minimal clinical differences are found, in fact, a lower age of admission is noted in cannabis users marking it as a precipitator of psychosis (1). Additionally, eleven percent of substance-induced psychosis cases from a study with 7,606 participants displayed progression to schizophrenia (2). Of these eleven percent, 18% were cannabis users and familial history of psychosis played a major role in disease progression (2). The study however displayed 89% of individuals that did not progress to schizophrenia suggesting that cannabis use is a potent precipitator in vulnerable individuals rather than a disease cause (2). Such discussions are explored in plethora of literature, as well in the DSM-V and ICD11 which explore in depth the psychiatric effect of various substances from intoxication to withdrawal.

However, perhaps due to public health efforts on reducing substances and alcohol dependency, there may be underassessment of other forms of addictions that can be equally distressing on patient life (3). Due to potent biological along with psychiatric side effects of substance misuse, there is profound research in that field, however behavioral addictions (BA) such as gambling that may have minimal biological implications, still impose significant psychological stress that may result in downstream biological manifestations. As well, literature states that 54.9% of given sample with BA have co-existence with substance abuse (3).

When explored in depth, there appears to be a neuroscientific overlap between substance abuse and behavioral addictions. Neuroscience of addictions regardless of subtype, affects the mesolimbic dopamine pathway which projects to nucleus accumbens, known as the reward center (4). The three-stage model of addictions describes an intoxication phase, withdrawal affect and anticipation (4). The intoxication phase consists of dopamine surge which leads to positive re-enforcement while the withdrawal affect creates a tolerance requiring higher future exposure (4). Anticipation or craving is described as the potential source of chronic relapse (4). According to literature review, there has been "overlap in multiple areas" of substance abuse and behavioral addictions including neurobiology (dopamine), course, comorbidities, and tolerance (4). Clinical literature explores in depth the etiology of dopamine surge in substance induced psychosis, yet there is lack of concrete supportive evidence behind behavioral addictions and their role in psychosis, despite both having a similar etiology. Although therapies and counselling services exist individually for various types of addictions, pornography addiction is yet to be recognised in the DSM-V as a mental health disorder. Although, the DSM-V was updated from *Substance related disorders to Substance-related and Addictive Disorders* (3), manuals such as ICD11 only recognise gambling and online/offline gaming as *Disorders of Addictive Behavior* (5). Whereas BAs ranging from shopping, work, computer, and sex/pornography coexist in substance abusing cohort at higher prevalence than gambling addictions (3). Thus, literature exploring correlation between behavioral addictions and psychosis may perhaps be a future exploration topic given the similar neurobiology with recognised disorders of substance abuse.

On the other hand, ICD11 does explore sexual behaviors such as masturbation and pornography under *Impulse control disorders*

specifically *Compulsive sexual behavior disorder* and outlines a thorough diagnostic criteria for difficulty controlling sexual impulses with unsuccessful efforts to reduce activity despite little satisfaction (5). It also specifies exclusion criteria where in the presence of substance use, this disorder cannot be diagnosed (5). This is where M.K does not fit into this diagnosis due to coexistence of substance misuse with behavioral addictions of gaming, music, pornography and masturbation. If his case is viewed from a black and white lens of substance induced psychosis, his symptoms of persecutory delusions, third person running commentary, and depersonalisation may be explainable from surplus of literature and ICD11 regarding cannabis induced psychosis (5). However, it is important to note that M. K's main psychological distress is caused by his intrusive sexual thoughts and paranoia of being viewed as a pedophile or having interest in beastiality. He denies sexual arousal by these individuals excluding a paraphilic disorder according to ICD11. Instead, the basis of his thought broadcasting is fear of having his sexual thoughts being exposed. Additionally, his tactile hallucinations of legs vibrating and passivity phenomena of being under "erotic hypnosis" suggest an underlying sexual obsession which may be explained with two years of daily pornography addiction rather than cannabis use alone.

In case of M.K where both daily cannabis and pornography consumption overlap to two-year history suggests a multifactorial presentation of psychoses and this should be reflected in his bio-psycho-social management plan. A management plan of antipsychotics and drug abstinence without adequate support for his behavioral addictions fails to create an individualised care plan catered to psycho-social presentation of this patient. In such a case, symptoms of sexual delusions could perhaps recur given that underlying addiction was not addressed, however such a claim is difficult to make due to limited research in this area. Perhaps future research into behavioral addictions and psychiatric manifestations with adequate screening tools may provide a more comprehensive individualised care plan for such cases.

*M.K is a fictional initial to protect patient confidentiality.

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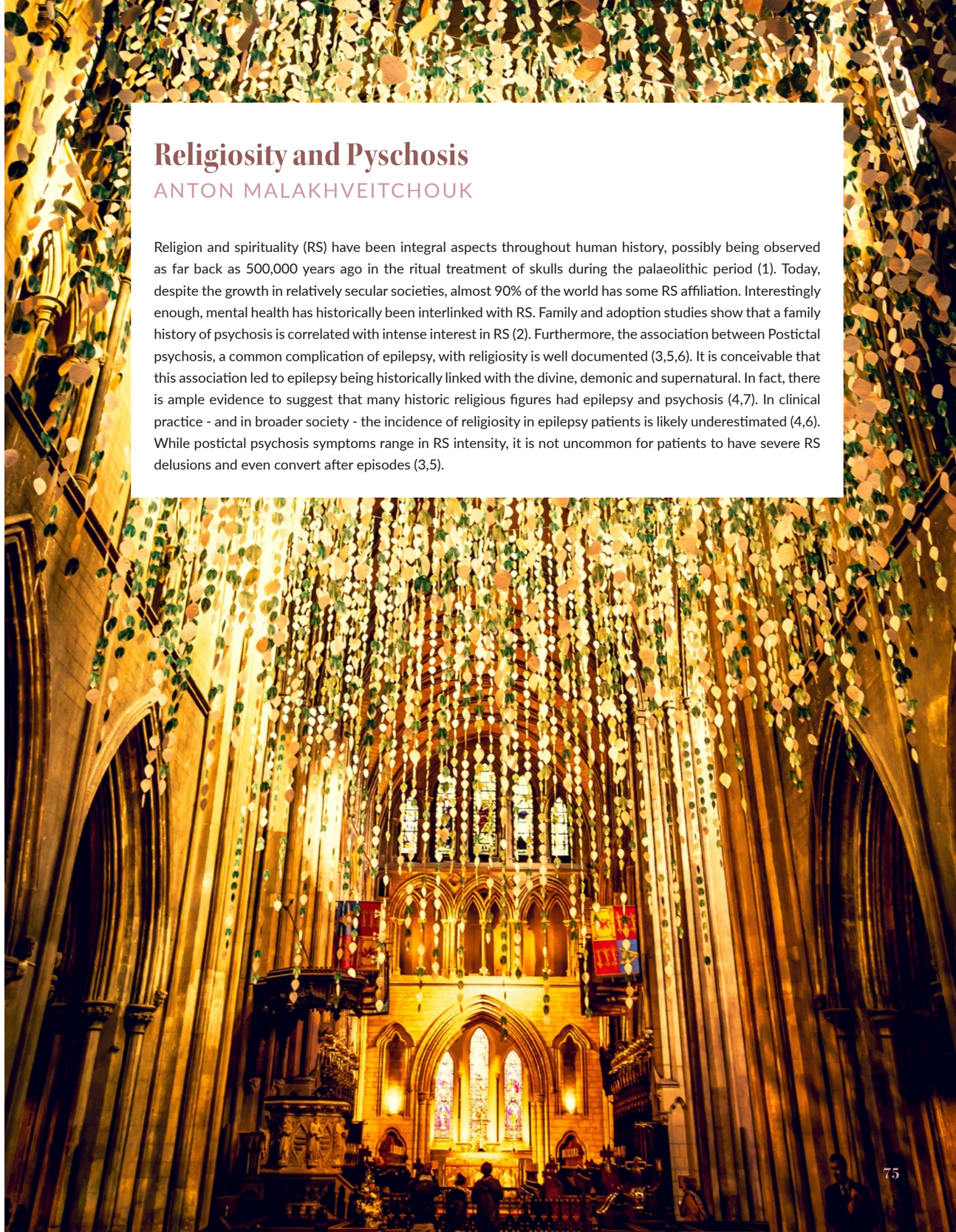
Religiosity and Pyschosis

ANTON MALAKHVEITCHOUK

Religion and spirituality (RS) have been integral aspects throughout human history, possibly being observed as far back as 500,000 years ago in the ritual treatment of skulls during the palaeolithic period (1). Today, despite the growth in relatively secular societies, almost 90% of the world has some RS affiliation. Interestingly enough, mental health has historically been interlinked with RS. Family and adoption studies show that a family history of psychosis is correlated with intense interest in RS (2). Furthermore, the association between Postictal psychosis, a common complication of epilepsy, with religiosity is well documented (3,5,6). It is conceivable that this association led to epilepsy being historically linked with the divine, demonic and supernatural. In fact, there is ample evidence to suggest that many historic religious figures had epilepsy and psychosis (4,7). In clinical practice - and in broader society - the incidence of religiosity in epilepsy patients is likely underestimated (4,6). While postictal psychosis symptoms range in RS intensity, it is not uncommon for patients to have severe RS delusions and even convert after episodes (3,5).



HOW CAN WE DISTINGUISH RELIGIOSITY BETWEEN A PSYCHOTIC EPISODE AND THAT OF AN RS EXPERIENCE?

To answer this question, we must first examine the similarities and differences between the two conditions.

There are many similarities between psychosis and RS experiences. Delusions of reference in psychosis may be analogous to the ideas held by RS believers that mundane experiences have a special and significant purpose (2). Furthermore, there are underlying commonalities between neurological pathways of RS experiences and the mechanisms of psychosis. A decrease in orbitofrontal cortex volume, for example, has been implicated in both people who fear God or in people with psychotic symptoms (1). Other associated areas include the medial frontal cortex, precuneus, posterior cingulate cortex and the caudate nucleus (1). More broadly, studies suggest that the right hemisphere is implicated in experiential and personality features related to the RS self, with the right temporal lobe involved in the experience of intense RS phenomena and the right frontal lobe involved in elements of personality, such as social, political and RS values (3). There is also evidence that implicates temporolimbic dysfunction in psychosis, especially with paranormal or spiritual events (8).

IS RELIGIOSITY AN EXPRESSION OF PSYCHOSIS?

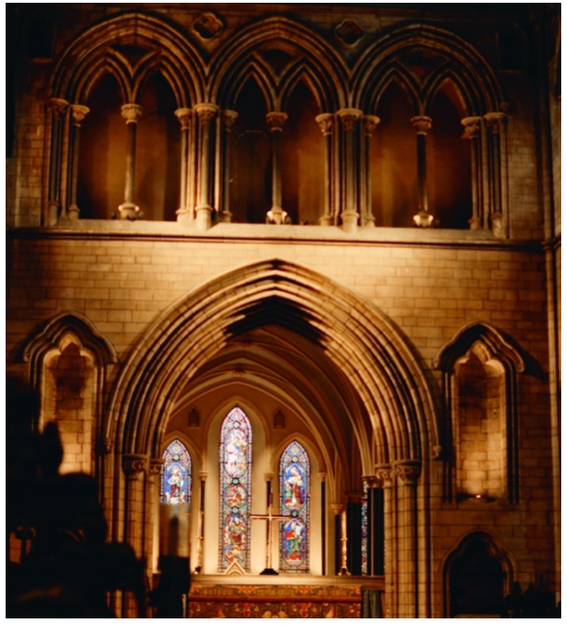
Not quite. A major difference between RS experiences and psychosis is a matter of insight. Ng (2007) suggests that psychosis may arise from varying contributions of abnormal perceptual experiences and abnormal interpretation of normal experiences, and RS is a common theme due to its intrinsic and cultural significance to humans (8). Thus, certain neurological pathways activated in a brain undergoing psychosis may trigger areas that experience and store RS information. Episodes of 'RS psychosis' triggered by major life events, psychoactive substance use or repetitive environmental stimuli tend to involve the deautomatization of habitual stimulus selecting and organising mechanisms (9). Analyses of autobiographical accounts from patients with mystic RS experiences indicated increased sense of inward attention, enhanced perception, and feelings of union with supernatural and divine powers while auditory hallucinations and thought disorder, common in psychosis, were not characteristic features (9). Therefore, a hallucination during a RS visionary experience is associated with a positive prognosis if insight is not lost because even though there may be a perception disorder, there is no associated judgement disorder (9,10). Similarly, Dein & Littlewood (2011) postulate that a difference between RS cognition and psychosis lies in the ability to attribute mental states to other people, enabling the individual to understand the behaviour of others and themselves (2). These findings would help explain the association between postictal psychosis and religiosity: some neuropathological process stimulates the areas responsible for RS. Furthermore, these studies suggest that a major difference between psychosis and RS experience has to do with the individual's level of introspection and insight.

IS RELIGIOSITY A PATHOLOGICAL FINDING IN PATIENTS WITH PSYCHOSIS?

Religiosity is one of the few enduring themes observed across cultures in postictal psychosis, however it appears that they have become less frequent over the last century, possibly reflecting a societal shift towards an increasingly secular world (8). These findings make sense when we consider that RS feelings exist within the psychosocial context of where individuals live (4,6). There is evidence that mental health professionals often overdiagnose psychosis during routine examinations of individuals with RS problems (9). RS can be a resource for enriching health and well-being. Among psychiatric patients, healthy RS practices and beliefs may reduce the fear, isolation and loss of control experienced during a psychotic episode (4). Koenig (2009) argues that religiosity is a powerful coping behaviour through which people can make sense of the world, their suffering and of the forces of nature - all while promoting social rules that facilitate cooperation and social support (4). Menzes and Moreira-Almeida (2010) suggest criteria in which a RS experience may be considered non-pathological: if there is an absence of psychological suffering, an absence of social or occupational impediment, the experience is of a short duration, there is a critical attitude regarding the experience, there is some compatibility of the experience with some RS tradition, the individual gains a more enriching understanding of their life and the individual is concerned with helping others (7).

The link between psychosis and religiosity leads us to many interesting questions that touch at a core part of the human experience. It would be fascinating to delve into the implications of RS regions in the brain and the complex interplay between RS as external factors that influence our internal thoughts and development, and in turn this internal development feeding back into the broader societal RS consciousness. RS, along with any modern ideologies, is likely part of the same evolutionary process that gave rise to abstract thought in homo sapiens that enabled their global dominance. Ideologies provide a framework for broader societal cooperation, the major advantage of our species. The triggering of an altered state of consciousness may provide diversity for selective pressures to act on and shape the course of history. What we can say for certain is that RS exists as an integral part of humanity and postictal psychosis and RS have numerous historical and biological associations with the same brain regions likely implicated in both processes. However, a major difference between RS experiences and psychosis is the unimpaired insight seen in the RS experiences, which may be a possible mechanism behind the benefits associated with RS experiences.

Finally, it is also important to mention that RS beliefs and delusions are increasingly difficult to define, which has implications on psychiatric practice. The rise in alternative spiritual movements, multicultural influences, new age mysticism and spiritual-but-not-religious attitudes make it difficult to access normality from false beliefs.9 It is, however, vital for psychiatrists to differentiate harmful beliefs from benign or beneficial RS to avoid harmful medicalization that can lead to stigmatisation. Clinicians should be aware of the RS lives of their patients and differentiate normative practices beneficial for healthy social functioning from distorted beliefs that limit the patient and contribute to their pathology (4,7). Fortunately, modern day attitudes in psychiatry towards RS have begun to shift, and some training schemes now incorporate training on RS factors that influence psychological development (4). Hopefully this trend continues as further research into the associations between psychosis and RS helps in the treatment of patients and in uncovering the bewitching complexities of the mind.



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Lille Erasmus Experience

LENA DABLOUK

I never expected my first full-time hospital placement to be in a foreign country where I wasn't completely fluent in the native language. The experience was equally exciting as it was daunting. It definitely brought with it various challenges, however I would absolutely do it again if given the chance.

The nerves were high on my first day of placement, so much so that I almost forgot it was my birthday. It was 8am as I walked into the << Hopital Salengro >> and made my way down to the emergency department. I was met by the noise of loud machinery and an overflow of patients being tended to by doctors and nurses. The first difference was immediately evident, the doctors and medical students were wearing white coats, a tradition which we seldom see in our Irish hospitals anymore.

The differences between the Irish and French health systems were unveiled over the next two months. One of the starkest differences is in the medical education system itself, something I noticed from my first day in the hospital. I spent a lot of my days of emergency medicine placement shadowing the << externs >>. These are 4th-6th year medical students who are extremely knowledgeable and carry out a lot of the work as the interns in Ireland do. It was easy to get the senior years mixed up with the doctors themselves, as they were confidently admitting patients and writing up their notes in the charts alone. This trust made for a great learning experience as I eventually got extremely comfortable examining patients and presenting histories back to the doctors. One of the most challenging things to adapt to during my time at the hospital was interpreting charts and getting used to the different medical abbreviations used in France. Although these are second nature when reading a chart here, acclimating to this while in Lille definitely took a few days.

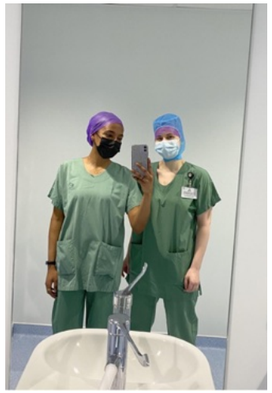
All of the charts in France are electronic, which is something we are yet to see be fully implemented by all departments in Irish hospitals. Patient notes are immediately typed into the computer software instead of being handwritten, allowing patient details to be accessed in any of the hospital departments immediately. This allows for smoother communication between specialties when referring patients. This was especially important in the << Centre Hospitalier de Lille >>, as the hospital complex was made up of ten separate hospitals, with each one containing departments for various specialties.

The weekends were a great time to explore the beautiful city of Lille, which is situated in the North of France, bordering Belgium. This means that Flemish architecture and traditions are prevalent throughout the city, permeating its culture and cuisine. << La Grand Place >> was our usual meeting point, a quaint market square with many restaurants and cafes surrounding it. <<Vieux Lille>> boasts cobblestone streets and red-brick buildings which made for a scenic backdrop every time we explored it. We spent hours in the << Vieille Bourse >>, which was the Old Stock Exchange located in the heart of the city that sells impressive artwork, books and posters.

Once it was time for a snack, we were spoiled for choice: traditional fries in <<Friterie Meuniere>> were always a popular choice. <<Meert>> sells delicious pastries and is located on a picturesque street in the city centre, where the façade alone would grab your attention and entice you to step inside. Here you'll find another one of Lille's delicacies known as <<gaufres>> or waffles, and Charles de Gaulle himself is known to have frequented the store in the past.

My second month was spent in the <<Institut Coeur Poumon>>, which is ranked second in all of France for cardiothoracic surgery. As someone interested in this field, I was extremely excited to spend a full month there. The hospital was extremely modern and students received frequent tutorials and teaching from various members of the team. My first time scrubbing into a surgery was a surreal experience and happened to be during my placement in Lille. I made my way into the <<Bloc Operatoire>> and scrubbed in to watch an aortic valve replacement, where the consultant guided me through the steps and allowed me to assist where possible.

I am extremely grateful to have been given the opportunity to spend two months abroad in the CHU de Lille as part of the UCC Erasmus + programme. The teaching I received over the two months was incredibly valuable, and so were the memories I made during my time there.



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