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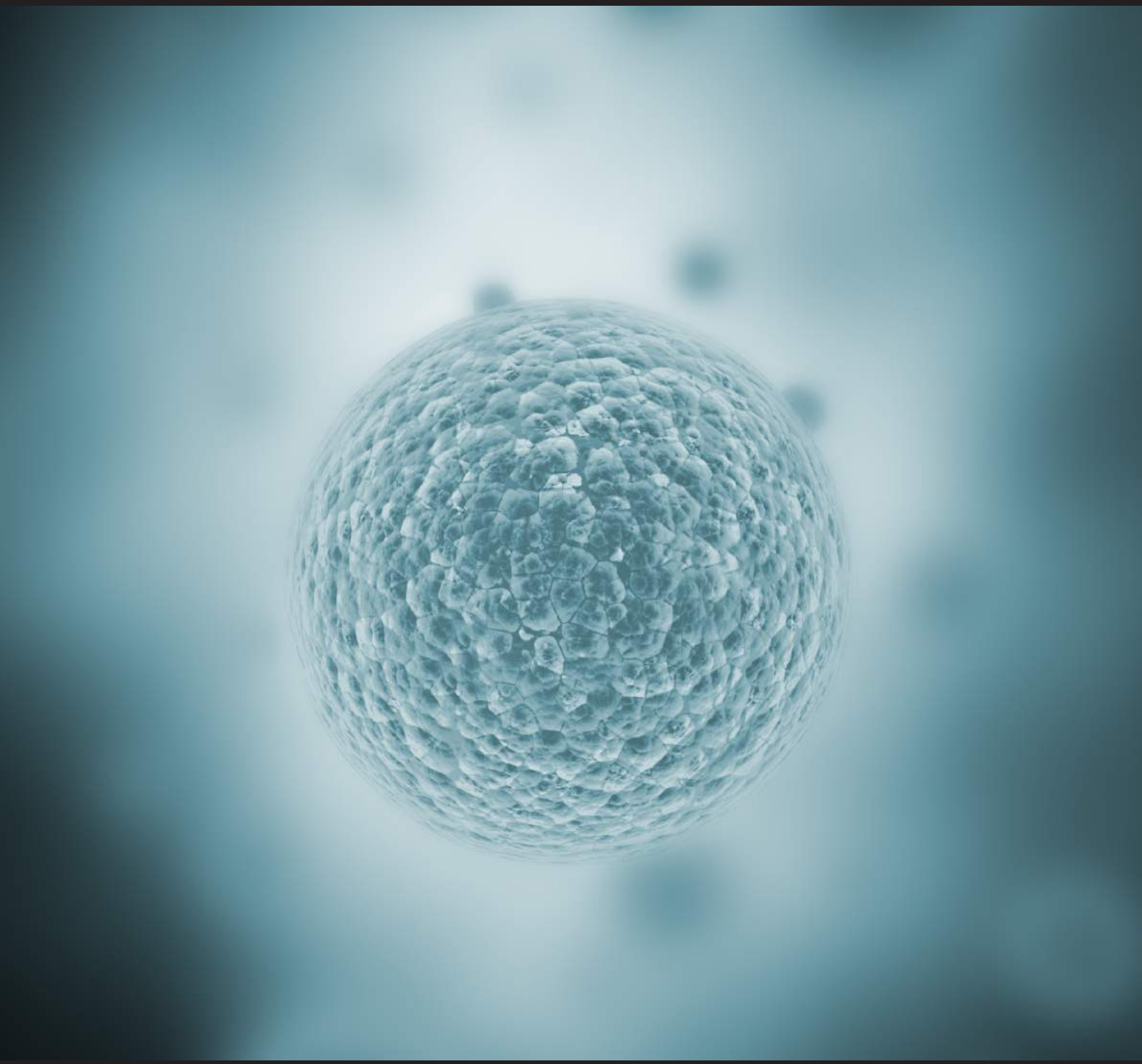
UCDsmj

student medical journal



ORIGINAL RESEARCH
LITERATURE REVIEWS
FEATURES

Expert article
Healthcare perspectives

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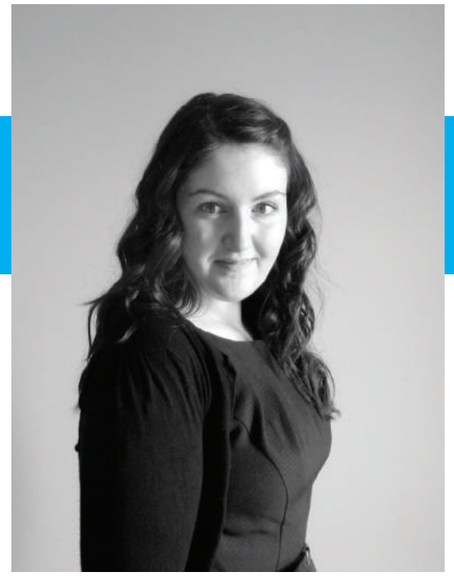
FOREWORD



Aileen Conway
Editor, Co-Founder.

Denise Connolly
Editor, Co-Founder.

Many of us enter medicine with the idealistic view that we can help people, that we can change and improve a healthcare system for the benefit of all patients. Whilst scoffed by some to be an unrealistic goal, one to be obliterated by 'real life', it is perhaps the one thing that we should hold on to as we navigate our way through our medical careers.

The pertinent question that always remains is: how can we affect change? It would appear that all change begins with something small, be it an experiment or a hypothesis put forward by an individual. These small actions raise awareness, generate discussion and challenge current best practices, eventually culminating in a change in patient management. Likewise, the UCDsmj began as a small step. More importantly, it now acts as a gateway to the exploration of the field of medicine, which will, we hope, drive future change. Within this journal we promote the publication of student-based laboratory and clinical research to demonstrate the evolutionary journey of a hypothesis to the generation of results. We also include review and expert articles that highlight new clinical developments as a means of encouraging students to gain contact with advances in evidence based practices and to promote the development of future clinicians that will hopefully place themselves at the forefront of the Irish healthcare system.

In medicine, there are inevitably some things we cannot learn from text books and lectures. The experiences of others around us can help develop our personal insight. Appreciating how an illness impacts on a patient's life, overall wellbeing and imposes itself upon a family and their environment can often be the 'pathology' that we easily, but unfortunately, miss. Some of our best teachers during our careers will be the patients and families that we meet along the way. Thus, we feel compelled to share their views through this journal. Written media is not the only forum through which we can encounter such experiences. We can also acquire these experiences for ourselves by organising medical electives, such as those reported in this volume, or through multimedia resources such as podcasts, which are becoming an increasingly important adjunct to our didactic learning as we progress through our degree.

It is almost two years since Norella Broderick gathered a small group of students with the aim of establishing UCD's own Student Medical Journal . Many people have helped us tremendously during this journey. Without their support, the publication of this first volume would have been very unlikely. In particular, we would like to thank Dr. Jason Last, Dr. Amanda McCann and Dr. Jane Dolan who have strongly supported the project from the outset. We would also like to thank Professor Powderly and the School of Medicine and Medical Science and the Societies' Office for their financial support and Bank of Ireland, Montrose for their sponsorship. Many thanks also to Mark Byrne of the School of Medicine and Medical Science for his guidance on the design and marketing of the journal. We would especially like to thank the students, both published and unpublished, who submitted their articles to us and endured many edits of their work. Without their committment, there would be no journal. We hope we have done them proud. Lastly, we would like to acknowledge the huge help, support and patience from our families during the past two years.

The path to the completion of this journal has been an arduous one. However, not once did we doubt the potential and positive impact that this could have on the current and future education of the students of School of Medicine and Medical Science. We hope that this potential is something that will become apparent to you all and something that we can share together.

We welcome any student who has an interest in medical research, writing, design, marketing, and information technology to join the UCDsmj team. This is a group-learning environment, whereby those with experience, share and reflect on this for the benefit of others. This is the ethos on which this journal was built, and upon this ethos it will grow.

Aileen Conway Denise Connolly

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The landscape of biomedical and clinical practice changes so rapidly that it is impossible for any medical education institution to accurately predict the factual knowledge to which our doctors, radiographers and scientists of the future should be exposed. This circumstance has led to an educational revolution that reduces the burden of fact, places greater emphasis on knowledge discovery and retains only those techniques, tools and tenants that remain fit for purpose. Juxtaposed to this knowledge explosion is the increasing clarity that the role of the doctor in caring, in healing and in patient advocacy remains steadfastly central to clinical practice. In turn, the role of the radiographer is increasingly advanced and diagnostic and that of the clinical scientist is exponentially pioneering. It is for these reasons that it is most gratifying to witness the UCD Student Medical Journal, emerging from this new generation of scholars, a testament to the importance of knowledge discovery, the central role of caring and the ambition to advance.

Within the pages of this volume are articles that have been penned or commissioned by students of the UCD School of Medicine & Medical Science, that explore perspectives inspired by both curricular activities and life experiences. Knowing the UCDsmj team, I am delighted but not surprised, to note that they have managed to craft a powerful vehicle for their peers, and have maintained a perfect balance between science, technology and personal reflection.

Having considered the local academic and social virtues of this entirely student-led initiative, let us not lose sight of the other fundamental values that this journal respects. In the context of the increasingly bleak economic environment, the temptation is to not invest in new knowledge but to batten down the hatches and ride the storm. Terms such as 'prioritisation' are used to blunt the reality of the cuts and the austerity society faces. The academic community is not immune to these cuts, nor should it be, but it is unfortunate that one key area of such prioritisation has been journal access in the Health Sciences library.

“Dort, wo man Bücher verbrennt, verbrennt man am Ende auch Menschen” *

Although this excerpt from a 19th Century play by Heinrich Heine is best known for its tragic and prophetic reality in association with the memorial to the Berlin book-burning of 1933, the significance of the statement may also be interpreted metaphorically. Just as the knowledge of the past may be used to build on new knowledge to the benefit of mankind, perhaps also the inaccessibility of past knowledge may lead to a lack of creation of new knowledge to the ultimate detriment of mankind. Thus, in an environment where we are narrowing our access to knowledge, the first edition of the UCDsmj is a beacon of light, widening access and providing an educational forum for the students of the UCD School of Medicine and Medical Science.

Considering once more the new UCDsmj, many have been involved, but I would like to pay tribute to Norella Broderick who helped initiate the venture and to the leadership and determination of both Denise Connolly and Aileen Conway who brought the UCDsmj to fruition. The future success of this journal is now dependent on the staff and students of the School, past, present and future, to maintain the momentum achieved in the production and publication of this foundation edition.

* “Where they burn books, they will, in the end, also burn people.”
Translated from Almansor: A Tragedy (1821) by Heinrich Heine

IDENTIFICATION OF A FUNCTIONAL HYPOXIA RESPONSE ELEMENT IN THE PROMOTER REGION OF THE DNA METHYLTRANSFERASE 3B GENE; POTENTIAL ROLE IN INFLAMMATORY, FIBROTIC & MALIGNANT DISEASE

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ABSTRACT

DNA methylation is the enzymatic addition of a methyl group to the carbon five prime position of a cytosine pyrimidine ring incorporated in a DNA sequence. This results in gene silencing that is maintained through cell division. De novo DNA methylation is associated with increased DNA methyltransferase 3b (DNMT3b) expression (3). As tissue hypoxia is a key component of inflammation, fibrosis and cancer, we propose here that hypoxic induction of DNMT3b plays a pivotal role in modifying epigenetic patterns key to the pathogenesis of hypoxic disease.

To elucidate hypoxic regulation of DNMT3b, a 250 base pair region of the DNMT3b promoter, which includes a putative Hypoxia-Inducible Factor (HIF) - 1 Response Element (HRE), was inserted into a pGL3 luciferase vector. HeLa cells were then transiently transfected with the pDNMT3b-Luciferase construct before being cultured in either hypoxic (1% O_2) or normoxic (21% O_2) conditions for 24 hours. After confirming hypoxic induction of luciferase activity, site-directed mutagenesis was used to mutate the putative HRE to assess its role in the hypoxic response.

Interestingly, hypoxia induced a mean 2.56 ± 0.32 fold increase in luciferase activity suggesting that the DNMT3b promoter is hypoxia responsive. Mutation of the putative HRE significantly reduced hypoxic induction by 40.2% ($P<0.01$).

These results describe for the first time that hypoxia induces DNMT3b promoter activity via a functional HRE in the 5' untranslated region. This suggests that HIF is a critical mediator of DNMT3b expression during hypoxic responses. Continued research could establish the link between hypoxia and its potential role in the epigenetic regulation of hypoxic disease.

Key Words: DNA methyltransferase 3b, hypoxia response element, promoter region, epigenetics, disease

INTRODUCTION

The field of epigenetics is on the rise, driven by the realisation that modifiers of chromatin are key regulators of biological processes in vivo. The three major classes of epigenetic effectors are DNA methylation, histone modifications (such as acetylation) and small noncoding RNAs (most notably microRNAs) (1).

Epigenetics

Epigenetics describes heritable changes in cellular phenotype that do not directly change the DNA sequence. These alterations have a major impact on a cell as they can regulate gene transcription and thus protein expression and therefore the cell's phenotype.

DNA Methylation

The emphasis of this project is on DNA methylation, which occurs through the enzymatic addition of a methyl group to the carbon five prime position of a cytosine pyrimidine ring already incorporated in a DNA sequence. The resultant epigenetic modification is associated with gene silencing (Figure 1) and constitutes a stable mark that is maintained through continuous rounds of cell division (2).

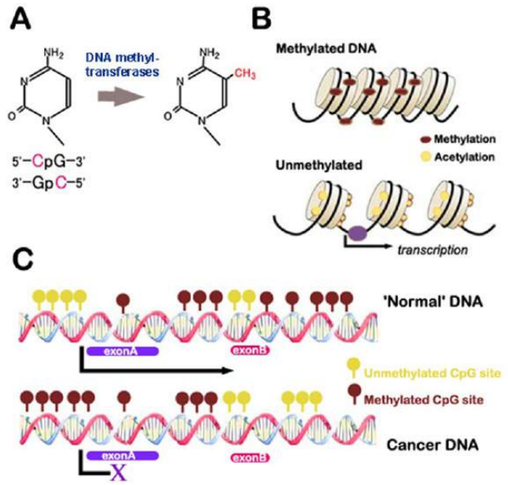


Figure 1. **[A] Mechanism of DNA methylation:** *cytosine is converted to 5 methylcytosine with the addition of a methyl group to the 5th carbon of the carbon ring. DNMTs (1, 3a and 3b) catalyse this reaction.* **[B] The reversible changes in chromatin organisation that influence gene expression:** *genes are expressed (switched on) when the chromatin is open (active), and they are inactivated (switched off) when the chromatin is condensed (silent).* **[C] DNA methylation directly impeding transcription factor binding.** *Image adapted from Rodenhiser, D. and M. Mann (3).*

This addition of methyl groups is catalysed by the enzyme DNA Methyltransferase (DNMT), of which there are 3 forms described in humans, DNMT 1, 3a and 3b. The focus of this project is on DNA Methyltransferase 3b (DNMT3b) which has been shown to be involved in de novo methylations and has been seen to be increased in disease states (3).

The majority of published epigenetic studies thus far have concentrated on the development and progression of cancer. It is emerging, however, that the epigenetic regulation of gene expression is important in many inflammatory and fibrotic diseases. For example, research involving patient lung tissue with idiopathic pulmonary fibrosis (IPF) has shown that there was loss of the fibroblast protein Thy-1 in areas of active fibrosis but it is expressed on fibroblasts in other areas of the lung. This loss of Thy-1 expression in the fibrotic foci of IPF lung tissue is the result of DNA Methylation of the Thy-1 promoter (4). The resultant decrease in Thy-1 expression has been linked to exaggerated fibrosis. However, to date, the processes controlling these changes are still not entirely clear. Work conducted in UCD has shown that hypoxia inhibits Thy-1 expression due to the promotion of Thy-1 promoter methylation (11). It is thus proposed that hypoxia may play a role in controlling gene expression via epigenetic regulation. The mechanism by which hypoxia regulates DNA methylation is unclear.

Thy-1

Thy-1 is a membrane bound protein with anti-fibrotic properties that is expressed in lung fibroblasts. Thy-1 knock-out mice have increased fibrosis in the lung. Fibrosis induced by radiation mimicking chemotherapeutic agent Bleomycin is also increased in these mice (5).

Hypoxia & HIF

Tissue hypoxia is a common occurrence in disease states and can induce epigenetic changes in the DNA of cells, which can be long lasting (6). It is generally accepted that hypoxia promotes Hypoxia-Inducible Factor (HIF) stabilisation within cells (7), thus making it our prime suspect in the association between hypoxia and changes in the epigenetic profile.

In normoxic conditions, HIF-1α is continuously produced and, in the presence of metabolic iron (Fe^{2+}) and oxygen, is continuously tagged for degradation through the Von Hippel Lindau protein (VHL)/Ubiquitin/Proteasome pathway (Figure 2). When oxygen becomes limiting, prolyl hydroxylase is inhibited and HIF-1α accumulates. After translocation and combination with the constitutive HIF-1β in the nucleus, the transcription factor binds to hypoxic response element (HRE) sequences to upregulate a number of downstream genes involved in tissue recovery, such as: EPO (erythropoetin) resulting in erythropoiesis, VEGF (vascular endothelial growth factor) leading to angiogenesis and glycolytic enzymes e.g. lactate dehydrogenase involved in anaerobic metabolism. In normal adaptive responses (for instance following ischaemic injury), these pathways are essential to enable tissue recovery. In the case of chronic inflammation however, maladaptive responses may significantly contribute to disease progression.

Association Between Hypoxia & Aberrant DNA Methylation in Fibrosis

As previously mentioned, there is evidence to support the hypothesis that epigenetic changes occur in fibrosis, however, the processes controlling these changes are still unclear. Research performed in the fibrotic kidney has shown links between increased methylation in fibroblasts and increased fibrosis (8). Interestingly, our laboratory has also similarly demonstrated increased global hypermethylation in primary lung and cardiac fibroblasts when they are exposed to hypoxia (11). Furthermore, we have shown increases in DNMT3b expression at both the mRNA and protein level in human primary lung fibroblasts cultured in hypoxic conditions (11).

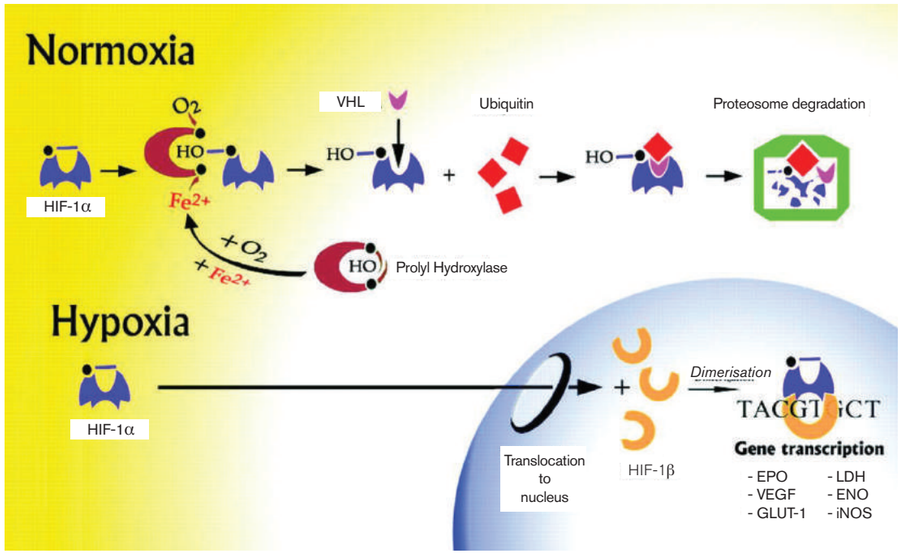


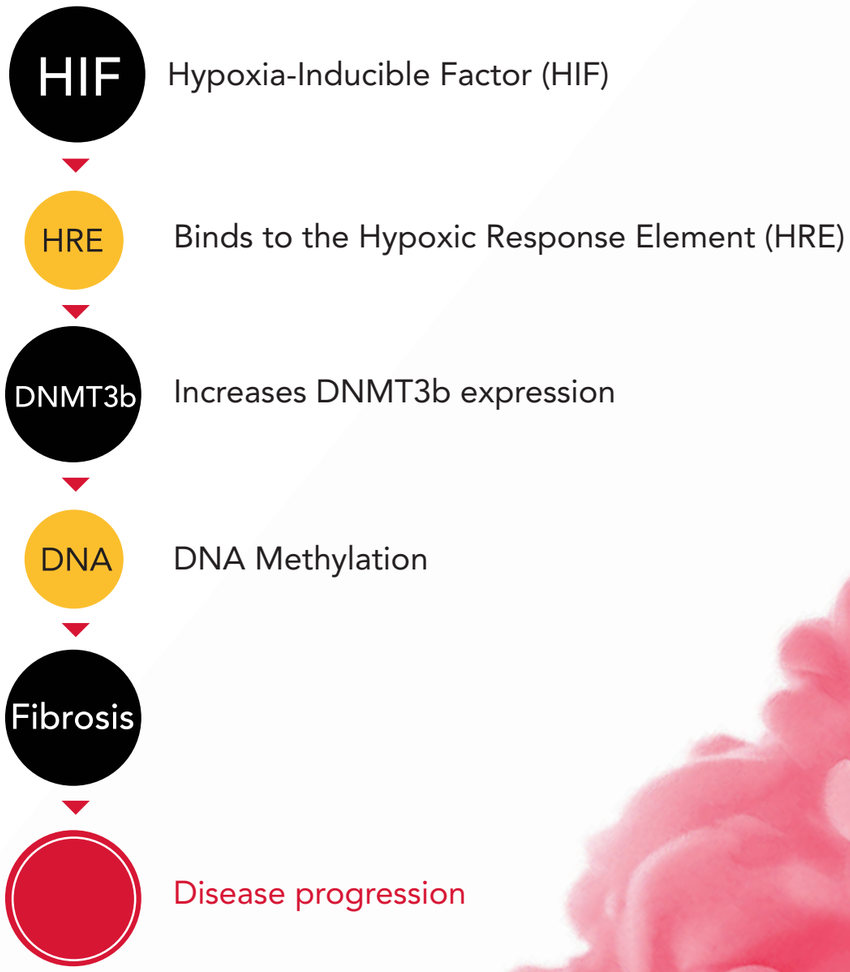
Figure 2. **Hypoxia Inducible Factor -1 Pathways in Normoxia & Hypoxia.** *(VHL: Von Hippel Lindau protein, EPO: Erythropoietin, VEGF: Vascular endothelial growth factor, GLUT-1: Glucose transporter 1, LDH: Lactate dehydrogenase, ENO: Enolase-1 and iNOS: Inducible nitric oxide synthase). (12)*

HYPOTHESIS

Investigation of the DNMT3b DNA sequence, revealed a putative HRE within the 5' untranslated region (5' UTR). We thus propose that it is hypoxia induced HIF binding to this putative HRE which increases the expression of DNMT3b.

Tissue hypoxia is a consequence of inflammatory cell infiltration, tissue damage and microvascular disruption and is a key component of inflammation, fibrosis and cancer. This hypoxic induction of DNMT3b may play a pivotal role in modifying epigenetic patterns key to disease pathogenesis, through its permanent effects on cell phenotype and their progeny post methylation.

This process is clearly visualised here:



OBJECTIVE

To prove our hypothesis, the main objectives of this project were:

1 - To subclone a fragment of the DNMT3b promoter into a pGL3 luciferase vector. Following this, to then quantify the hypoxic regulation of the DNMT3b promoter activity by performing luciferase assays on cells transfected with the pDNMT3b-luciferase construct (Figure 3) and subsequently, exposing the cells to either normoxia or hypoxia

2 - To use site-directed mutagenesis to mutate the putative HRE within the DNMT3b promoter and hence test its functionality and assess its role in the hypoxic regulation of DNMT3b expression.

MATERIALS & METHODS

pDNMT3b-Luciferase Vector/Construct

A cloned 250 base pair (bp) fragment of the DNMT3b promoter (pDNMT3b), which includes the putative HRE (Figure 3), was excised from the pCR4 TOPO Vector using restriction enzymes (XhoI & HindIII) and subsequently underwent electrophoresis on a 1% agarose gel. Upon visualisation with a UV Transilluminator, the identified 250bp DNMT3b fragment was extracted from the gel using a Gel Extraction Kit (Qiagen, cat # 28704). Finally, it was ligated into the XhoI & HindIII sites of a pGL3 Luciferase Vector (Promega, cat # E1751).

pGL3 Luciferase Reporter Vector:

The pGL3 Luciferase Reporter Vectors provide a basis for the quantitative analysis of factors that potentially regulate mammalian gene expression. The assay of this genetic reporter is rapid, sensitive and quantitative.

The reporter gene and the gene of interest are placed in the same DNA construct to be inserted into the cell or organism. This is usually in the form of a circular DNA molecule called a plasmid.

Commonly used reporter genes that induce visually identifiable characteristics usually involve fluorescent and luminescent proteins. The luciferase reporter vector makes use of the enzyme luciferase, which catalyses a reaction with luciferin to produce quantifiable amounts of light.

The sequence of events following the design of this DNMT3b-Luciferase construct follows this concept:

1 - Hypoxia induced HIF binding on the HRE of our construct/vector (Figure 3) causes,
2 - Transcription of Luc+ gene (Figure 3), which ultimately produces luciferase.
3 - Luciferase catalyses a bioluminescent reaction to produce quantifiable amounts of light.
4 - This light is directly proportional to the amount of luciferase produced and hence allowing us to accurately measure DNMT3b promoter activity.

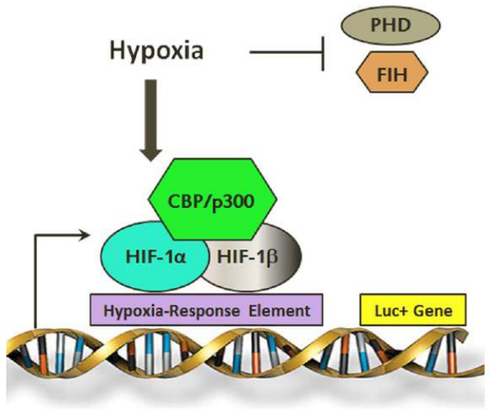


Figure 3. **The pDNMT3b-Luciferase Vector/Construct.**
Hypoxia induces HIF-1 transcription factor binding to the HRE, resulting in expression of the Luc+ gene and hence luciferase production. (p300: EIA binding protein p300; CBP: CREB-binding protein; HIF: Hypoxia Inducible Factor; PHD: prolyl hydroxylase; FIH: factor inhibiting HIF).

Transfection of pDNMT3b-Luciferase Vector/Construct

HeLa (human cervical epithelial cancer cells) cells were transiently transfected with the pDNMT3b-Luciferase vector using FuGENE HD (Promega, cat # E2311) in 12-well cell culture plates. HeLa cells were used because of their high transfection efficiencies. A FuGENE HD:DNA Ratio of 3:1 was used (3.3µl FuGENE HD:1.1µg DNA). Transfection was carried out in antibiotic and serum free medium (Modified Essential Medium, Gibco, cat. # 31095), supplemented with 10% FCS (Gibco, cat. #10270) and penicillin/streptomycin (Gibco, cat. # 15140) diluted 1:100).

Cell Culture

After five hours, each well of transfected cells was split equally into two wells of separate 24-well cell culture plates; this ensured identically transfected cell populations. Following an overnight incubation of both 24-well cell culture plates in normoxia (21% O_2), one plate was then placed in hypoxic conditions (1% O_2) while the other was left in normoxia (21% O_2). The duration of this incubation was 24 hours.

Luciferase Assay

After 24 hours, each well of cells was lysed with 40µl of Passive Lysis Buffer (Promega, cat # E1941). The lysate from each well was then transferred to separate microcentrifuge tubes and vortexed for 15 seconds followed by centrifugation at 12,000 x g for a further 15 seconds. 10µl of this 'processed' lysate was pipetted into 50µl of Luciferase Assay Reagent (Promega, cat # E1500). The readings were taken using a GloMax 20/20 Luminometer (Promega, cat # E5311). A 10 second measurement read for luciferase activity was used. Results were expressed as fold induction, i.e. the ratio of normalised luciferase activity of transfected HeLa cells in normoxia to those in hypoxia.

Site-Directed Mutagenesis

Site-directed mutagenesis was used to mutate the putative HRE within the pDNMT3b-Luciferase vector.

Putative transcription factor binding sites and specific mutations were identified using MatInspector software (www.genomatix.de). The putative HRE was then mutated using the QuikChange II Site-Directed Mutagenesis Kit (Stratagene, cat # 20052) and the following primers:

ΔHRE sense 5'-GCTCCGCGGCCGCAGACCGAGTGGACGCTCCGAGC-3'

ΔHRE anti-sense 5'-GCTCGGAGCGTCCACTCGGTCTGCGGCCGCGGAGC-3'

Two cytosine bases within the putative HRE were replaced with adenine bases (Figure 6). The mutant vectors (ΔHRE) were sent for Sanger DNA sequencing (Source Bioscience) to confirm the presence of the mutation.

Western Blotting

Western Blot was performed on HeLa cells that underwent a 3-day time course in hypoxia (1% O_2). Nuclear proteins were extracted using a Nuclear Extraction Kit (Thermo Scientific, cat # 78833), and quantified using a BCA Protein Assay Kit (Thermo Scientific, cat # 23227). They were then run on a 10% SDS-PAGE gel and transferred onto a PVDF membrane (Immobilon-P Transfer Membrane, Millipore, cat.# IPVH00010). The PVDF membrane was subsequently blocked for one hour using blocking buffer (2.5g dry milk (Fluka, cat.# 70166) in 50ml TBS-0.25% Tween (Tris saline buffer with 0.25% Tween pH 7.8) with 1ml of goat serum on a rotating holder to reduce non-specific binding to the membrane. Following this, the PVDF membrane was then incubated for another two hours with 8 µL (1:500) of mouse monoclonal anti-HIF-1α antibody. After washing with TBS-0.25% Tween for three times, of ten minutes each, the PVDF membrane was exposed to 0.5 µL (1:10,000) of Horseradish Peroxidase (HRP) - Conjugated anti-mouse IgG and visualised by chemiluminescence using HRP substrate (Supersignal Wes Pico Chemiluminescent Substrate, Pierce Biotechnology, cat.# 3480). 2µL (1:2,500) of anti-α-Tubulin antibody was used as the α-Tubulin loading control.

α-Tubulin as a loading control

In a western blot, the size of the bands are indicative of the amount of protein present. α-Tubulin is a structural protein, that should not change between samples. The amount of target protein is normalised to the structural protein to control between groups. This practice ensures correction for the amount of total protein on the membrane in case of errors or incomplete transfers.

Statistical Analysis

Five separate experiments (n=5) were repeated to assess the fold induction of luciferase activity in hypoxia. A further twelve experiments were conducted to determine if the hypoxic induction of luciferase expression was HRE dependent. Results were expressed as means ± SEM (standard error of the mean). Differences between groups were tested for statistical significance using the Student's t-test. Prior to this, the data was assessed for Gaussian distribution. A P value of 0.05 ($P < 0.05$) was used to determine statistical significance and were two-sided. The analysis was performed with GraphPad Prism 5 Software (GraphPad Software Inc., San Diego, CA).

RESULTS

Western Blotting for HIF-1α in HeLa Cells

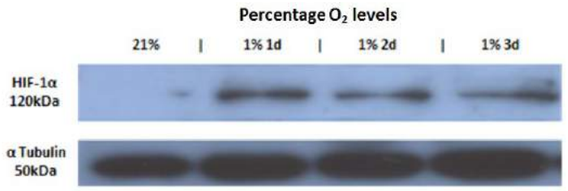


Figure 4. **Western blotting analysis showing stabilisation and accumulation of HIF-1α protein in HeLa cells incubated in hypoxic conditions (1% O_2) for 1, 2 and 3 days.** *α-Tubulin was used as a loading control in normoxic conditions (21% O_2); n=1.*

To ensure that HIF-1α is stabilised when HeLa cells are placed in hypoxic conditions, we cultured them in hypoxia (1% O_2) over a three day time course. As expected, Western blotting analysis (Figure 4) showed stabilisation and accumulation of HIF-1α from Day 1 onwards. This confirms the hypoxia-induced upregulation of HIF-1α in the HeLa cells used by this group.

Fold Induction of Luciferase Activity in Hypoxia

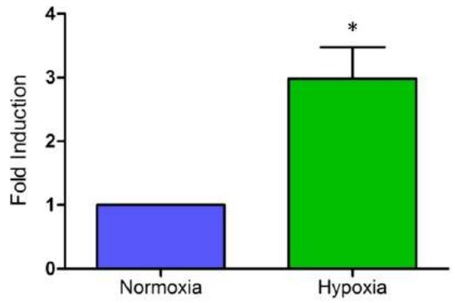


Figure 5. **Luciferase expression was induced by hypoxia.** *Comparison of luciferase activity in HeLa cells transfected with a pDNMT3b luciferase vector in both normoxic (21% O2) and hypoxic (1% O2) conditions after 24 hours incubation. (Fold induction = the ratio of normalised luciferase activity of transfected HeLa cells in normoxia to those in hypoxia). (*P < 0.05). The values represent means ± SEM for n=5 (representing the 5 pairs – one set of normoxic and hypoxic – of HeLa cells that were compared after being placed in their respective conditions.*

As the graph clearly illustrates (Figure 5), luciferase expression was induced by hypoxia, indicating that the DNMT3b promoter is hypoxia responsive. This is represented by a mean 2.98 fold induction of luciferase activity (and hence DNMT3b promoter activity) observed after a 24 hour incubation of transfected HeLa cells in hypoxia (1% O_2).

Site-Directed Mutagenesis

It was now established that the pathway responsible for hypoxic induction of DNMT3b promoter activity was intact in the transfected HeLa cells. Whether this putative HRE was functional, however, remained unclear. This hypothesis was tested by using site directed mutagenesis to mutate the putative HRE within the pDNMT3b-Luciferase vector to show that this putative HRE is indeed functional in the DNMT3b gene.

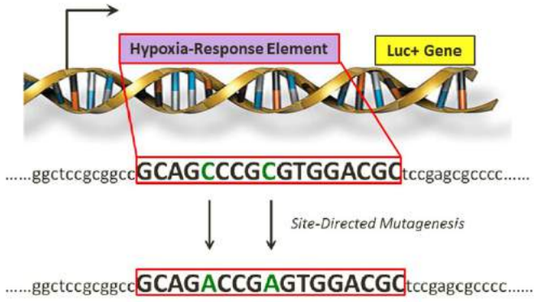


Figure 6. **Site-directed mutagenesis was used to mutate the putative HRE within the pDNMT3b-Luciferase vector;** *two cytosine bases within the HRE were replaced with adenine bases.*

To create the mutant, two cytosine bases within the putative HRE were replaced with adenine bases (Figure 6). This mutation specifically disrupts transcription factor binding sites on the putative HRE. Bioinformatic analysis confirmed that this mutation would block HIF binding with minimal disruption to other putative binding sites in neighbouring regions of the sequence.

Hypoxic Induction of Luciferase Expression is HRE Dependent

After confirming the presence of the mutation through the use of DNA sequencing, identical hypoxic luciferase reporter assays were repeated in cells that were transfected with the wild type pDNMT3b-Luciferase vector or the mutant vector alone.

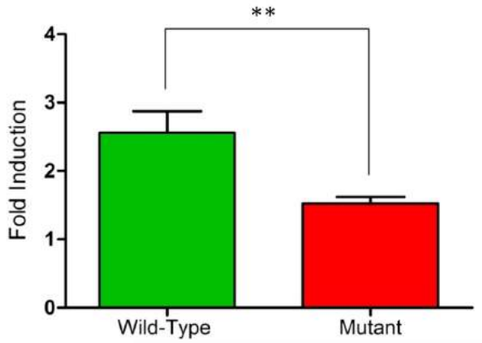


Figure 7. **Hypoxia induced DNMT3b promoter activity is dependent upon a functional HRE in the 5'UTR.**
*Comparison of luciferase activity in HeLa cells transfected with a wild type and mutant pDNMT3b-Luciferase vector in hypoxic (1% O_2) conditions after 24 hours incubation. (Fold induction = the ratio of normalised luciferase activity of transfected wild type HeLa cells versus transfected mutant HeLa cells in hypoxia). (**P < 0.01). The values represent means ± SEM for n=12 (representing the 12 pairs of HeLa cells – one with wild type DNA, one with mutant DNA - that were compared after being placed in hypoxic conditions (1% O_2).*

It was observed that after a 24 hour incubation period in hypoxic conditions (1% O_2), a mean 2.56 (95% CI: 0.34-1.72, t=3.11, P<0.01) fold induction of luciferase activity (and hence DNMT3b promoter activity) was observed for HeLa cells transfected with the wild-type pDNMT3b-Luciferase vector. For HeLa cells transfected with the mutant vector only a mean fold induction of 1.53 ± 0.10 was seen (Figure 7). Mutation of the putative HRE significantly reduced the activity of the DNMT3b promoter by 40.2%.

Based upon this mutational analysis of the HRE, we are confident that hypoxia induced DNMT3b promoter activity is dependent upon a functional HRE in the 5' UTR of the DNMT3b gene and that HIF is likely to be a principal regulator of its activity.

DISCUSSION & CONCLUSION

This study illustrates for the first time that the DNMT3b gene contains a functional HRE in the 5' UTR that is predominantly responsible for the hypoxic induction of DNMT3b promoter activity. And thus, we propose that HIF is a critical mediator of DNMT3b expression during hypoxic responses. It should be noted that further investigation into the residual activity of the mutant vector may also be warranted (see Continuing Experiments).

Although the link between various disease states and hypoxia is evident, it is less clear how hypoxia contributes to disease progression. DNMT3b has the ability to change the methylation pattern of DNA in a cell. This alteration in the epigenetic profile is associated with, and can possibly drive, the pathogenesis of certain disease states. For example, in chronically hypoxic benign prostate epithelial cells (a characteristic feature of the aging prostate), there is a marked increase in DNMT3b expression. This can promote and maintain the expression of regulatory genes and adaptive pathways that instigate tumour development (9). Preliminary data from our laboratory has also shown this same increase of DNMT3b expression at the mRNA and protein level in human primary lung fibroblasts cultured in hypoxic conditions (11).

With these new results, there is good precedent for continued research into this area, which could further establish the link between hypoxia and its potential role in the epigenetic regulation of hypoxic disease.

Implications for the Patient in Clinical Practice

Unlike genetic alterations, epigenetic modifications influencing gene expression are reversible, thus providing an area of therapeutic interest. Currently, there are inhibitors for DNA methylation available, for example: 5-aza-2'-deoxycytidine (Decitabine) which has been approved by the FDA for the treatment in myelodysplastic syndrome (10). Therefore, it is known that targeting DNA methylation is a viable treatment option in diseases.

We thus propose that developing DNMT3b inhibitors to block aberrant methylation or inhibiting HIF-1 activity may be novel therapeutic strategies for inflammatory and fibrotic diseases (E.g. Idiopathic Pulmonary Fibrosis) which also has important implications in the treatment of certain cancers.

Continuing Experiments

Additional experiments are required to address some shortcomings in this research due to the limited timeframe and also to build on observations from the results obtained:

1 - From this study it is clear that one of the principal regulators of DNMT3b expression during hypoxic culture is likely to be HIF. In order to confirm that HIF specifically induces DNMT3b promoter activity, it would be necessary to utilise a HIF-1 overexpression vector to over-express HIF-1 during normoxic culture. This would be done via a co-transfection scenario with the wild type or mutant vector. Results will be determined via luciferase analysis as carried out in this study.

2 - Challenge the hypoxic mutant with Mithramycin A (eliminates Sp1/Sp3 binding) to see if it abolishes residual mutant activity.

A Role for Sp1/Sp3 in Hypoxic Induction of DNMT3b? :

Analysis was carried out on the potential transcription factor binding sites proposed by MatInspector. Interestingly, it was found that there were a total of six potential Sp1/Sp3 binding sites on our 250bp DNMT3b fragment. Sp1/Sp3 are hypoxic responsive transcription factors that are thought to facilitate transcriptional activation. Studies have also shown that the transcription factors Sp1/Sp3 are closely linked to transcriptional regulation of DNMT3b. Could the hypoxic induction of DNMT3b be also due in part to the hypoxic upregulation of Sp1/Sp3?

3 - Investigate the effect of Mithramycin A on endogenous DNMT3b expression in cells that have been placed in hypoxic conditions.

4 - Challenge the hypoxia-induced DNMT3b response with SiRNA (small, interfering RNA) that targets HIF. Si HIF induces the degradation of HIF RNA, preventing its translation. When cells are treated with Si HIF and placed in hypoxia, it is expected that there should be no increase in DNMT3b expression. This would corroborate well with the results of this study.

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THE RESEARCH EXPERIENCE / ACKNOWLEDGEMENTS

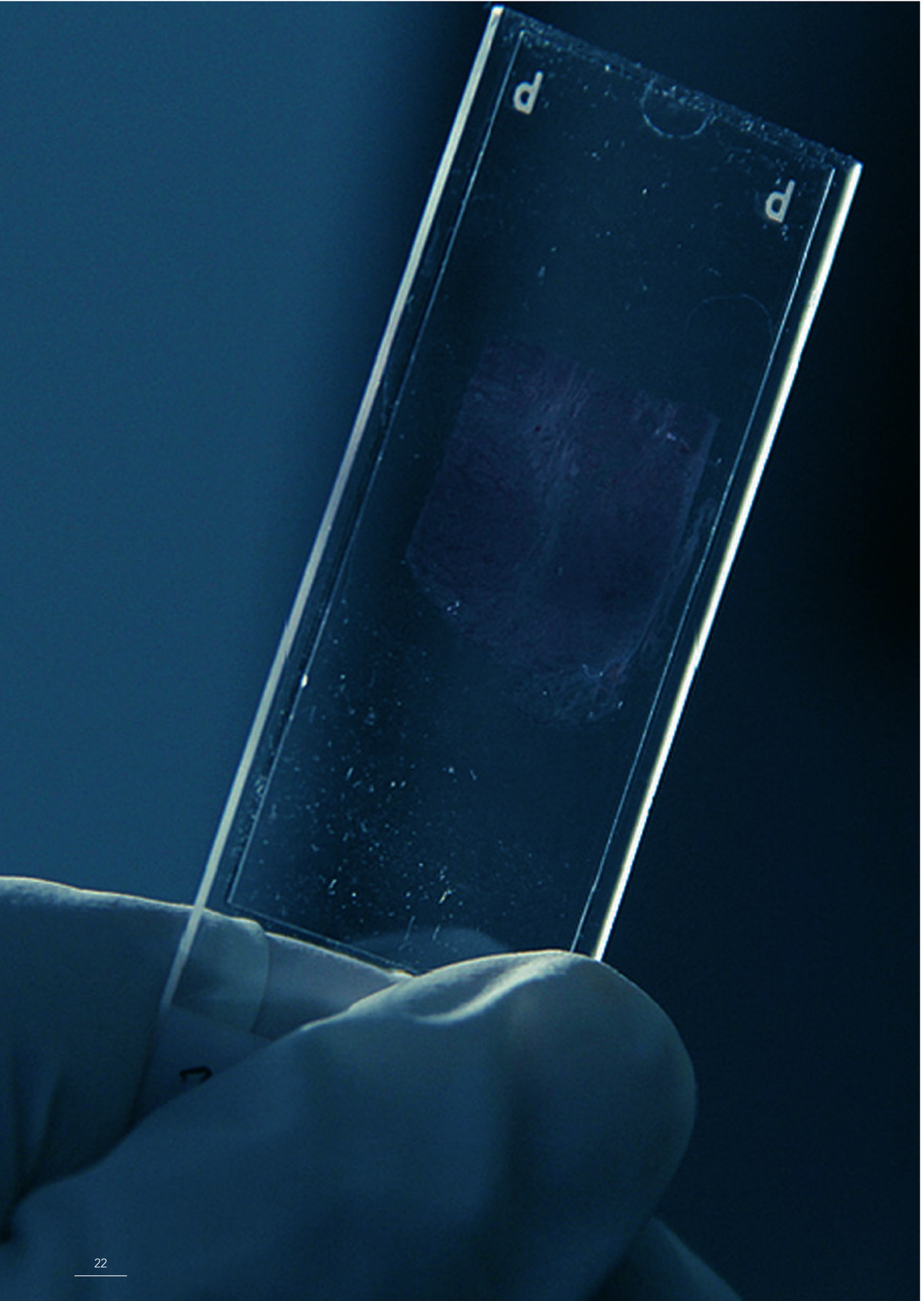
I relish the challenge that all research demands, especially the need to think critically and adapt to diverse and rapidly changing situations. I was also eagerly looking forward to be further introduced into research. I believe some research experience is integral to giving me a better understanding of pathology and the path needed to generate new treatment or biomarkers. Ultimately, I hope this experience will help me become an even better clinician. However, when I initially decided to sign up for the UCD Summer Student Research Program, I was rather hesitant as I knew that the experience I would have was up to me as much as the supervisor I had and the research team I would be working with. As it turns out, I managed to find a project in an area I was interested in, and a supervisor, whom I had enjoyed lectures from previously. Both of these were crucial to making this project a success.

Before the project began, I had huge concerns about not being able to produce results with the limited experience I had. Of course, all this was soon put to rest. Being able to work with and feel part of a research team has truly been an enjoyable experience. Everyone was both knowledgeable and friendly. This helped me to settle into the lab as I was both able to lean new techniques and grasp a thorough understanding of what I was doing. They took my "lower-level" input without any fuss, and even took the liberty to explain in detail any questions I had regarding laboratory techniques or the research itself.

From this experience it is my opinion that the toughest part in research is not learning laboratory techniques. Contrary to popular belief, I think that's the easy part. The invaluable skill, is knowing when it's the right decision to continue and when to end experiments. There were many times during the course of the research that experiments would continue to fail. Being able to tell if this was a problem due to poor experimental design or a problem with the materials or techniques was something that I could not do. Only the insight of an experienced researcher could have known what the next best course of action was.

With the above in mind, I'd like to especially thank Dr. John Baugh (supervisor) and Claire Robinson (Ph.D. Student) for their continued support, guidance and most importantly patience during this project. Their advice and counsel has been invaluable to its success and my excellent summer research experience. I am definitely considering doing more research down the line. Thanks also go out to all in the lab who put up with my silly questions and to Dr. Amanda McCann for making all this possible.

Finally my appreciation goes out to the Pathological Society of Great Britain & Ireland for supporting me through the Undergraduate Bursary (UE 2011/04/38) which helped greatly to ease my burden on expenses this summer.



GENOMIC MEDICINE, BIOMARKERS AND THE PROGRESS TOWARDS PERSONALISED MEDICINE: THE HEPATITIS C PARADIGM

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Disclaimer: The views expressed within this article are the author's own and do not necessarily reflect those of the National Virus Reference Laboratory or UCD

ABSTRACT

Hepatitis C virus (HCV) exerts a profound burden of liver disease with global estimates suggesting over 200 million people are infected. Current therapy for chronic HCV infection employs pegylated interferon-α (IFN) and the nucleoside analogue ribavirin (RBV). Treatment success is dependent on many factors; including viral genotype, gender, ethnicity, liver histology, baseline viral load and pre-treatment levels of interferon-stimulated genes. Genes influencing treatment response have been identified in cohorts comprised predominantly of the "difficult to treat" HCV genotype 1 and a number of groups independently described genetic variation adjacent to the interleukin 28B gene (IL28B) - encoding interferon-λ3 (IFN-λ3) which was associated with treatment response and also with spontaneous clearance without treatment. From a clinical point of view, these findings have created great excitement as it has opened the possibility for determination of the IL28B genotype status of patients presenting with chronic HCV infection. Conceivable clinical algorithms have been suggested where individuals with haplotypes associated with HCV spontaneous clearance might be monitored longer whereas patients with haplotypes associated with viral persistence might receive therapy during the acute period and be monitored for a shorter period prior to treatment. Remarkably, new HCV treatment modalities promise a cure with the advent of direct acting antivirals, such as the protease and polymerase inhibitors, despite issues of cost and development of resistance. Nevertheless IL28B genetic testing and prediction of HCV treatment response to IFN/RBV serves to illustrate methodologies which will likely become increasingly common in clinical practice in the future where the treatment is tailored to each patient rather than a one size fits all approach.

Key Words: hepatitis C treatment, biomarkers, IL28B gene, genomics, interferon-stimulated genes

INTRODUCTION

Following the completion of the human genome project in 2003, expectations were raised that this landmark event in human history would herald the rapid translation of new treatments for human genetic disease, a deepening of the understanding of the interaction between the human immune system and pathogenic agents and a revolution in personalised medicine where treatments would be tailored to the individual patient (1). Personalised medicine and the use of biomarkers - definable as quantifiable indicators in the patient of normal biological or pathogenic processes or responses to treatment and/or other therapy - gleaned from advances in basic research offer an alternative to the empirical (trial and error based) treatment approaches.

HEPATITIS C VIRUS

Hepatitis C virus (HCV) was first described in 1989 by Michael Houghton and co-workers (2) and is a hepatotropic member of the family Flaviviridae within the genus Hepacivirus. The Flaviviridae includes the arboviruses (arthropod-borne viruses) dengue and yellow fever virus from which the family derives its name, as the jaundice is characteristic of the latter infection (flavus is Latin for yellow). The viral particle consists of an envelope surrounding a positive polarity RNA genome of some 9600 nucleotides which encodes a polyprotein that is processed by cellular and virally-derived proteases. Staggeringly, 3% of the human population, over 200 million individuals, are estimated to be chronically infected with HCV (3). This epidemic exerts a profound burden of liver disease and pressure on health care systems particularly as the majority of infections are in the developing world in Africa and Asia. Egypt is a particularly striking example where approximately 14% of the population have serological evidence of previous exposure (anti-HCV antibody) with almost 10% of the population chronically infected (viremic) (4). This is sadly linked to efforts to eliminate schistosomiasis. HCV positivity strongly correlates with antischistosomal injection treatment before 1986, which unfortunately involved the reuse of contaminated needles. HCV genotype 1 is the predominant genotype in Europe and the United States, so intense research interest has been focused at determining viral and patient-specific markers as well as environmental factors that increase the predictive value of determining the likelihood of treatment success at baseline. One clue was the differences in ethnic responses to treatment where Africans responded less than Europeans who, in turn, had poorer response rates than Asians to standard therapy. This also correlated with rates of clearance in the absence of treatment (so called spontaneous resolvers).

THERAPY FOR HCV INFECTION AND THE SEARCH FOR TREATMENT PREDICTIVE BIOMARKERS

Unlike hepatitis B virus (HBV) no vaccine is currently licensed for HCV. Primary infection with HCV in about 30% of cases results in spontaneous clearance where strong HCV-specific innate and adaptive immune responses control and eliminate the infection. Following establishment of a persistent infection, some 20% of individuals have progressive hepatitis and a further subset progress to worsening liver function with increased fibrosis, cirrhosis and ultimately hepatocellular carcinoma necessitating transplantation.

There are major differences in response rates to standard therapy which currently consists of weight-based pegylated interferon-α (PEG-IFN-α) and the nucleoside analogue ribavirin (RBV) for patients chronically infected with HCV. Less than 50% of individuals achieve a sustained virological response (SVR), as determined by the absence of viral RNA six months after treatment cessation with "difficult to treat" HCV genotypes (HCV genotypes 1 and 4) (5). In contrast, individuals infected with "easy to treat" HCV genotypes 2 and 3 typically achieve SVR rates between 70 and 90% (6). Hepatologists predicting treatment response and defining treatment duration have relied on clinical and laboratory-derived parameters such as the viral genotype, as described above, the baseline viral load determined by quantitative polymerase chain reaction (qPCR), on treatment viral kinetics, the degree of fibrosis, the extent of hepatosteatosis, the association of female gender with higher clearance rates than males, lack of co-morbidities such as HIV-coinfection, low initial IFN stimulated gene (ISG) expression, and also ethnicity (7).

IFN-α has been used for more than two decades for treatment of HCV infection, however, therapy is associated with significant adverse side effects, is prolonged in duration and expensive. This has stimulated the search for biomarkers that could potentially be employed to predict an individual's likelihood of achieving a successful treatment outcome prior to commencement of therapy. In order to explain the recent ground-breaking advances in prospects for personalised therapy for HCV-infect-

ed patients we must first discuss the technology and how it has lead to the development of biomarkers to predict treatment response that ultimately improve patient care.

GENOME WIDE ASSOCIATION STUDIES

The complete DNA sequence of a genome from person to person is thought, by various estimates, to differ by approximately 0.1%, which accounts for some 3 million genetic differences within the 3 billion base pairs of the haploid human genome. This inter-individual variation is largely thought to be of a neutral nature whereby the vast majority of this genetic variation does not alter amino acids within proteins and has no discernible phenotypic effect. However, a smaller subset of this variation between individuals can occur within genes leading to changes in the encoded protein (non-synonymous mutations) within regulatory regions, such as promoters, that influence gene expression or splicing or other post-transcriptional effects. When this variation is greater than 1% within a human population the site is termed a single nucleotide polymorphism (SNP).

Rather than studying genetic variation in a single gene derived from a list of plausible gene candidates between cases and control groups, or a small subset of potential target markers, there are alternative strategies which analyse susceptibility loci without a priori hypotheses which by virtue of their essentially random nature offer the potential to uncover new pathways into the aetiogenetic pathogenesis of disease. Genome wide association studies (GWAS) are one such unbiased approach, which analyse gene frequencies of SNP's across the genome in individuals with the disease or trait of interest by statistical comparison with a suitable control group. GWAS's have been increasingly used in medical research since the sequencing of the human genome to analyse complex, polygenic groups of human disease such as rheumatoid arthritis, atherosclerosis, Crohn's disease and type 2 diabetes mellitus. The general theme of the results from these diverse areas of study is a complex interaction of small effect genetic susceptibility loci (host genotype) with, of course, environmental factors that lead to disease (host phenotype).

INTERLEUKIN 28B (IL28B) AND HCV CONTROL

In this regard, a notable exception was the strong and unambiguous genetic association uncovered by four independent groups in 2009, conducting research into host genetic factors influencing treatment response rates in chronically HCV-infected patients (8-12). Their cohorts were comprised predominantly of the "difficult to treat" HCV genotype 1 and strikingly, each study described SNP's adjacent to the interleukin 28B gene (IL28B) -encoding interferon-λ3 (IFN-λ3) on the long arm of human chromosome 19 (19q13.13). This was associated with clearance following standard therapy and also, importantly, with spontaneous clearance without treatment. In this landmark study, Ge and colleagues (8) compared the allele frequencies of 600,000 SNPs by GWAS from 1,137 individuals of differing ethnic backgrounds (African, Hispanic and European) with persistent HCV who were treated for 48 weeks with standard therapy and then followed for 24 weeks after discontinuation of treatment. A remarkably strong genetic influence was found for individuals homozygous (CC) for the rs12979860 SNP and treatment-induced clearance. No other SNP's outside the IFN-λ cluster approached genome-wide significance for predicting treatment response. Crucially this protective effect of the C allele was seen across the three different ethnic groups and when combined data for the three groups was analysed together (Figure 1) individuals carrying the CC genotype had a sustained virological response (SVR) almost three times higher than the minor homozygote TT poor response genotype (78%/28%). Furthermore, the effect of the T risk allele appeared to be dominant as heterozygosity (CT) lead to a >2-fold decrease (38% SVR rate) in the likelihood of achieving a post-treatment SVR compared to CC homozygotes. The authors compared the known baseline predictors for patient treatment response with the magnitude of this newly uncovered effect for IL28B genotype and concluded that genotyping of this SNP is associated with the most substantial influence on treatment response.

This seminal work was quickly followed by three other studies and the association of genetic variation near IL28B on predicting treatment response rates was further strengthened. Tanaka and colleagues (9) analysed by GWAS the genetic influence of 142 Japanese individuals infected with HCV-1, comprising of 78 treatment non-responders and 64 who achieved an SVR; and identified a risk allele (GG) for the SNP rs8099917 8.9kb upstream of the IL28B gene strongly associated with null virological response. Specifically, homozygous carriers of rs8099917 G allele had >2 fold more likelihood to fail to achieve an SVR compared with heterozygotes and the TT major homozygote responder genotype. Suppiah and co-workers (10) in a larger study also identified rs8099917 as the statistically most significant SNP influencing treatment response in 293 Australians of European ancestry, made up of 162 non-responders and 131 with SVR. They validated their findings with 555 patients from Germany, Italy, Australia and the UK. Finally, the Swiss HCV and HIV cohort study (11) analysed 465 individuals including individuals with HCV genotypes 1-4 and again identified rs8099917 as a predictor of treatment response, with the strongest effects in HCV genotypes 1 and 4 infected patients. The lowest carriage rate of the rs8099917 risk allele GG (24%) was in individuals with spontaneous clearance, in 32% of chronically infected who responded to treatment and in 58% who did not clear the virus on treatment. An important technical point to note is that that the three studies above describing the association of rs8099917 with SVR did not contain or had limited representation of the rs12979860 SNP unearthed by Ge and co-workers but the latter SNP has since been replicated independently by a number of other centres and these markers are in strong linkage disequilibrium (i.e. the genotypes at each locus are not independent of one another).

As many of the SNPs identified by the GWAS studies are located upstream of the IL28B gene - rs12979860 for example lies 3kb upstream of the gene start within a CpG island promoter - this immediately suggested that individuals with the poor response alleles may have lower levels of expression of interferon-λ3 (since the IL28B gene encodes for interferon-λ3). This has indeed been demonstrated by two groups (looking at the rs8099917 SNP) in peripheral blood mononuclear cells (9,10). This suggests that an attenuated antiviral response to HCV infection could lead to the establishment of a persistent infection, however, Ge and co-workers found no significant association between the rs12979860 SNP and IL28B expression (8). Interestingly, baseline levels of the downstream media-

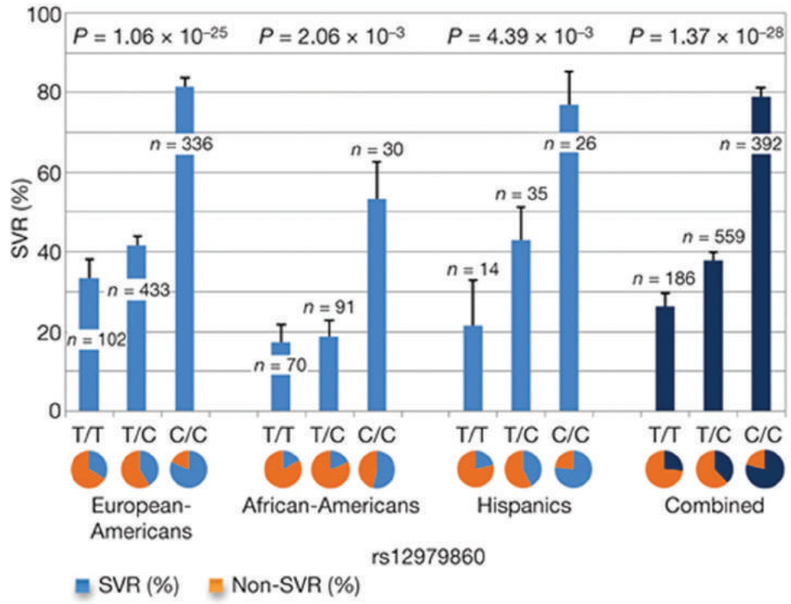

Figure 1. Genetic variation in IL28B predicts HCV treatment-induced viral clearance.
Data are presented for each group as percentages ± standard error of the mean. Reprinted from Nature 2009, 461:399-401, copyright 2009 with permission from MacMillan Publishers Ltd (8)

tors of the interferons, the ISGs (interferon stimulated genes), have been inversely correlated with treatment success. That is to say that, paradoxically, individuals with higher ISG expression pre-treatment have poorer treatment success rates so the genetic effects of the risk alleles could be associated with higher ISG expression (13,14). Even more bizarrely, the good treatment response markers also correlate with higher pre-treatment HCV RNA levels (8). However, based on a previous study showing that ISG expression may modulate the response to PEG-IFN-α (15), this is potentially explicable by patients with higher baseline viral loads having lower basal levels of hepatic ISG expression. These patients with low pre-treatment ISG levels when stimulated with standard therapy then show greater upregulation of ISGs from basal levels and hence better treatment response. In contrast, patients with higher basal levels of hepatic ISG expression gained no benefit from treatment with PEG-IFN-α (8).

Clearly all the GWAS studies point to IFN-λ as central to the control of HCV infection (16). There are considerable similarities between the modes of action of IFN-α and the IFN-λ's and the downstream signalling pathways in particular (17, Figure 2). However, while IFN-α receptors which are broadly expressed on most cell types, including leukocytes, IFN-λ receptors are largely restricted to cells of epithelial origin (18). Furthermore, the IFN-λ's have been shown to inhibit the replication of HCV, HBV and the influenza A virus in vitro (19,20,21) and this has stimulated interest in the IFN-λ's as potential therapies in the infectious disease and cancer settings (22).

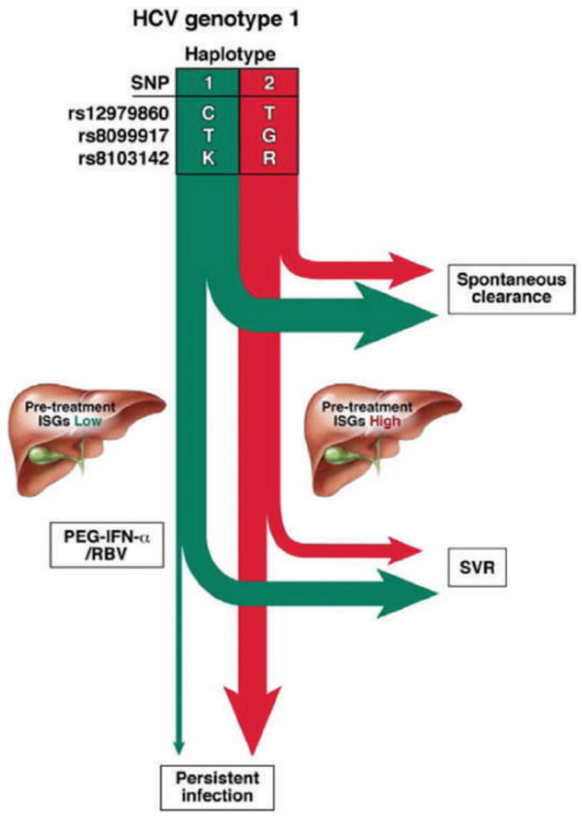

Figure 2. Predicted natural course of HCV infection in the absence of therapy and treatment response based on IL28B haplotypes.
Employing IL28B SNPs alone or together allows allele and haplotype tagging associated with a greater likelihood of HCV treatment response spontaneous clearance (depicted by the green arrows) or viral persistence (in red). Reprinted from Gastroenterology 2010, 139:1865-76, copyright 2010 with permission from Elsevier (17)

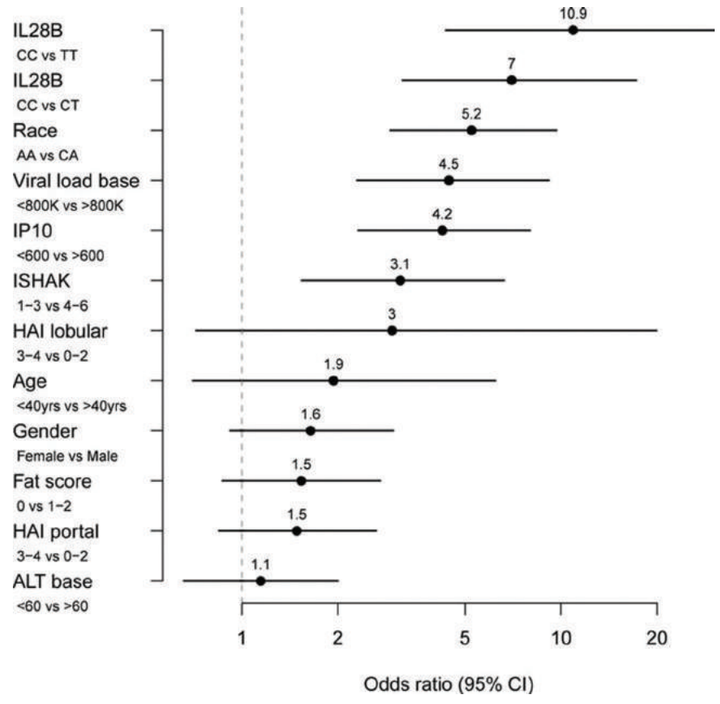

Figure 3. Predictors of sustained virological response to PEG-IFN-α and ribavirin therapy.
Odds ratios were calculated from a logistic regression model including IL28B genotype and baseline (pretreatment) measurements of IP-10, HCV viral load, fibrosis stage (ISHAK), age, gender, alanine transaminase (ATL), steatosis (fat score), and portal and lobular histologic activity index (HAI). Reprinted from Hepatology, 2011, 53:14-22, copyright 2011 with permission from John Wiley and Sons Inc. (23)

CLINICAL VIEWPOINT

From a clinical point of view, these basic research findings have created great excitement among clinicians and scientists alike as it has opened the possibility for determination of the IL28B genotype status of patients presenting with chronic HCV infection. Determination of IL28B genotype in replication studies has shown this locus to be the highest independent predictor of treatment response eclipsing viral genotype, pre-treatment (baseline) viral load, liver histology and ethnicity (23, Figure 3). Conceivable clinical algorithms have been suggested where individuals with a haplotypes associated with HCV clearance in the absence of therapy might be monitored longer, since they are more likely to spontaneously clear the virus whereas patients with haplotypes associated with viral persistence might receive therapy during the acute period and be monitored for a shorter period prior to treatment (17). The association between kinetics of HCV response to IFN treatment and IL28B genotype might also be used to identify patients that require shorter durations of therapy. The finding that individuals with poor response alleles have lower expression of IFN-λ suggests that a direct protein based replacement therapy of IFN-λ in individuals with the poor response alleles may be advantageous. The highly related cytokine, interleukin 29 (IL29, interferon-λ1) is in clinical trials (16). This drug has demonstrably lower toxicity than the current IFN-α based regime and importantly exhibited robust antiviral effects (24). This is likely due to the expression pattern of IFN-λ receptors, which are predominantly restricted to cells of epithelial origin such as hepatocytes, keratinocytes and bronchial epithelial cells (25). The decreased side effects, such as reduced bone marrow destruction and flu-like effects common with current standard therapy, is likely attributable to the lower levels of expression of type III (IFN-λ) receptors in the bone marrow and in the brain (25).

The recent licensure of HCV protease inhibitors (telaprevir and boceprevir) and other direct acting antivirals (DAAs) in the pipeline (such as further protease and polymerase inhibitors) may render IL28B testing obsolete as they operate independently of IL28B haplotype. However, as with HIV-infected individuals on antiretroviral therapy, the emergence of resistant mutants will need to be evaluated (26). In the short term, due to the high initial cost of the currently licensed DAAs, IL28B testing may be utilised to identify individuals carrying alleles predicting poor response to standard therapy for treatment with the new agents, whereas the favourable response allele carriers may obtain existing PEG-IFN-α/RBV. Furthermore, the knowledge that this form of genetic test highly predicts treatment response can help patients to be encouraged to commence therapy and reassure them during a long treatment course with side effects. A new polymerase inhibitor (PSI-7977) has shown 100% virologic response rates when taken with RBV or three separate PEG-IFN-α regimes (27) suggesting a cure for HCV could be on the near horizon only shortly over two decades since the agent's original identification, which is a remarkable achievement for the medical and scientific communities. The likelihood is that PEG-IFN-α/RBV-free DAA-only regimes will go through Phase III clinical trials in 2012.

What is clear is that personalised genomics will become increasingly common in the future in many areas of medicine. Whole genome DNA sequencing technology is still prohibitively

expensive and technically demanding for the widespread application in the clinical setting. This is expected to change rapidly, however, and the benchmark of the '$1,000 genome' may well have already been reached (28). This is almost beyond comprehension when you consider that the entire Human Genome Project started in 1990, was finalised at 99.99% coverage in 2003 and cost $3 billion. The advent of genomic medicine is thus fast approaching and its direct impact on patient care is already here, where diagnostics and therapy-related diagnostics (so called theranostics) will be revolutionised by the advances in basic science.

Thomas S. Kuhn argued in The Structure of Scientific Revolutions (1962) that the accepted model of scientific advance involving a slow, linear, incremental accumulation of knowledge was inconsistent with the facts and an episodic, distinctly non-linear model of scientific innovation where revolutions occurred within disciplines was more reflective of the actual workings of the scientific enterprise (29). Kuhn coined the term paradigm shift to denote this model and there appears some similarities to what we are seeing now with the advent of genomic and more personalised medicine. The translation of the vast amounts of genetic information obtained from genomics into medical practice and improving patient care is the ultimate rationale underpinning the human genome project and will become increasingly more common in clinical practice in the future.

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CLINICAL INFORMATION AND VISUAL SEARCH PATTERNS AS FACTORS WHICH INFLUENCE DETECTION OF ABNORMALITIES IN RADIOGRAPHS: A REVIEW OF THE LITERATURE

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ABSTRACT

Purpose: The search for abnormalities in radiographic images is difficult due to the complex anatomical structures represented, which often mask or decrease the conspicuousness of lesions of interest. This review article aims to consolidate the existing literature relating to two factors which influence detection of abnormalities, the provision of clinical information and the visual search pattern employed.

Method: A MEDLINE search was carried out and extended by a search of reference lists. Articles were selected which examined the effect of clinical information and visual search patterns on detection of abnormalities on radiographs.

Results: The majority of studies related the effect of clinical information to an increase in detection of abnormalities with the presence of clinical indications, in addition to a number that showed a concomitant increase in false positives. A minority of studies claim no significant increase in detection. Research findings arising from visual search pattern studies have categorized error as errors of search, recognition and decision making. Reduced fixation time on abnormalities, and the presence of multiple abnormalities are predictive of detection failure. To a lesser degree of expertise is also predictive of detection failure due to differences in search strategies employed.

Conclusions: The presence of clinical information increases the detection level of abnormalities during radiological image interpretation, although this may be accompanied by an increase in false positives. Specific search pattern characteristics have been shown to increase abnormality detection success. These results provide a framework for an increased detection of abnormalities in radiographic images.

Key Words: visual search patterns, error, clinical information, radiographs

INTRODUCTION

Radiographic images contain a range of perceptual ambiguities that contribute to a statistically significant error rate in diagnosis (1). Small pulmonary nodules are often missed because of poor lesion conspicuity caused by superimposition of hilar and mediastinal structures, blood vessels, clavicles, or ribs. In patients diagnosed with lung cancer, it is not unusual to discover significant abnormalities in previous radiographs when viewed in retrospect (2). These rather worrying findings illustrate that the reading of radiographic images is complex. This review aims to consolidate the existing literature that examines two factors that influence detection of radiographic abnormalities, namely, the provision of clinical information, and the visual search pattern employed.

THE INFLUENCE OF CLINICAL INFORMATION

Detection of abnormalities is rooted in the cognitive schema that the observer brings to bear on the image data collected by the retina. The two major components of this schema are *knowledge* of how anatomy and pathology map onto radiographic images and *expectations* about the image to be seen (3). Available clinical information can conceivably alter expectations about the image about to be seen and it is here that the potential lies for this information to alter detection.

Benefits of Providing Clinical Information

A number of studies claim an improvement in observer performance when tests are read with clinical information. Schreiber (4) and Potchen et al. (5) both concluded that including a clinical history increased the rate of detection of pathology where present, i.e. true positives. Rickett et al. (6) observed a number of emergency department doctors and radiologists, showing them the same set of images six months apart, once with a clinical history and once without. Both groups of observers improved their performance with clinical details. Fippona et al. (7) looked at the detection of focal liver lesions on CT and found that knowledge of the clinical history significantly improved the accuracy ($p = 0.02$) of the detection of lesions with a diameter of less than 1cm. This was however accompanied by an increase in false positive reporting of malignancy. Ehara et al. (8) reported similar findings when assessing the importance of clinical information for the detection of non-displaced paediatric wrist fractures. The detection of the fractures was significantly improved with clinical information. The main reason for this was an increase in the true positive fraction. Houssami et al. (9) examined the influence of knowledge of clinical information on the accuracy of mammography in women referred for investigation of breast symptoms. ROC (receiver operating characteristic, a measure of accuracy) curves for both radiologists in the study found that reporting mammography with knowledge of clinical information resulted in a small (about 2%) but significant improvement in overall test accuracy.

A number of mechanisms have been proposed to account for the increased detection rate in observer performance tests in laboratory settings, e.g. participants interpreting the image findings with a different degree of care, or anticipating a higher than normal rate of abnormal images. Doubilet and Herman (10) therefore, examined previous claims of improved performance within the clinical environment. Test films were added to the normal workload of resident radiologists on night-time coverage of the Emergency Department, who reported the images blind. Each image was read eight separate times, four times with an accompanying history suggestive of the pathology which they contained, and four times with an unrelated clinical history. Radiology readings were reviewed, and altered as appropriate by a consultant radiologist in the morning. The study concluded that providing a suggestive clinical history increased the rate of true-positive readings in a realistic clinical setting. Again however, there was a concomitant increase in false positive reporting in this study. The increase in sensitivity, therefore, comes at the expense of lowering the specificity of the test (Table 1).

Neutral or Negative Effects of Providing Clinical Information

A number of studies have reported that providing a clinical history or prompting the reader to search for certain pathologies has no effect. Cooperstein et al. (11) examined the effect of clinical history and of prompting the reader to search for interstitial disease, nodules or pneumothorax on interpretation of chest radiographs in a digital reporting environment. No significant differences in the detection of abnormalities for any of the individual radiologists in the study, or for the group as a whole were found. Good et al. (12) sought to generalise these findings to a realistic clinical environment. Test cases, with and without clinical histories, were designed and incorporated into the daily workload of consultant radiologists who reported them blind.

		Suggestive Hx	Non-suggestive Hx
Resident	True Positives	23/32 (72%) *	5/32 (16%)*
	False Positives	9	0
Resident and Consultant	True Positives	27/32 (84%) †	12/32 (38%) †
	False Positives	9	0

* Difference is statistically significant ($p<0.01$)
† Difference is statistically significant ($p<0.01$)

Table 1: Effect of Suggestive History (Hx) on the Detection of Radiograph Abnormalities
Table adapted from Doubilet et al. (10) (Hx = History)

Radiologist confidence ratings of the presence or absence of one or more of the following abnormalities: interstitial disease, nodule, and pneumothorax were recorded. Across all participants no statistically significant difference was found and the researchers concluded that knowledge of clinical history has no effect on detection.

An increase in false positive rates can potentially place an increased burden both on the healthcare system and the patient. Croswell et al. (3) investigated the effect of false positives in chest radiographs and CT examinations reviewed as a screening tool for lung cancer in smokers. They found that a person's cumulative probability of one or more false-positive CT examinations was 21% after one CT screening and 33% after two. The rates for chest radiographs were 9% and 15%, for one and two radiographs respectively. A total of 7% of participants with a false-positive low-dose CT examination and 4% with a false-positive chest radiography underwent a resulting invasive procedure.

THE INFLUENCE OF VISUAL SEARCH PATTERNS

The field of view in humans extends over 180° but it is only the centre of this visual field that provides sharp detailed vision. Consequently, eyes move to bring interesting features into this centre. The pause over the point of interest is known as a foveal fixation. Fixations are characterised by their multiple (clustering) nature when observers dwell extensively on a location, as the eyes do not remain stationary for long before losing sensitivity (13). Eye-tracking experiments assume that fixations represent the location of conscious attention of the viewer. The eye movements of an observer over an image can be tracked with remote, infra-red pupil-corneal reflection cameras which group these fixations into search patterns (Figure 1). The use of visual search patterns provides an organisational framework for studying basic perceptual processes that can be applied to the understanding of abnormality detection. It is useful for classifying detection failures and has suggested methods for improving perceptual performance (14). The following paragraphs outline two important aspects of visual search, namely, aspects leading to failure and where in the sequence they occur, and aspects leading to success by examining the visual search patterns of experts.

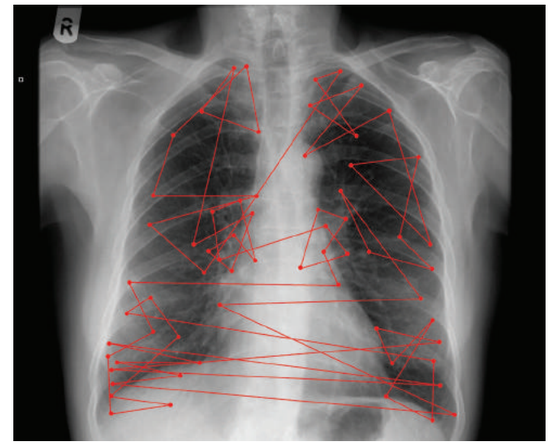

Figure 1: An example of fixations across a chest radiograph and the visual search pattern constructed by them. *Image courtesy of the Adelaide and Meath Hospital, Dublin.*

Sources of Error in Searching

Kundel et al. (15) put forward that there were three categories of error for false negative reports, based on how long they are dwelled on or fixated upon. This study tracked the eye movements of four observers searching a set of 60 chest radiographic images, 24 normal and 36 abnormal, for the presence of pulmonary nodules. Error rates, scanning patterns and the dwell time of fixation clusters on normal and nodule-containing areas of the film were studied. Errors were categorised as follows:

1. Search error or sampling error is where the observer never fixates the lesion with high resolution foveal vision and thus cannot begin to process the information. Visual attention is given to a particular area by repeated fixations in that area, grouping together in what is known as a fixation cluster. Maps of fixation clusters have shown that they are unevenly distributed over a chest image. It is estimated that it takes approximately eighteen fixation clusters to adequately sample the area of a chest radiograph (16).

2. Recognition error is where an abnormality is fixated upon but not reported. Looking at a target does not guarantee that it will be recognised. It has been shown that fixating upon a region for one third of a second is sufficient for a negative decision, but a deeply embedded target can require a cluster of fixations lasting up to three seconds (17).

3. Decision-making error is where camouflaged objects are detected, but the viewer decides that they are normal variants rather than the target. These errors are relatively easy to identify in the eye-movement record because there is an increase in the number of fixations clustering on the target site caused by the increased visual scrutiny. This is the most prevalent type of error (15).

Studies focused upon lung nodule detection have shown that 10% of misses were due to search error, 30% were due to recognition error, and 60% were due to decision making error (18).

In the case of multiple nodules, a further important source of error is "satisfaction of search". This is where one abnormality interferes with the detection of other abnormalities in the same radiograph. It is possible that detected abnormalities distract the reader from identifying other abnormalities, or that the detection of an abnormality causes an early halt of a search. Samuel et al. (1) reported that indeed nodule detectability was lower on native abnormality-containing images than it was on normal images. They also concluded that it was due to both of the aforementioned factors. This finding was replicated by Ashman et al. (19) when examining the effect of multiple abnormalities on detection of nodules in skeletal radiographs. Thirteen orthopedic surgery residents were shown in random order 15 cases in which one abnormality was present and 15 cases in which either two or three abnormalities were present. Where two or more abnormalities were present, there was a statistically significant decrease in detection rates.

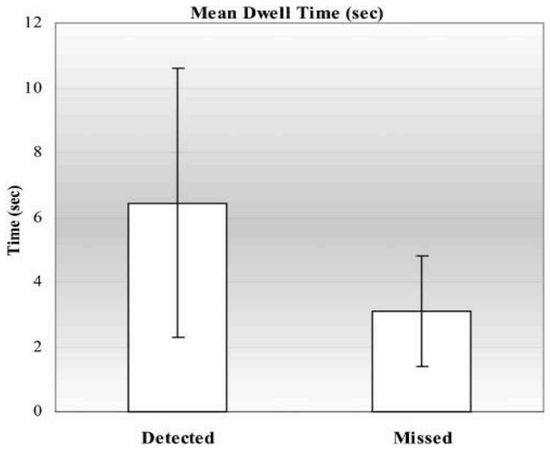

Figure 2: Mean dwell time for nodules fixated and missed. *Image reproduced with permission from Manning et al. (21)*

Dwell Time of Missed Nodules

There are characteristic search patterns that result in higher success rates. The amount of dwell time an observer spends fixating on a potential target can be instructive. It has been put forward that 0.9 seconds dwell time at a location is the minimum period required for detection of an abnormality to occur (20). Manning et al. (21) showed that with radiologists, confidence scores on positive decisions correlated well with fixation time. This study also showed that nodules that were missed were fixated on average for less than half the time of detected nodules (Figure 2).

The Visual Search Patterns of Experts

Along with the time spent fixating on a potential targets, expert observers are also known to adopt different search strategies to novices. Kundel and LaFollette (22) reported on the visual search patterns of consultant radiologists compared to that of radiology trainees and suggest that trainees use a "forward reasoning" strategy whereby all clinical features are examined in one view before proceeding to the next. This behaviour entails the use of a "mental protocol" in which a list of features are checked and ruled in or out. This is in contrast to experts who are better at gathering information from the initial holistic representation, effectively taking a global view of the image before proceeding. These search strategies are more efficient and less time is devoted to fixating non-informative areas of the image (23). Cave and Batty (24) suggested that this may be due to the experts' utilisation of information at the pre-attentive stage. This stage involves the subconscious accumulation of information from the environment. This is supported by Taylor (25) who concludes that it is this collection of information from the pre-attentive stage, rather than in the assessment of the features present in the image, that gives the expert the advantage.

Gunderman et al. (26) put forward that although radiology experts can point out and name more anatomical features than the novice, what really sets them apart is their ability to integrate structures into three-dimensional maps. These maps provide information more relevant to the diagnostic decision. In theory this would result in the novices looking almost indiscriminately at all image features whereas the experts' conceptual knowledge guides them to key features. It is possible, therefore, that with a reduced ability to holistically gather information from an image, and with less knowledge of the pertinent features, novice observers may be more susceptible to influence from clinical information.

SUMMARY

Research investigation of everyday clinical practice reported that the diagnosis 'lung cancer' was not made on the chest radiograph initially in one-fifth of the cases, even though in retrospect the lesions had been visible (27). It is thus clear that there is scope for a methodical approach to improve the success of radiographic image interpretation. Clinical information and the search pattern employed by the reader are both significant predictors of success in detection of abnormalities in radiographic images.

Generally, the provision of clinical information has resulted in higher detection rates, however this must be looked at in the possible context of a concomitant increase in false positives. An initial blind read, followed by a reading of the history is one solution put forward (28). However, it would seem that the consequences of a missed abnormality are sufficiently consequential to justify the current practice of reading radiographs with the clinical information present.

Studies have elucidated the points at which detection failures occur in the visual search of radiographs. This can be at the stage of searching the image, recognising abnormalities, or deciding whether these abnormalities represent pathologies. Analysis of this kind can guide our intervention to the appropriate part of the search algorithm to minimise failures. Analysing the search patterns of experts, the importance of the pre-attentive stage and three-dimensional mental schemas become apparent. The causes of failure and success are particularly relevant to radiology trainees in particular, medical trainees in general and those responsible for radiographic image interpretation education. The increased awareness of educators has the potential to enhance teaching with the inclusion of search pattern models to improve reader success and accurate diagnosis.

These findings have implications for both the practice of reading radiographic images and also for radiological education. Further studies are needed to examine the interaction between clinical information and visual search patterns, in particular with a focus upon the impact of providing clinical information at different stages in the image search process, whilst the current evidence-base provides a baseline framework for strategies which increase detection of abnormalities on radiographic images.

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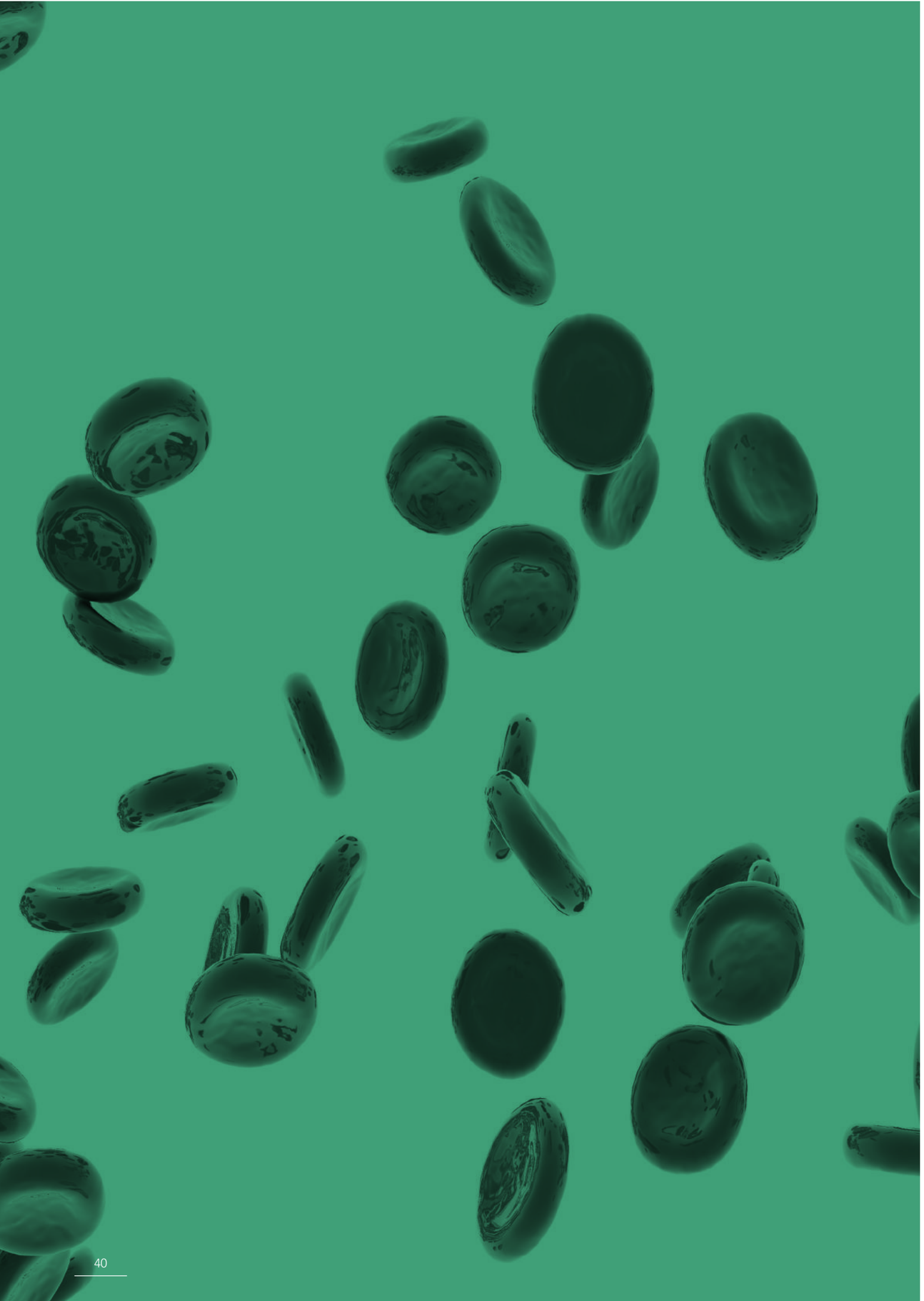

MAJOR ADVANCES IN THE MANAGEMENT OF TRAUMA - LESSONS FROM THE MILITARY

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ABSTRACT

The numerous casualties of war facilitate the collection and evaluation of data on trauma management. Massive haemorrhage is the underlying cause of mortality in a significant proportion of military personnel killed or fatally injured on the battlefield. Retrospective analysis of the management of these casualties combined with experimental research in animal models resulted in the advocacy of Damage Control Resuscitation (DCR), which includes the use of hypotensive resuscitation, the prevention and treatment of elements of the lethal triad of hypothermia, acidosis and coagulopathy, and an innovative approach to infusion of blood products. Guidelines have been drawn up for the identification of those on whom DCR confers the best chance of survival. Technological advances have facilitated data analysis and audit of case management in these casualties, leading to evidence-based alteration in clinical practice and improved patient outcome.

Key Words: damage control resuscitation, coagulopathy, acidosis, haemorrhage, hypothermia

INTRODUCTION

Throughout history, wars have taught us invaluable medical lessons particularly in the area of trauma. The American Civil War brought about the development of field hospitals due to the discovery of the link between immediate treatment and survival rates. In World War I psychological trauma was recognised for the first time as a serious illness and blood banks were pioneered, with blood transfusions becoming common practice. World War II saw innovation in the surgical field, particularly in orthopaedics, due to the number of emergency procedures which were required to take place. During the Korean War, mobile hospitals were developed so that an injured soldier was never too far from medical attention, while the Vietnamese war saw helicopters emerge as a way to transport critical patients (1). In more recent years, the wars in Iraq and Afghanistan have led to some of the most important discoveries in trauma medicine of the 21st century, particularly in the region of resuscitation. Such experiences from the military are important as they are often subsequently incorporated into civilian practice.

WHY ARE THESE ADVANCEMENTS MADE DURING TIMES OF WAR?

It is presently thought that approximately 7% of combat casualties are in need of massive transfusion (2). This has influenced the practice of resuscitation enormously. In civilian medicine, life-threatening trauma constitutes a very small proportion of patients attending a large number of geographically distinct emergency departments (3) leading to difficulty in accumulating and analysing data. The reason such rapid advancements are made in times of war is due to the large number of casualties and the collection and evaluation of data. The use of this data has been optimised in the 21st century due to innovations in technology which allow for accurate and rapid analysis.

American casualties in Afghanistan pass down a specifically structured chain of management, further facilitating data audit. The first response to a casualty is from the combat medic who travels as a part of each team on manoeuvres. A helicopter retrieves the wounded from their position within an hour and takes them to the closest resuscitation unit from where they are transferred to a military base hospital in Germany within two hours. If more sophisticated care is required the patient is flown to one of two major US military hospitals. This definitive chain allows for excellent standardisation of data collection. The data is analysed and improvements are made, providing a continuous loop of quality analysis and quality improvement in medical care.

DAMAGE CONTROL RESUSCITATION

Uncontrolled haemorrhage has been identified as the cause of death in approximately 40% of trauma related cases (4). Clearly, the management of trauma was unsatisfactory and so research was devised to identify how management of patients with exsanguinating haemorrhage could be made more effective. The result was Damage Control Resuscitation (DCR), which is the protocol now used to treat all severely injured battlefield casualties. It evolved during the Afghanistan war due to the large number of patients requiring massive transfusions in an attempt to manage both the risk of haemorrhage and coagulopathy (5). DCR includes the use of hypotensive resuscitation; the prevention and treatment of hypothermia and acidosis and an innovative approach to blood transfusion. DCR can be divided into two phases, hypotensive resuscitation and haemostatic resuscitation.

Hypotensive Resuscitation

The body's natural defences against exsanguination are considerable and should be recognised in treatment. For example, if an artery is completely transected, the resulting hypotension due to initial blood loss along with vasoconstriction and coagulation can be extremely efficient in stopping haemorrhage. Traditionally in cases of haemorrhagic shock, intravenous (IV) fluids were given until a blood pressure of 120mmHg systolic pressure was achieved. However, this works against the body's natural defence mechanism which allows blood pressure to remain low in order to minimise blood loss. Therefore, in the Iraq and Afghanistan wars it has become standard to aim for a systolic blood pressure of approximately 90mmHg and a conscious, responsive patient. This is thought to maximise the resuscitation benefit to the mitochondria while minimising the chances of "popping a clot" and causing even further bleeding (5). Though this approach is supported by a significant number of studies and has proven to be effective, it is important to remember that the evidence from these wars is based on young, fit and healthy soldiers and is therefore not an accurate repre-

sentation of the population at large. For example if a sixty year old man with coronary artery disease presented with haemorrhagic shock it would be inadvisable to follow this course of action as a myocardial infarction could result from maintaining this patient's blood pressure at 90mmHg systolic. In much the same way, a patient with carotid artery disease could suffer from a stroke in these circumstances. It is very important, therefore, to implement sound clinical judgement when using this form of resuscitation.

The Lethal Triad and Haemostatic Resuscitation

The 'lethal triad' refers to a combination of hypothermia, acidosis and coagulopathy and is seen in patients who have suffered severe trauma. It is associated with a considerable increase in mortality rates (6). It is further complicated by the fact that each component of the triad can exacerbate the other two. The second phase of DCR – haemostatic resuscitation – is concerned with the lethal triad and attempts to minimise acidosis and hypothermia. DCR recognises the fact that coagulopathy is the most easily treatable element of the triad and therefore aims to immediately correct it.

Acidosis

Hypovolaemia and peripheral vasoconstriction cause inadequate perfusion of peripheral tissues, causing anaerobic cellular respiration which leads to an accumulation of lactic acid and consequent metabolic acidosis. How acidosis negatively affects the coagulation cascade is not fully understood, however studies have shown acidosis to have harmful effects on the prothrombinase complex, platelets, thrombin generation and fibrinogen concentration (7, 8). It can be difficult to reverse acidosis in a patient who is still haemorrhaging as its reversal is dependent on reperfusion of peripheral tissues. Currently, studies are being carried out to ascertain whether the administration of exogenous bicarbonate or tris-hydroxymethyl aminomethane can combat the negative effects of acidosis on the coagulation system (9, 10).

Considering the difficulties in reversing acidosis, it is extremely important to avoid any actions which can worsen this condition. An important example is that of hypoventilation which is easily managed by adequately ventilating the patient. Also of importance when considering acidosis, is the choice of resuscitation fluid. Normal saline, which is one of the most commonly used isotonic crystalloid fluids, has a pH as low as 4.5 and has been proven to contribute to metabolic acidosis in patients suffering from shock (11).

Hypothermia

Hypothermia refers to the drop in core temperature to below 35 degrees Celsius at which point bodily functions and metabolism cannot be carried out as normal. The effect hypothermia has on survival rates is clearly described and severe trauma-related hypothermia (<32 degrees Celsius) has been associated with 100% mortality (12). The coagulation cascade is an enzymatic pathway and is therefore sensitive to changes in temperature. Hypothermia affects coagulation in two ways; moderate hypothermia (32-34 degrees Celsius) directly reduces coagulation factor activity by approximately 10% for each temperature decrease of one degree, while significantly reducing the activity of platelets (13, 14, 15, 16). Hypothermia also shifts the oxygen-dissociation curve, making oxygen less readily available to tissues for cellular respiration (14). This leads to anaerobic respiration and consequent acidosis.

Trauma patients at war are susceptible to hypothermia for a number of reasons. In general, wounding occurs outdoors, severe injury renders the casualty immobile, and loss of blood slows down metabolic activity. All of which contribute to a drop in core body temperature. Due to the association of hypothermia with a decreased survival rate, a new protocol was established by the US military in Iraq with an emphasis placed on pre-hospital management of the patient regarding hypothermia prevention. Combat medics were trained in basic principles on how to avoid hypothermia such as the rapid control of external haemorrhage, limited removal of patients' clothing, and the use of thermal blankets and in-line fluid warmers, for example, the Thermal Angel. These in-line fluid warmers keep blood and fluids warm – between 37 and 42 degrees celcius – by delivering warm fluids at rates of up to 5000ml per hour. The Thermal Angel is particularly useful in trauma situations as it is disposable, lightweight and completely portable. Easy to set up, it provides warm fluids within 45 seconds. This protocol has been highly effective and the number of casualties arriving to military hospitals with hypothermia is now decreased from 7% to 1% (17).

Coagulopathy and Blood Transfusions

Coagulopathy is a defect in the body's ability to form clots leading to bleeding diathesis. It was thought up until recently that coagulopathy occurred primarily due to the dilution of clotting factors after the infusion of crystalloids. However, new research has concluded that a large percentage (38% in one study), of casualties were already coagulopathic on admission to hospital before the administration of any fluids (18). Traditional methods of resuscitation involve administering IV crystalloid and blood at a ratio of 3:1. Fresh frozen plasma and platelets – which provide the clotting factors – were only given after 8-10 units of blood, due to the fact that many patients were not identified as coagulopathic at this stage of treatment.

There is a significant scientific basis for the early administration of coagulation factors for patients requiring massive transfusion (19, 20, 21, 22) and recently studies have recommended a 1:1:1 ratio of packed red blood cells, fresh frozen plasma and platelets - this is the protocol now followed by the US and UK militaries which is slowly being translated to civilian practice. There are a variety of positive effects associated with this new transfusion protocol. Firstly, superior clotting ability is observed as the clotting factors are administered earlier, decreasing the possibility of coagulopathy and consequently the amount of blood lost. The 'Michelin Man Effect' refers to peripheral oedema and is a result of crystalloid infusion. As crystalloids are not transfused as part of this protocol there is a decrease in the 'Michelin Man Effect'. Orthopaedic surgeons in the American military hospitals have commented on how much easier their surgeries are due to this decrease in oedema and have noted that they see less post-operative complications in patients without the 'Michelin Man Effect'.

The benefits of freshly donated blood have also become apparent in recent years. Donated blood has a shelf life – approximately 5 weeks for white blood cells and 6 weeks for red blood cells – but the older the blood is, the less clotting factors will be present. This may not be important in patients who do not require significant quantities of blood. Where it is not necessary to use the most recently donated blood, blood banks will send out older blood so as to minimise waste. However it has now become protocol in the US military to use the most recently donated blood for patients requiring massive transfusion. This has translated into civilian trauma care in the US and a special request for recently donated blood from the blood bank can be made when a patient in need of massive transfusion arrives at the emergency department.

In the US military, every soldier is aware of their blood type and therefore fresh whole blood can be donated on scene in emergency situations when component blood is unavailable. Between March 2003 and July 2007, over 6000 units of warm fresh whole blood were transfused in Afghanistan and Iraq by US medical service providers to patients with life-threatening traumatic injuries with haemorrhage (23). Rapid screening assays are performed to ensure the blood is safe and free from infectious diseases. In patients who don't stop bleeding despite all conventional treatments, fresh whole blood has frequently been seen to work. This is thought to be due to the fresh clotting factors. The effective use of fresh whole blood by the military has led to renewed interest in its use in civilian practice. Studies carried out on animals have had positive results, and human studies have shown that in emergency situations the risk: benefit ratio of fresh whole blood favours its use (23, 24). Randomised trials are now being set up to determine whether the use of fresh whole blood could be favourable to that of component blood even when both are available.

In Ireland, individual hospitals each have separate protocols. However in general, patients requiring an emergency transfusion are initially administered a crystalloid, for example normal saline or Hartmann's solution, and are then given colloid (plasma-type solutions) until the crossmatched blood becomes available. Alternatively these patients are given O-negative blood. Doctors attempt to keep blood pressure within normal limits - unlike the hypotensive resuscitation in the US - and keep haemoglobin greater than 8g/dL. After two litres of blood loss, fresh frozen plasma and platelets are given to replenish coagulation factors (25). In time it is likely that protocols in Irish hospitals will evolve to incorporate aspects of damage control resuscitation, sound clinical judgement will be imperative in deciding when this should be used.

Identification of Patients who Require Damage Control Resuscitation

It is important to identify patients in need of damage control resuscitation as opposed to patients requiring 'standard' resuscitation because theoretically patients with less severe injuries could manifest hypercoagulability if subjected to DCR. This needs to be assessed quickly and is based on rapidly obtainable clinical parameters. On the battlefield, approximately 95% of casualties present with penetrating injuries which has led to the identification of certain patterns of injury that reliably predict the need for massive transfusion and DCR (26). These patterns include patients with multiple proximal amputations (particularly thigh level), truncal haemorrhage combined with a proximal amputation and abdominal evisceration with hypoten-

sion (26). Other measurable parameters which predict a need for DCR are: systolic blood pressure below 90 mmHg for a soldier or below 110 mmHg for a civilian, a base deficit of less than 6 mEq/L, a haemoglobin level of less than 11 g/dL, temperature of less than 35 degrees Celsius and a weak or absent radial pulse. Patients matching any of these clinical parameters should be immediately identified as in need of DCR.

CONCLUSION

Times of war have always precipitated innovations in medicine. One of the most important of these advances in the 21st century is the development of damage control resuscitation. DCR advises hypotensive resuscitation as opposed to conventional normotensive resuscitation and emphasises the importance of prevention and treatment of all three elements of the 'trauma triad of death'. It is important to note that most data currently available concerning DCR is from retrospective observational studies and more definitive tests are needed to prove and further define the beneficial properties of DCR. Data analysis has been facilitated by the technological advances of the 20th and 21st centuries. The Trauma Audit and Research Network (TARN) actively produces evidence-based changes in treatment as result of auditing and research. There are elements in trauma management that require further research such as the use of fresh whole blood and the administration of exogenous bicarbonate or tris-hydroxymethyl aminomethane to reverse acidosis. With ongoing experimental work and data collection, the scientific community continues to improve the treatment of severe trauma injuries.

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FLUID REPLACEMENT FOR THE TREATMENT OF HAEMORRHAGIC SHOCK; A 21st CENTURY PERSPECTIVE

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ABSTRACT

Haemorrhagic shock is a common and life-threatening consequence of trauma, gastrointestinal bleeds, and other pathologies. The main goals of treating haemorrhagic shock are to achieve haemostatic control and restore intravascular volume. The most common fluids currently used in fluid resuscitation are crystalloids, colloids, hypertonic solutions, allogeneic blood, and artificial oxygen carriers. While blood is the best form of fluid replacement, especially in severe haemorrhagic shock, its limited availability necessitates the use of other fluids. Crystalloids are the most commonly used fluid replacement class in the pre-hospital setting but must be delivered in large quantities and can therefore cause pulmonary oedema and other complications. Artificial oxygen carriers are a relatively recent development and show promise of providing optimal oxygen delivery to tissues until blood transfusions are available. Since World War II, the accepted treatment approach for haemorrhagic shock has been to restore physiological parameters as soon as possible with aggressive fluid replacement. While this strategy may be beneficial for patients with controlled haemorrhagic shock, recent research shows that patients with uncontrolled haemorrhagic shock may require hypotensive resuscitation to achieve the optimal balance between haemostasis and perfusion of vital organs. If intravenous administration of fluids is impossible, alternate routes include intraosseous needles, intraperitoneal delivery, nasogastric tubes, and rectal delivery. Finally, under-resuscitation can be avoided by monitoring more accurate end-point markers of fluid replacement, such as oxygen debt, lactate, and inflammatory markers.

Key words: haemorrhage, shock, colloid, crystalloid, transfusion, artificial oxygen carrier

INTRODUCTION

Shock is a state of circulatory failure that leads to life-threatening hypoperfusion of vital organs (1). Initially, in 1934, Alfred Blalock identified 4 categories of shock: hypovolemic, vasogenic, cardiogenic and neurogenic (2). The commonest of these, hypovolemic shock, results in a loss of circulating blood volume due to either haemorrhage (internal or external) or increased vascular permeability and dilatation (1). Haemorrhage is regularly encountered by physicians in emergency departments, operating rooms, and intensive care units and requires rapid medical intervention (3). The estimated blood volume for a 70 kg person is approximately 5 L and represents 7% of total body weight (3). Haemorrhage is divided into 4 classes (see Table 1), with Class I (blood loss <750 mL) being a non-shock state, while Class IV (blood loss >2 L) is characterised by signs of severe shock (eg, tachycardia, hypotension, tachypnea, anuria, lethargy) and requires immediate therapy (3).

The most common cause of haemorrhagic shock is trauma, which in turn is the leading cause of death worldwide in people between the ages of 5 and 44 years (5). Particularly important mechanisms of trauma include lacerations, penetrating wounds to the abdomen and chest, and ruptured major vessels (3). Other common causes of haemorrhagic shock include gastrointestinal bleeding (eg, from oesophageal varices), massive internal bleeding from long bone fractures and solid organ injuries, antithrombotic therapy, coagulopathies, obstetric/gynaecologic causes (eg, ruptured ectopic pregnancy), pulmonary causes (eg, lung cancer), ruptured aneurysms and retroperitoneal bleeding (3). The pathophysiological consequences of prolonged hypoperfusion include metabolic acidosis, loss of cell membrane integrity (3), and an aggressive inflammatory response that can result in irreversible multi-organ failure (5). Therefore, basic therapeutic goals for haemorrhagic shock are to provide fluid resuscitation as well as control bleeding, coagulation support, and maintenance of normothermia (5). In recent years, there have been many important advances in fluid resuscitation with regards to classes of fluid replacements available, strategies for fluid administration, and therapeutic end-points for which to monitor (3).

	CLASS			
PARAMETER	I	II	III	IV
Blood loss (ml)	<750	750 - 1500	1500 - 2000	>2000
Blood loss (%)	<15%	15 - 30%	30 - 40%	>40%
Pulse rate (beats/min)	<100	>100	>120	>140
Blood pressure	Normal	Decreased	Decreased	Decreased
Respiratory rate (breaths/min)	14 - 20	20 - 30	30 - 40	>35
Urine output (ml/hour)	>30	20 - 30	5 - 15	Negligible
Mental status	Normal	Anxious	Confused	Lethargic

Table 1. Classification of haemorrhage. *Modified from Committee on Trauma (4).*

CLASSES OF FLUID REPLACEMENTS FOR HAEMORRHAGIC SHOCK

The most common types of fluids used in the treatment of haemorrhagic shock include crystalloids, colloids, hypertonic solutions, allogeneic blood, and artificial oxygen carriers (2).

Crystalloids

For the past 40 years, the gold standard for treating trauma victims in haemorrhagic shock has been to infuse large volumes of crystalloids early and rapidly, especially when blood products are not available (5). The most commonly used crystalloids are lactated Ringer's solution and normal isotonic saline solution (5). Lactated Ringer's solution, a mixture of salts that is isotonic with blood, is relatively safe and inexpensive, and equilibrates rapidly through the extracellular compartment, restoring the extracellular fluid deficit that accompanies haemorrhage (2). Another advantage of lactated Ringer's solution is the generation of bicarbonate from the metabolised lactate, which buffers against metabolic acidosis associated with haemorrhagic shock (2). Unfortunately, lactated Ringer's solution has negative effects on the immune response to haemorrhagic shock, including increased neutrophil superoxide burst activity, increased neutrophil adherence, and increased cytokine activation (eg, IL-1, IL-6, and TNF) (2). The large volumes of crystalloid required for adequate resuscitation can lead to decreased intravascular oncotic pressure (2) and subsequent pulmonary oedema (5). Furthermore, a study using an experimental haemorrhagic shock model in pigs showed that fluid resuscitation with lactated Ringer's solution was inferior to blood or gelatine (a colloid) at improving mucosal tissue oxygenation of the small intestine (6).

Colloids

Because crystalloids were known to cause pulmonary oedema ("shock lung"), in the 1970s, focus was placed on the development of hyperosmotic solutions called colloids (eg, gelatins, dextrans, and hydroxyethyl starches) with a primary goal of improving pulmonary function during fluid resuscitation for haemorrhagic shock (5). Their use has since been advocated because unlike crystalloids, colloids remain in the intravascular compartment and so a lower volume is required to attain hemodynamic stability (2). On the other hand, colloids are expensive, lower calcium and immunoglobulin levels in the blood, and may deplete the extracellular fluid volume (2). Furthermore, a recent Cochrane review assessing fluid resuscitation in critically ill patients with trauma, burns, or following surgery found no reduction in mortality with use of colloids compared to crystalloids (7). There is also concern that colloids can cause hyperoncotic acute renal failure, but a recent study using an experimental model of severe haemorrhagic shock in rabbits showed that 6% hydroxyethyl starch either alone or in combination with Ringer's lactate improved renal glomerular function and did not have a harmful effect on the kidney (8).

Hypertonic solutions

The use of hypertonic solutions (eg, hypertonic saline) has been investigated since the 1980s, and while their use in humans has not yet been approved by the US Food and Drug Administration, they show promise as a fluid replacement therapy (5). Experimental models of haemorrhagic shock in animals have shown that small volume hypertonic saline is as effective as large volume crystalloids in expanding plasma volume, improving cardiac output and microcirculation, restoring renal function, and reducing acute lung injury and red blood cell injury (5). Hypertonic saline is shown to be of particular benefit to trauma patients with combined head injury and haemorrhagic shock, because it increases cerebral perfusion while decreasing intracranial pressure and cerebral oedema (5). Furthermore, a recent study comparing the ability of hypertonic saline plus dextran (HSD) and crystalloids to prevent harmful immunologic effects in haemorrhagic trauma patients found that HSD significantly inhibited neutrophil activation, inhibited proliferation and cytokine production of pro-inflammatory monocytes, and stimulated proliferation and cytokine production of anti-inflammatory monocytes (9).

Allogeneic blood

Allogeneic blood transfusion is invaluable for the pre-hospital and in-hospital treatment of severe haemorrhagic shock because blood is currently the only substance that can fully restore oxygen-carrying capacity (3). A recent study using an experimental haemorrhagic shock model in dogs showed that while blood, Oxyglobin (an artificial oxygen carrier), saline (a crystalloid), and 6% hetastarch (a colloid) were able to restore microvascular and systemic function, only blood was able to restore oxygenation changes to pre-haemorrhagic levels (10). Unfortunately, blood is not readily available in the pre-hospital setting due to the necessity of refrigeration and blood typing (3).

In patients with no known risk factors, blood transfusions should be delivered when blood loss from haemorrhage exceeds 30% of blood volume (Class III haemorrhage) or when the haemoglobin level drops below the threshold of 7-8 g/dL (3). However, blood transfusions should be used to maintain haemoglobin at 10 g/dL in patients who are actively bleeding, the elderly, and those at risk for myocardial infarction (3). If type and cross-matched blood is unavailable, O-negative blood should be given (3). Despite the advantages of allogeneic blood, it is in limited supply and carries multiple potential adverse effects (5). These include infectious complications (eg, hepatitis, HIV, and bacterial contamination), immune reactions, metabolic complications (eg, hyperkalemia, hypocalcemia, and citrate toxicity) and mistransfusion (5).

Artificial oxygen carriers

Artificial oxygen carriers are a relatively new development and show promise of providing a better oxygen carrying capacity than crystalloids, colloids, and hypertonic solutions while avoiding the problems of storage, compatibility, and disease transmission associated with blood transfusions (2). The three types of artificial oxygen carriers currently under investigation are perfluorocarbons, haemoglobin-based oxygen carriers, and haemoglobin vesicles (5).

Perfluorocarbons are synthesized by halogenating cyclic or straight-chain hydrocarbons (5). They have a long shelf life, minimal infectious or immunogenic effects (2), and are an effective initial replacement for blood transfusions in patients with haemorrhagic shock (5). However, perfluorocarbons undergo rapid plasma clearance (2) and are known to be associated with neurological complications and postoperative ileus (5).

Haemoglobin-based oxygen carriers (eg, PolyHeme) are derived from human or bovine sources and are thought to act by scavenging nitric oxide, increasing release of endothelin, and stimulating endothelin receptors and adrenoreceptors (5). While they have a high oxygen carrying capacity and prolonged shelf life, some disadvantages include hypertensive effects, immunogenic effects, and potential renal toxicity (2). Moreover, a recent study in patients with haemorrhagic shock revealed a significantly higher risk of myocardial infarction associated with fluid resuscitation using PolyHeme plus blood (3%) compared to crystalloid plus blood (1%) (11).

Lastly, haemoglobin vesicles consist of purified human haemoglobin encapsulated by phospholipid vesicles (5). While their use has been limited to experimental studies of haemorrhagic shock, haemoglobin vesicles have been shown to maintain systemic oxygenation without producing hypertensive or immunogenic effects (5).

TREATMENT STRATEGIES FOR FLUID RESUSCITATION

Aggressive versus hypotensive resuscitation

From the time of World War II until recently, the accepted therapeutic dogma for the treatment of haemorrhagic shock has been to replenish blood volume rapidly and to attain normal physiological parameters (3). This view was reinforced during the Vietnam War due to the observation that aggressive fluid resuscitation with red blood cells, plasma, and crystalloid solutions appeared to improve survival in trauma patients (2). However, this dogma is now being challenged by clinical trials and experimental animal models that have differentiated between controlled and uncontrolled haemorrhagic shock and identified key differences in their responses to aggressive fluid replacement (2).

In controlled haemorrhagic shock, fluid resuscitation is aimed at normalising haemodynamic parameters (2) because the source of bleeding has been occluded (eg, by the formation of a clot) (3). In contrast, uncontrolled haemorrhagic shock is characterised by ongoing bleeding, and therefore a failure to achieve haemostasis (2). As such, attempts to normalise vital signs in someone with uncontrolled haemorrhagic shock may lead to volume overload that prevents clot formation at the site of injury and leads to renewed bleeding (2).

It is now becoming clear that using smaller doses of fluid replacement—hypotensive resuscitation—achieves optimal survival in uncontrolled haemorrhagic shock (2). A recent study using an experimental uncontrolled haemorrhagic shock model in guinea pigs compared the efficiency of aggressive fluid resuscitation, low-volume fluid resuscitation and permissive hypotensive resuscitation therapy approaches using crystalloids and

colloids and found that survival time was significantly higher in the permissive hypotensive resuscitation groups (12). In the pre-hospital setting, it is recommended that trauma victims with uncontrolled haemorrhagic shock receive repeated aliquots of 250 mL of lactated Ringer's solution during evacuation, with a goal of maintaining a systolic blood pressure of 80 mmHg as well as controlling bleeding (2).

Immediate versus delayed resuscitation

Related to the dogma of aggressive resuscitation for haemorrhagic shock during World War II and the Vietnam War was the belief that early resuscitation was key to survival (3). For many years, the thinking of trauma surgeons was dominated by the concept of the "golden hour", the time period in which shock could be reversed and organ damage prevented (3). However, it was shown that in patients with haemorrhagic shock from penetrating truncal injuries, a higher discharge rate and fewer complications were seen in those who received delayed resuscitation with lactated Ringer's solution compared to those who received immediate resuscitation (3). Findings such as this indicate that patients with moderate hypotension from modest bleeding may benefit from postponing heavy fluid replacement until arrival at a definitive care facility (3). However, early provision of intravenous crystalloids, colloids, or blood products can be life saving in patients who are in severe haemorrhagic shock (3).

Routes of delivery of fluids

While large-bore peripheral intravenous lines are the most widely used method of administration of fluid replacement, alternate routes must be sought when haemorrhagic shock and compensatory vasoconstriction make intravenous access exceedingly difficult (13). A common route of fluid replacement in the paediatric setting is via an intraosseous needle, which is rapid but requires special equipment and sterile conditions and carries a risk of osteomyelitis and compartment syndromes (14). Intraperitoneal fluid replacement is used to improve organ perfusion and like intraosseous needles, requires special skills and equipment, sterile conditions, and increases the risk of peritonitis (15). Nasogastric tubes allow easy administration of fluids and do not require sterile conditions, but the amount of fluid necessary to reverse haemorrhagic shock increases the risk of vomiting and aspiration (16). A relatively new route for administration of fluid resuscitation and anti-shock drugs is across the rectal mucosa, which is relatively easy, painless, efficient, and does not require special skills or sterile conditions (17). A study using an experimental model of hypovolemic shock in rabbits showed that compared to no treatment, administration of 0.9% sodium chloride solution via the rectum resulted in a significantly raised mean arterial pressure (17).

MONITORING END-POINTS FOR FLUID RESUSCITATION

The goals of fluid resuscitation are to normalise blood pressure, heart rate, and urine output while keeping central venous pressure in an adequate range (8-15 mmHg) (5). If blood pressure and urine output alone are used as end-points for fluid replacement in haemorrhagic shock, up to 85% of patients may be under-resuscitated (3). This occurs because measured normalisation of gross physiologic parameters does not necessarily correspond to reperfusion of cells via the microvasculature (3). Normalisation of oxygen transport variables, oxygen delivery, cardiac index, oxygen consumption, lactate, base deficit, and mucosal gastric pH represent more accurate therapeutic end-points (3). Some of these variables are currently monitored indirectly, but experimental and clinical studies using new technologies are uncovering more direct and accurate methods (eg, transcutaneous partial oxygen tension) of monitoring patients receiving fluid resuscitation (5).

Other important end-points are pro-inflammatory cytokine levels (eg, TNF-alpha, IL-1, and IL-6), as excessive inflammatory reactions in ischemic tissues have been shown to adversely influence prognosis in patients being treated for haemorrhagic shock (5). New biotechnological methods are being utilised to identify immune cell surface markers (eg, HLA-DR), expression of pro- and anti-inflammatory mediators, and genes for cytokine expression (5).

CONCLUSION

Haemorrhagic shock is a common and life-threatening consequence of trauma, gastrointestinal bleeds, and other pathologies. The main goals of treating haemorrhagic shock are to stop the bleeding and restore intravascular volume. While blood is the best form of fluid replacement, especially in severe haemorrhagic shock, its limited availability necessitates the use of other fluids. Crystalloids are the most commonly used fluid replacement class in the pre-hospital setting, but the risk of pulmonary oedema and other complications has motivated the development of new classes such as hypertonic solutions and artificial oxygen carriers. The old dogma of early and aggressive fluid resuscitation for all haemorrhagic shock victims is now being challenged by an evolving understanding of controlled and uncontrolled haemorrhagic shock, with the latter requiring hypotensive resuscitation to achieve the optimal balance between haemostasis and perfusion of vital organs. Ongoing research into the optimal routes of delivery and end-point markers of fluid resuscitation show promise of improving outcomes for distinct populations of patients suffering from haemorrhagic shock.

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Photo taken of John McCarthy at the UN Convention on the Rights of the Disabled, New York, 2006. Image reproduced with permission by Tom Olin.

John McCarthy

LIFE & LOVE

FOREWORD / Aileen Conway

This article, 'Life and Love', was originally printed in the Cork Independent on the 25th of August 2011 in John McCarthy's column called 'The Human Condition' (1). John lived with Motor Neuron Disease (MND) for the last two years and chronicled his journey of living with MND and mental illness in his column since 2009. 'Life and Love' was later recorded by John and broadcast on Newstalk 106-108fm (2) and generated a huge response from the general public and media alike. 'Life and Love' is a very honest and emotional account of how an illness such as MND can affect not only every aspect of a person's own life, but also the impact a chronic illness can have on the family as a whole.

John, in his own words, lived, not suffered with mental illness for many years and was an outspoken and often controversial advocate for the rights of those living with what he termed 'the normality of madness'. He was the founder of Mad Pride Ireland, a lobby group for mental health issues. Mad Pride Ireland has organised many events to generate debate and reduce the stigma of mental health issues in Irish society, such as the Mad Pride Festival which was first held in 2008 and was held in Tullamore, Portlaoise and Cork in 2011. John had also recently been appointed to the Implementation Group for the National Disability Strategy by Minister Kathleen Lynch, Department of Health and Department of Justice, Equality & Defence, with responsibility for Disability, Older People, Equality & Mental Health. Sadly, John passed away at home in Cork on the 10th of January 2012, aged 61. He is survived by his wife Liz, his children David and Jill and his grandchildren.

The editors of the UCD SMJ would like to thank the Cork Independent for allowing us to reproduce 'Life and Love' here and would especially like to thank John's family in giving us permission to reprint John's article.

"Pain in the body can be handled so much easier than pain in the spirit"

I have been thinking about writing this for a bit.

When friends ask me how I am, "I'm grand" is the stock in trade answer, with a smile. Or "as good as it gets". A few ask how do I retain the humour? A few suggested I write it down, warts and all. So here it is.

If I don't complain or crib, then I will not feel so bad. It works; it works very well. Most of the time!

We in this house are going through hell right now.

I'm dying.

We know how hard it is for all of us, but we smile most of the time. We remain calm, most of the time. I am stuck in the bed, most of the time. Outings are rare and tiring. Taking a leak, a nightmare! Putting on socks, underwear, pants, exhausting. Sitting up in a chair - exhausting. Not able to walk. Sponge baths! Losing the use of my hands. Basically, my life is gone to shite.

This motor neurone is relentless. The old name is so much more descriptive - creeping paralysis. That is what it is does, it creeps over your body, like a rapist. I cannot stop this disease destroying my body but I can prevent it destroying my mind and spirit.

You know there is an opportunity in everything, and in every situation. We as a family are closing ranks and showing our strength. Growing!

I would suggest, even, that this family is getting stronger. There is a lot of love in the air in the house, there is a lot of sadness.

When I need to, and I do, I cry, scream and vent. Alone, when they are out, they know it.

I tell them, and they say it helps to know.

I have learnt to be very open about my feelings whether they're good or bad. Some cannot handle that level of truth. My family can. And they do me the great honour of telling me "will you cop on?" when I run away with myself.

So, I just cry - not too often mind you - but I do it, and it helps. It does not help as much as a deep breath and a smile when my beautiful wife returns, gently opens the door and asks "You all right?"

I can smile and say "I'm grand, you?"

"Me?" she always lies, "I'm fine, cup of tea?" and we get on with this shite.

Liz, being Liz, walks the pressure off in her beautiful garden and vents in her space.

She rarely speaks of her pain, but the story is in her face at those brief moments when I see her and she can't see me. If she catches me glancing, that beautiful smile magically reappears.

This paralysis is nearly as disabling for Liz. Her whole life has been shagged up, she is on a new, unexciting, heart-rending, heart-breaking career, love is forcing this choice on the woman I love, she is a carer now. I so hate that, I so hate seeing my beautiful wife under this strain.

We all make an effort to smile through the strain, and we all see the strain through the smiles.

My family, the four of us, have sat at the kitchen table, looked into each other's eyes and hearts and agreed to stay united through this.

Love at its best. They are doing for me what I have done for them. They are looking after me and I am making it as easy as possible for them to look after me.

My daughter and my son are in their mid-thirties. They're great, and they have their own families to distract them, thank God. I know it helps them. They give me huge strength, giving too much of their time but I am greedy for it.

I have an extended family now, grandsons! One side with a nana and a granddad, one with a granddad.

It breaks my heart that I will not know them as men. I get pangs of jealousy, when I see the other two granddads, playing, walking with the boys.

I have great friends who visit, and we have talked this out. I think most of them call to drink my stock of gin! But I really hope the gin does not run out, I love the company.

Choice

So I have a choice: I can allow all this to overwhelm me or I can smile. I can only smile if I keep the mind busy. So I work in the bed for eight - ten hours a day. I write, make calls and stir the media and political pot to keep this fight for human rights of the mad community on the boil.

I love it. I love the progress we are making.

I cannot imagine having something like creeping paralysis in the old days when a cripple was a cripple, before computers, mobiles, skype, all of that contact with other human beings from the bed.

How and where did those in the past find the strength to live with this cruel way of dying?

Mad Pride is working hard to get its autumn national radio campaign in place to stop force by scrapping mental health laws.

What is going on in my spirit? I am simply experiencing the beautiful side of madness, I have a sense of peace, because I have found that essential unselfish way of loving myself.

The normality of madness! Being quietly confident!

Having spiritual disquiet, depression is the most crippling method of destroying a human being. Pain in the body can be handled so much easier than pain in the spirit.

I will take the last few years I have with creeping paralysis, but with my spirit growing, rather than 20 years with depression and my spirit dying.

I have been there, that place of self isolation.

That was truly awful.

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THE SCIENCE OF RESPECT;
A SIBLING'S PERSPECTIVE

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"Yes, my older brother has Down syndrome, but does he "suffer" from it?"

Ever since my high school biology class I've known that going into science was going to be a hard road. It was the first time I was taught that someone with an extra 21st chromosome "suffers" from Down syndrome. And it was not the last.

I remember sitting in that class and being shocked. I wondered: does my brother "suffer" from Down syndrome? Yes, my older brother has Down syndrome, but does he "suffer" from it? The teenager at home who loves to sing and dance, play Nintendo, and go hiking and camping? The sports fanatic who keeps up-to-date on all his favorite teams – the Mariners and Seahawks – and enjoys competing in Special Olympics? Sure, he struggles at some things, but don't we all? And, sure, his struggles may be with counting out change at the store while I'm struggling with pre-calculus, but does that mean he "suffers"? The image of Down syndrome painted by my biology teacher just didn't fit with the brother I know and love. I remember wondering what to do.

And that was just the first time. I've taken numerous science classes since – both in my first degree and now here in medical school – that have included negative and outdated language and images. And every time I hear it, I feel awkward and alone, wondering if I'm the only one who notices. And every time I wonder what to do. Do I want to be the sibling who objects to every offending word and phrase, and gets labelled as being too "politically correct" when all I really want is language that shows respect for people with disabilities? And what can I do to make people understand that people with disabilities are just like us and that they deserve respect?

Well, what I can do right now is tell you a bit about my brother. His name is Travis and he's older than me – by three years. We went to the same middle school and high school and all the teachers knew him; he's the social butterfly of the family and he'll talk your ear off – the complete opposite of me. He was a Boy Scout and got his Eagle Scout in 2002 – it took him a few years longer than most scouts, but he made it. He's always enjoyed going hiking and camping with the family and he loves watching movies and playing Nintendo. Since 2005 he's lived in a group home with four other guys with various disabilities and their care provider. He thoroughly enjoys the independence of living away from home and the camaraderie with his housemates. He also works two part-time jobs – one re-shelving books at the local library and the other bagging groceries at a grocery store. His social calendar is filled with various sporting events, dances at a local community center, dinner at the mall with friends, church on Sundays, and going to the movies. He sounds pretty much like you or me or anyone, doesn't he?

So this is what I think of when I sit in class and hear "suffers from" or "Down syndrome baby" or other phrases that sound archaic and negative. Is my brother a "Down syndrome" or a person? He's a person first and foremost in my mind, which is why I always prefer "person with Down syndrome." And it is not just from the lecturers and doctors that I hear negative language. I can't count how many times I have sat in class waiting for a lecture to begin and heard the word "retarded" flung about. Anytime I hear it used in a derogatory manner, it feels like a kick in the gut. And I hear it far too often.

So here I am, years down the road from that high school biology class and I'm still faced with the same dilemma. What to do? How much to do? Can just one person make an impact? Well, maybe it doesn't have to be just one person. Maybe you can help me. The next time you talk about someone with a disability take a moment to think. Ask yourself if the language you choose shows respect. Or next time you hear someone say something negative or grating, speak up and help me change the conversation. Do it for me. Or better yet, do it for Travis, for his housemates, and for his friends. Do it for the over 200 million people around the world with intellectual disabilities.

AN IRISH EMERGENCY MEDICINE ELECTIVE - A GERMAN MEDICAL STUDENT'S PERSPECTIVE

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INTRODUCTION

Electives form an integral part of undergraduate medical education in Germany. During a six-year program, consisting of five years of medical school and 12 months of internship, students must complete at least four months of elective rotations. The German Board of Examination encourages students to take at least some electives abroad to gain insight into different healthcare systems. Students are free to choose electives that reflect their personal interests, but are expected to spend at least one month in an ambulatory setting of a hospital or in clinics.

EMERGENCY MEDICINE IN GERMANY

Traditionally, emergency medicine in Germany is focussed on the pre-hospital environment. German ambulance services are operated by paramedics, who are joined by an emergency doctor at the scene. Hospital-based emergency medicine also differs significantly from the Irish model; German emergency departments are commonly divided into “trauma” and “medicine” sections, which operate independently from each other. A triage system is also not used in Germany (1). The emergency departments are staffed by junior doctors in early stages of their specialty training (mostly surgery, general medicine). Lacking a formal, board-recognised training scheme in emergency medicine, electives abroad in this specialty are a valuable experience for German medical students. This report will detail the experiences of two German 4th year medical students during an emergency medicine elective at St. Vincent’s University Hospital (SVUH) in the summer of 2010 (2).

AN EMERGENCY MEDICINE ELECTIVE: TEACHING

Although not referred to as a “sub-internship” by the consultant in charge or the department administrator, an elective rotation at SVUH’s emergency department (ED) fulfils all necessary criteria for a sub-internship, as listed in the model curriculum of the International Federation of Emergency Medicine. Major educational criteria include, but are not limited to, basic and advanced life support skills, airway management, the ABC approach, toxicology, etc. as well as clinical decision making in an emergency environment, focused physical exam and others (2,3).

The elective was based on three “columns”: a 1) formal taught program, 2) experience on the floor and 3) interaction with senior staff. The taught program was divided into attendance at regular lectures for house staff and residents as well as student-only conferences, which were tailored to the student’s needs and interests. Formal teaching was provided once a week by the senior house officers and a consultant, along with registrars from other departments. Students were encouraged to participate actively during these sessions. Lectures covered various common topics in emergency medicine and the so-called “radio-rounds” helped both house staff and students to familiarise themselves with common x-ray findings in an emergency medicine setting. New insights from research were discussed in a journal club.

Two senior registrars were in charge of the student-only conferences. In these daily, two-hour long seminars, which were either held as a lecture or at skills stations, common diseases and patient conditions in an ED were discussed. These conferences covered topics such as basic and advanced life support, advanced trauma life support, airway management, burn care, suturing and a brief introduction to FAST scanning.

FAST (“focused assessment in severe trauma”) ultrasound is a fast and reliable method to diagnose free intra-abdominal fluid in trauma. The FAST scanning introduction was a highlight and was highly appreciated by the students, as this is an integral part of trauma assessment in Germany. It should be added to the in-house curriculum of SVUH’s ED elective (4).

THE ELECTIVE EXPERIENCE

The clinical program slightly differed from the usual curriculum, because both visiting students stayed for more than four weeks. This left time for a ‘step-by-step’ introduction to a typical Irish Emergency Department. In the first week, each visiting student worked under the direct supervision of an experienced registrar, who assessed the student’s competencies and to whom the student presented the patients he had seen. Common procedures were taught if the student was not able to perform them. The supervising doctor provided a brief introduction to the computer system and the Irish way of documentation in the patients’ files, which proved to be very different to its German equivalent. During the following weeks, the students were allowed to see patients first and present their findings to the supervising doctor while rotating through the different parts of the ED. Every patient was discussed and in most cases a ‘bed-side-teaching’ took place, where an emphasis lay on differential diagnosis and diagnostics. In major situations, such as cardiac arrests or trauma, every student was assigned a task and closely supervised. We must point out that every aspect of teaching and interaction with the senior staff happened in a warm, friendly and encouraging way.

A VALUABLE ELECTIVE

A medical elective abroad in emergency medicine is a valuable experience and will dramatically increase the level of knowledge as well as the clinical skills of a medical student. Being exposed to an ED environment helps the student to focus on the important details and improves one’s skills in clinical decision-making in emergency cases. For a German medical student in particular, it is important to gain insight into a modern ED, as the concept of multidisciplinary EDs is on the verge of replacing the current practice of EM in Germany in the near future. Currently, in Germany ongoing and very controversial discussion about introducing emergency medicine as a board recognised specialty is taking place (5). We can only encourage every visiting student to take the opportunity to go abroad.

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MEDICAL STUDENTS OVERSEAS RELIEF ELECTIVE - MALAWI

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Until we went to Malawi, being a medical student had involved lectures and a short introduction into hospital life. Malawi brought us many firsts. There, we got our first true taste of what it is to be a doctor. It was there that we first saw the birth of a baby. It was there we had our first real experience of the death of a patient. It was the first time we'd been asked questions not to test our knowledge, but because someone wanted our opinion. It was the first time we got a taste of what is it to be responsible for a patient.

HOLY FAMILY HOSPITAL

MALAWI

We spent the first four weeks of the summer of 2010 in the Holy Family Hospital in Phalombe, southern Malawi. "Holy" is tucked into the foothills of Mount Mulanje on the Mozambique border and is one hour away from the nearest main road. It is the only hospital in the region, serving an official population of about 350,000, not counting the many patients travelling from neighbouring Mozambique. It was first established in the 1970s by Dutch nuns and is now Malawian-run by the local diocese. It is a private hospital and few locals can afford to pay their healthcare costs. However, as most patients are referred to the hospital by the local medical centre, the government covers the costs. It has one qualified doctor. The rest of the care is delivered by clinical officers, as is the norm in most of Malawi. Clinical officers have the same responsibilities as non-consultant hospital doctors (NCHDs) in Ireland. However, clinical officers spend just three years training before being qualified to work in all fields of medicine, including the performance of most surgical procedures in the hospital. Once a month, a gynaecologist from a large hospital in the nearest city comes to host a clinic.

A typical day

Our day began at the very civilised hour of nine with the morning handover, a meeting attended by all medical and nursing staff. It was the time to discuss any patients who were critically ill or who had passed away during the night. We would then divide up onto the four wards - male general, female general, paediatrics and maternity. We did rounds with the clinical officers and helped carry out any procedures that were necessary, such as lumbar punctures and chest drains. Lunch in the hospital was a leisurely affair, lasting about three hours!

Our afternoons were mainly spent in clinic or in minor theatre. There were many HIV clinics, at which the patients' CD4 counts were monitored and treatment was adjusted accordingly. Malawi has quite a good HIV treatment programme, with maintenance treatment being provided free of charge by the government. In minor theatre we debrided, sutured and dressed wounds, and set broken bones. We were informed that the plaster of paris requirement increases exponentially in October as all the children start climbing trees in mango season!

The healthcare profile of Holy Family Hospital

HIV is endemic in Sub-Saharan Africa and affected at least half of the in-patient population. Meningitis, TB, pneumonia and falciparum malaria were among the most common adult presentations to the hospital. The paediatric ward was filled with children with problems ranging from serious burns (most cooking is done on open fires) to snake bites, fractures, respiratory distress and malnutrition. We worked in the hospital during a measles epidemic, emphatically illustrating how important vaccination is.

Comparing Malawi to Ireland

Our experience of Malawian hospitals was worlds apart from what we've become used to at home.

In sharp contrast with Irish hospital wards, elderly patients were few and far between. Many of the sickest patients were close in age to us. The hospital's investigation facilities stretched to gram stains, full blood counts, malaria blood films and an x-ray machine. The neonatal resuscitation equipment was simply a single lightbulb to warm the sickest and smallest premature babies, despite the fact that the hospital was the referral centre for complicated deliveries presenting to the primary care clinics. We had expected to be confronted with a lack of resources but the biggest differences, and some of the most challenging, were the cultural ones. In Malawian hospitals, their own family cares for patients' non-medical needs. They are fed, washed and dressed by the family members who fill the open-air courtyards cooking on open fires and sleeping outside in order to care for their loved ones. The Malawian people are very relaxed, illustrated best by the fact that there is no Chichewa (the language of Malawi) word for emergency - the concept does not exist. If a patient deteriorates while everyone is on lunch break, it was next to impossible to get someone to help. Urgency is simply not a feature of life, and death was readily accepted as an everyday part of life.

"Our experience of Malawian hospitals was worlds apart from what we've become used to at home."

The influence of the sangoma (traditional witch-doctor) was everywhere, most patients would present to the hospital only

Locals in Phalombe, Malawi

The UCD 'azungus' with some of the local children

after exhausting their options with him. As a result people presented in the advanced stages of their illness with clinical signs that you might read about in books, but hope never to see.

Malawi is proudly known as the warm heart of Africa, and the warm heart of Malawi is the people. Being the only "azungus" (white people) for miles, we generated a lot of attention everywhere we went. Children came charging through the fields to wave or walk with us. People were eager to welcome us to Malawi, and delighted in bombarding us with questions about the previously unheard of Ireland. There was never any other agenda than curiosity and the desire to make us feel welcome.

The Likhulesi Project

We met Gemma Brugha, an Irish ex-nurse who worked in Holy in the seventies and eighties, teaching in the nursing school on the grounds of the hospital. Twelve years ago she stopped teaching and set up the Likhulesi Project, a fantastic grassroots initiative based in the neighbouring village which provided practical support to AIDS patients living in the community. Volunteers visit the patients - their neighbours - a few times a week to help with everyday tasks that they are unable to manage like fetching water from the well or chopping firewood. The project also supplied the patients with a sleeping mat (the houses all have mud floors and walls), a bar of soap and paracetamol if it was available. The project supports AIDS orphans to stay in school and assists families who take in these orphans with a little extra grain. The project also aims to raise awareness about HIV – ensuring to educate the children as early as possible about HIV prevention by performing plays and songs around the theme through their drama group.

It was sad to leave Holy, after spending a fantastic four weeks there. We would always enthusiastically recommend anyone to visit Malawi as a medical student. Clinically, our skills improved. After being confronted with countless cases of pneumonia, auscultation couldn't but come on in leaps and bounds! And more importantly, we were lucky enough to experience a completely different way of life as we lived in the warm community that was Holy village.

The Likhulesi project's community drama about AIDS awareness

TOP TEN PODCASTS FOR MEDICAL STUDENTS

Podcasts are free downloadable audio files which users can subscribe to, receiving regular updates and weekly episodes. They are normally used with Apple iTunes, and are great for listening to on the move or in a spare 15 minutes between our busy schedules. Here is a review of some of the best medical podcasts available, some to aid revision, others for frank ethical discussions and some for general news articles and topics of interest. They can all be easily found by searching on iTunes. Website addresses are included for reference.

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1

Student BMJ Podcasts
http://feeds.bmj.com/student/podcasts

These podcasts are extremely helpful tools to learn bite-sized scenarios. They really aid learning and understanding, everything is explained clearly and is delivered in a calm simplified manner. Podcasts such as 'How to scrub in', 'How to tie a surgical knot', and 'Shortness of breath' all go a long way to crystallising difficult subjects and suddenly I find myself thinking "maybe medicine isn't all that hard".

2

BMJ Podcasts
http://podcasts.bmj.com/

Leading on from the student section is the slightly more doctor focused BMJ site. The website is well organised, and the content interesting and relevant. It often features topical interviews and debates with key players in the medical field. The podcasts are divided into numerous categories including Emergency Medicine Journal , Heart podcast, STI treatment, Gastroenterology, among many. If you are looking for something slightly more in-depth these podcasts are the way to go.

3

Surgery 101
http://surgery101.libsyn.com/

Dr. Jonathan White, a professor and surgeon at the University of Alberta, decided to make ten to thirty minute podcasts of his lecture material in order to reach his students at different levels. This series of thirty-five entertaining, and concise lectures are seriously good. They cover subjects ranging from Diverticular Disease to Aortic Dissections. Can't recommend them enough.

4

Pod Medics
http://www.podmedics.com/

Podmedics is a useful site, namely for its vid-casts, podcasts with video as well as its audio files. It is usually ranked number one in video medicine and two to three out of 'all' (audio and video) medicine on iTunes. It is a new site but has over one hundred podcasts already up. They are mostly clinical revision pieces and with such a large choice, a podcast can be found to suit whichever topic interests.

5

Scientific American
http://www.scientificamerican.com/podcast/

A broad knowledge base of science and technology and the current advances in these fields is important for us to have as future doctors. This podcast series is perfect for anyone with an interest in research, oncology, microbiology and pharmacology as it is often in these fields the most exciting advances are being made and this podcast covers them with gusto. 60 Second Health gives a weekly one-minute report on the latest health and medical news.

6

MedPod 101
http://www.medpod101.com/

The hugely popular MedPod101 is described on its site as being 'Designed by chic doctors for the busy medical student looking to enrich her clinical acumen'. It delivers clinical medicine titbits with a healthy dollop of cheesy humour. It is intended to be for more entertainment purposes than cramming sessions but the fun of it all is refreshing, and it definitely helps stick those facts in your head. It is rife with stereotypes and what some would consider bad taste. Avoid if easily offended.

7

RCSI Surgery Now
www.rcsi.ie/surgerynow

This impressive podcast was released to a bare Irish market a few months ago and although more advanced than what Irish medical students require, anyone with a penchant for surgery should subscribe immediately! The thirty minute magazine style podcast delivers the latest information about clinical practice and surgical technology with its focus on Ireland's current trends. It features a case and image of the week, Journal club, and spotlight on surgery and surgical technology. Interestingly, surgeons can receive CPD accreditation for viewing the podcast and submitting assignments based on the case of the week, showing the technological direction medical education is headed.

8

The Lancet (Standard and Student)
http://podcast.thelancet.com/lancet-student.xml

The Lancet is one of the world's best known, oldest, and most respected general medical journals and with these podcasts comes a review of each week's most important peer-reviewed articles with discussions and debates on the tenuous points made. The depth of content and speed of deliverance is on the mark. The student section specifically deals with global health, sociology and life at the coal face of medical practice through interviews from a brilliant range of subjects. These include first-hand accounts from junior doctors, foreign students, and students on elective, aid-workers, authors and professors amongst its forty-seven pieces.

9

ACC Conversations
http://conversations.acc.org

When writing this piece I questioned my North American counterparts on podcasts they listened to and this was suggested by many. Produced by the American College of Cardiology, Dr. Adolph Hutter asks the tough questions in these twenty minute topical discussions. Each conversation is a fast-paced discussion between leading experts in cardiology. Although more targeted at practicing doctors this podcast received unanimous praise. Upon listening, it's obvious the lively content is at the top of its game.

10

Medical School Podcast
Search in iTunes

This series of podcasts by Dr. Goljan was my favourite of the list. The main aim of these podcasts was to publish recordings of mastermind experts in various medical education fields and also educate students and doctors on the topic of burnout. The listener is recruited into an active role to increase awareness about medically underserved populations, sources of stress in medical students and interns, and efforts to remedy the imbalances. It's important for us all to strike a good work life balance, something which can fall by the wayside when studying most hours of the day. This podcast reminds us there is more to life than one's job and to keep a healthy mind in the midst of attaining our medical degree.

MSOR

Medical Students Overseas Relief, MSOR, is a voluntary society run each year by Stage 4 UCD medical students which gives students a chance to experience life as a doctor in a developing country. These students travel to one of many developing countries in Africa and around the world for short 4-6 week placements. In return for the teaching and experience they receive, students work hard during the year to raise much needed funds for these under-resourced hospitals.

EVERY CENT raised goes to the hospitals they support.

Many of these hospitals receive no or very little funding from their government and the MSOR volunteers provide money for their maintenance and development. These projects help make these hospitals more self-sustainable, more stable and help secure a better future for them and the rural populations they serve, often receiving no payment from their impoverished patients.

All the money donated to this society will go straight towards those who need it most, so please give generously!

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You can also find more details about our society on our new website page at

www.msorucd.com

Thank you!

MSOR