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- Clinical Development of Experimental Therapies for Malignant Glioma
- Improving Road Safety through Deterrence-Based Initiatives - A review of research
- Trends and Challenges in Pathology Practice - Choices and necessities
- Asthma Control in Oman - National Results within the Asthma Insights and Reality in the Gulf and the Near East (AIRGNE) Study
- Vitamin D Status in Pregnant Omanis - A disturbingly high proportion of patients with low vitamin D stores
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Sultan Qaboos University Medical Journal
Indexed in WHO EMR Index Medicus

February 2011 Volume 11 Issue 1

Sultan Qaboos University Medical Journal is a nationally and internationally peer reviewed multidisciplinary biomedical journal. It publishes original articles in both print and on line editions, the latter with free public access to full text articles. Its aims are: 1) to be a leading regional medium of biomedical and allied scientific communication with international recognition and acceptance; 2) to encourage and stimulate medical research and scientific publication within Oman and the Gulf area, while attracting contributions from further a field; 3) to create awareness of developments in medicine and allied fields among health professionals in and outside Oman. It is a forum for the exchange and dissemination of medical knowledge and research among health professionals within Oman, the Gulf region, the Middle East, North Africa and Asia.

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SQUIMJ is published quarterly with a print run of 2,500 copies and distributed free of charge to medical colleges and institutions in Oman, the Gulf countries, the Eastern Mediterranean Region, India, USA, Canada, Australia and the UK

All SQUMJ articles are freely available for inspection and download on the Journal website

Published by: Sultan Qaboos University with the support of College of Medicine & Health Sciences
PO Box 35, Al-Khod 123, Muscat, Oman
Email: mjourn@squ.edu.om & squmjournal@gmail.com; Website: http://www.squ.edu.om/squmj
Phone: (+968) 2414 3457, Fax (+968) 2441 3419

Edited, designed and typeset by the Editorial Office, College of Medicine & Health Sciences, Sultan Qaboos University

Printed at Sultan Qaboos University Press.

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ISSN (print edition): 2075-0513; ISSN (internet edition): 2075-0528

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Medical imaging using X-rays is like a double-edged sword. On the one side, we cannot do without it because of its enormous benefit in medical diagnosis, but on the other side, we risk exposing ourselves to potentially harmful low-dose radiation. Typically, two-thirds of all medical imaging procedures involve the use of ionising radiation, either X-rays in diagnostic radiology or gamma rays in nuclear medicine. Thus there is an urgent need to educate medical staff on the appropriate utility of this diagnostic tool, and for the creation of a regulatory body controlling and monitoring its use. This is of particular urgency in Oman.

Since the introduction of computed tomography (CT) scanning, diagnostic accuracy has significantly improved in clinical medicine, but concomitantly radiation exposure to human subjects has also significantly increased. By far, the CT scan results in much higher radiation exposure to the patients than does plain radiography, typically 40 to 50 times more.¹ A vivid example is the CT of the abdomen versus plain film. A plain X-ray of the abdomen results in about a 0.25 milligray (mGy) dose to the stomach which is about 40 times lower than the 10 mGy dose from an adult abdominal CT and the much higher 20 mGy dose in the case of the neonatal abdominal CT.¹

Fazel et al. reviewed almost a million non-elderly adults between 2005 and 2007, and discovered that CT scanning and nuclear imaging accounted for over 75% of the cumulative effective dose to the population; however, CT scans and nuclear medicine together accounted for only 21% of total procedures in that study.² Other studies have also shown that CT scans of paediatric patients result in alarming radiation doses.³,⁴,⁵ This is a source of concern given that such a population is still at a tender age.

Such studies show that physicians need to restrategise significantly how they request medical imaging studies, and especially CT scans, in the paediatric population. The increase in the numbers of medical imaging scans involving ionising radiation is alarming. In the United States, the number of CT scans in 2006 was 62 million, and since then it has been increasing at an unprecedented rate, both in United States and elsewhere.⁶ In 2010, of the 5 billion medical imaging procedures worldwide, two-thirds utilised ionising radiation,⁷ thus the need of discernment on our part in requesting diagnostic imaging. We need to look into the clinical indications for CT more critically and adhere more closely to the guidelines and “Appropriateness Criteria” set by the American College of Radiology (ACR) and other national radiological institutions.⁸ Radiation is much more damaging to the growing child who has a longer life expectancy than to an adult. We, as physicians, therefore need to consider alternative imaging methods as much as possible and increase our use of ultrasound and magnetic resonance imaging (MRI) in place of CT scans and other radiation-dependant...
modalities whenever possible. Perhaps, more significant in increasing the unnecessary radiation exposure of the public is the repetition of medical imaging studies. It is not uncommon for patients to resort to ‘doctor shopping’ which often entails the repetition of imaging procedures. This may be triggered by a lack of faith in the initial images or by the financial interests of the clinics involved. Unnecessary repetition of imaging studies is today a major source of unnecessary radiation exposure for patients.

Physicians need to be educated about the dangers of radiation and the benefits of its wise use. They also need to take the initiative to learn more about the risks of their orders. For example, every CT scanner clearly shows the dose from a particular scan to a patient, but unfortunately, very few radiologists ever bother to review the dose they have given to their patients. Modern CT scanners will give the CT dose index (CTDVol) per slice, expressed in mGy per volume, and also the dose length product (DLP) which represents the total exposure dose to the length of the area imaged (slice dose x length). DLP is expressed as mGy–cm.9 In the case of mammography, the mean glandular dose (MGD) is also available. We need to make greater use of these exposure figures in appreciating how much radiation dose our patients receive at each imaging session.

Physicians should consider joining the “Image Wisely” organisation and take the pledge to be acutely aware of the radiation dose to the patients. There they will get educated about the necessary precautions. “Image Wisely” is an organisation sponsored by the ACR, the Radiological Society of North America (RSNA), The American Association of Physicists and other reputable organisations to promote judicial use of radiological science for diagnoses.10

There is also a growing momentum to get patients involved in radiation awareness and protection. One way would be to inform the patient each time they have an imaging study about the radiation dose to which they have been exposed. Unfortunately, the difficulty will be for the physicians to explain to the patients what that dose or their cumulative dose means as we have limited knowledge about the biological effects of various doses of low-dose radiation exposure. Perhaps we should also consider giving patients electronic “Radiation Registry Cards” to carry around so that each radiation exposure dose is added and an ongoing record kept since radiation is cumulative throughout one’s life. The X-ray machines and CT scanners of the future will have to be modified so that with each exposure the scanner can register the dose on the patients’ “Radiation Registry Card”. If this were to be implemented, it would be one of the steps to come to grips with the emerging problem of rising radiation exposure.

What then is known about the tissue damage caused by low-dose radiation exposure? There is a significant amount of data known about high-dose radiation exposure and this is mainly from the survivors of the two atomic bombings of Japan in 1945 and the Chernobyl Nuclear Plant accident in Ukraine in 1986. Unfortunately, while the effects of high-dose radiation exposure have a linear relationship with the dose this cannot be extrapolated down to the lower dose or to zero. At higher doses, the biological effects are generally “deterministic” whereby the severity of the effect is dose-dependent and there exists a threshold dose above which the effects occur.11 At lower doses, the effects are “stochastic”, whereby the probability of an effect occurring is dose-dependent, but there is no threshold dose below which we could be relatively certain that no adverse effect will occur.11 The best known stochastic effect is cancer production from radiation exposure. There is a significant amount of epidemiological data which indicates that exposure to low-dose radiation may result in cancer including cases of leukaemia, as well as thyroid nodules and other cancers.12

At the 2010 UN General Assembly, The United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) presented the radiation risks estimate for cancer and for hereditary effects. They defined a low-dose to be 200 mGy (or millisievert) or below or 0.1 mGy (or 0.1 mSv) per minute for exposure from external sources such as X-rays.13 They pointed out with respect to radiation-induced cancer, that the long delay (often years or decades) between exposure and disease presented a major difficulty in attributing specific diseases to low-dose radiation exposure. This is compounded by the anyway high spontaneous incidence of disease associated with ageing. Besides epidemiological data, there are experimental studies that show the dose response...
relationship with cellular and sub-cellular elements exposed to radiation. The target appears to be the chromosomal DNA molecule. If the radiation damage to a group of genes is not repaired, the cell will die, but even if repaired the surviving cells may show DNA mutations that may affect cell behaviour in the exposed individual. Even a minor degree of mutation can result in development of cancer.12

There are also several radiation-associated non-cancer diseases, which are a result of radiation exposure. The best example is congenital disorders from exposure to a developing foetus, the neurological system being the most susceptible.12 The risk will depend on the dose and the timing of exposure during pregnancy. UNSCEAR also pointed out that recently there is increased evidence of cataract development after low-dose radiation exposure.12 Until recently, cataracts have been related only to high-dose exposure.

The third group of disorders related to radiation exposure is the heritable effects of radiation. UNSCEAR presented evidence that damage to DNA of germ cells resulted in heritable diseases. Unfortunately, these may be passed on to offspring and to several future generations. The evidence is clearer with higher than lower doses, but there is experimental evidence in animals that mutations induced by radiation can indeed appear in several generations.12

For the optimists in the medical profession, there is a group who believe in radiation hormesis.13 This is the hypothesis that a small dose of radiation may actually be beneficial to living tissues. Mice exposed to low dose of radiation became resistant to the effects of future exposure to radiation and also resistant to certain diseases.13 It is my wishful thinking that this be true, but unfortunately the evidence is not yet strong enough.

Perhaps in Oman at this stage, we more importantly need good regulations to control the handling and uses of ionising radiation. Unfortunately, there are currently no regulations in Oman that control X-ray machines, and only lax regulations for handling radioactive substances. Thus the use of low-dose radiation in Omani medicine is potentially dangerous to our community. We need tougher regulations on the purchase of X-ray machines and on the running these machines. In some private clinics in Oman, unqualified technologists run X-ray machines as there are no specific licensing regulations. The training requirements for radiological technologists are also limited. In addition, there are no training requirements for physicians who use X-ray machines. Unfortunately, there are physicians who actually run fluoroscopic machines without adequate licensing procedures. Not all radiological procedures and cardiac catheterisation procedures are adequately monitored from the equipment point of view, or by the physicians or technologists involved. The good news is that there is a move in the government towards setting up such a regulatory authority.

It should be mandatory for any physician handling radioactive material or using X-ray machines to attend a course on radiation safety and the use of these materials and machines to be certified prior to any use. Likewise, the technologists or nurses involved must have a similar course and certification. In addition, Oman needs to recognise medical physics as a specialised profession. Medical physicists are needed in all radiology and nuclear medicine departments to be in charge of quality control and assurance and as radiation safety officers.

What we need in Oman now is education of medical doctors on the dangers of medical imaging that utilises ionising radiation, in particular CT scanning and cardiac catheterisation studies. We also need continuing medical education on the appropriate criteria and clinical indications for the uses of these diagnostic tools.14 Most importantly, and perhaps of great urgency, is the creation of an independent regulatory body to regulate the safe use of ionising regulation in medicine and otherwise. This governmental body needs to be free of the control of any ministry, and has to have authority to license the use of radioactive materials and of X-ray-producing equipment, as well as regulatory control over the education of staff handling both low and higher dose radiation sources in medicine and elsewhere. We need to respect and control low-dose radiation to safeguard ourselves and our future generations.
References

Glia-derived neoplasms of the brain (gliomas) of the histological grade 3 (anaplastic astrocytoma, anaplastic oligoastrocytoma, and anaplastic oligodendroglioma) and grade 4 (glioblastoma multiforme, [GBM]) according to the morphology-based classification of the World Health Organization (WHO), represent different stages of the same fatal neoplastic disease of the central nervous system (CNS). WHO grade 3 and 4 gliomas are also known as malignant gliomas and are the most frequent intrinsic brain tumours in adults.

According to the US central brain tumour registry, the annual rate of primary tumours of the CNS is...
14.1 per 100,000 persons, of which more than 36% are malignant gliomas. Malignant gliomas typically arise in the lobar white matter or in the deep grey matter of the brain and are characterised by diffusely infiltrating growth. These gliomas consist of highly proliferative and exceptionally migratory tumour cells with a variety of acquired genetic alterations which make them extremely resistant to antiproliferative therapies. There is no clear demarcation of tumour from normal surrounding brain tissue to allow the surgeon to completely remove a glioma. Neuroradiological imaging such as computed tomography (CT) or magnetic resonance imaging (MRI) can only visualise areas with a high percentage of tumour cells and strong neoangiogenesis, but not those with a low density of invading glioma cells and neoplastic capillaries. Gliomas expand both by mitotic activity of tumour cells and by migration of these cells away from the initial tumour mass and into the surrounding brain. It is now generally accepted that widespread migration of glioma cells is a very early feature of these tumours, and by the time of clinical manifestation and histological diagnosis cells have spread throughout the CNS and are located beyond the extent of any feasible surgical resection. There is evidence to suggest that the density of infiltrating malignant cells decreases according to the distance from the initial tumour mass, and there is a tendency of tumour cells to migrate along defined anatomical structures such as white matter tracts. Supporting the notion that malignancy declines with distance from the main tumour, the fact is well known that most malignant gliomas will recur within a few centimetres of the walls of the resection cavity or the visible tumour mass, respectively. Simple diffusion of cells could potentially explain this observation. On the other hand, recent insights in glioma cell biology suggest that more malignant and actively proliferating cells might be less migratory and less invasive, while less malignant cells may tend to invade more remote areas of the brain which surround the tumour mass.

Survival of patients with malignant glioma has remained essentially the same in the last three decades, despite a quantum leap in medical and surgical technology and major advancements in understanding the natural history and biology of the disease, and the molecular genetics of glioma cells. Median survival time for malignant gliomas varies according to different studies, but is generally agreed to be 18–20 months for anaplastic astrocytomas and 8–14 months for GBM. There are no curative treatments for malignant glioma at present and no treatment able to prevent tumour progression or recurrence. Even the most radical surgical resection is unable to cure a malignant glioma, although it may significantly prolong survival and improve quality of life.

The current standard therapy of newly diagnosed malignant gliomas consists of surgery, preferably a gross total resection of contrast-enhancing areas, subsequent fractionated external radiotherapy up to a total dose of 60 Gy, and concomitant and adjuvant chemotherapy in WHO grade 4 tumours, with various combinations of these three approaches. The use of chemotherapy, in addition to surgery and radiation, was considered somewhat controversial until recent studies demonstrated significantly improved outcome for patients with previously untreated GBM receiving radiotherapy plus concomitant and adjuvant chemotherapy after surgery. However, in all clinical studies, significant improvements in overall survival are demonstrated mostly in selected subpopulations of patients with somewhat better prognostic factors, such as a higher Karnofsky Performance Status (KPS), younger age, specific molecular markers, complete surgical resection of tumour, and others.

Perhaps the most prominent feature, which renders malignant glioma a preferred target for tumour selective drugs and local treatments, is the unique combination of high mitotic activity of tumour cells against the background of the mostly post-mitotic environment of the adult brain. In addition, malignant gliomas do not metastasise systemically, but are extremely aggressive in their local environment within the closed compartment of the CNS. Multidrug and radiation resistance genes are upregulated in primary and recurrent glioma and allow tumour cells to escape the toxicity of the standard treatment modalities and to continue growing even during radio- or chemotherapy.

Distinct biological features common in malignant gliomas, but unusual in normal brain tissue, have been the subject of many research studies. Differential expression of specific proteins
Nikolai G Rainov and Volkmar Heidecke

**Review**

and clinically relevant approaches to local intracerebral therapy of malignant gliomas. All of these are currently employed in an adjuvant setting, in addition to the above mentioned and well established standard modalities of surgery and fractionated external radiotherapy with chemotherapy, and not as their replacements. We will focus mostly on therapeutic approaches that have demonstrated safety, feasibility, and potential anti-tumour efficacy in previous and ongoing clinical studies.

**Interstitial Chemotherapy with Biodegradable Polymer Wafers (Gliadel®)**

Sustained and controlled local delivery of therapeutic agents to a brain glioma is one of the recent strategies for increasing toxicity to tumour cells while reducing systemic side effects. Gliadel® (MCI Pharma Inc., Bloomington, MN, USA) wafers were developed as a means for controlled release of a chemotherapeutic agent from biodegradable polymer wafers to the surroundings of surgically resected malignant glioma. Their efficacy was investigated in several preclinical and clinical studies. Gliadel® wafers are currently approved for use in patients with primary and recurrent glioblastoma as an adjunct to standard treatment modalities.

**Figure 1:** Handling and implantation of Gliadel® wafers during brain tumour surgery. A: A single wafer is removed from the sterile aluminium foil protective packaging. B: All 8 Gliadel® wafers belonging to a complete pack are removed from the packaging and prepared for intracerebral implantation. C: Gliadel® wafers lining the wall of a glioblastoma (GBM) resection cavity. Note that the relatively small tumour resection cavity can only accommodate 5 wafers. Slight overlapping of wafers is acceptable. D: Implanted wafers are covered with oxidised cellulose (Surgicel®, Ethicon, Hamburg, Germany) to prevent dislocation and intracavitary movement.

in malignant glioma versus the normal brain and aberrant activation of signal transduction cascades in tumours seems to provide an opportunity for selective molecular targeting of tumour cells despite the molecularly targeted agents being given mostly systemically, thus associated with less toxicity to the patient and with a higher rate of tumour cell killing. All these features make gliomas a model disease for the design and testing of novel treatments with tumour-selective toxicity and a local rather than systemic mode of administration.

Several novel approaches have been introduced into clinical practice in a continuous effort to improve the outcome of malignant glioma. In general, such approaches can be categorised as systemic or local, the latter being intratumoural, peritumoural, intracerebral, or combined. Novel systemic approaches demonstrating potential in early clinical studies are chemotherapy drugs with molecular selectivity mechanisms (such as receptor tyrosine kinase inhibitors), and active (e.g. dendritic cells) or passive (e.g. antibodies) immunotherapies. Novel local intracranial approaches include intracerebral delivery of standard chemotherapy drugs (interstitial chemotherapy) by slow release polymers, recombinant targeted toxins administered by convection enhanced delivery (CED), or genetically modified viruses with molecular selectivity for glioma cells.

This review will summarise the most recent
delivery of the well-known and frequently used chemotherapy drug, 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU or carmustine) began in the early 1990s. BCNU was initially chosen due to proven efficacy against glioma cell lines. For local delivery of BCNU, biodegradable polymer wafers were used so as to deliver the drug to the tumour resection cavity in a controlled-release fashion over time, which should circumvent the short in vivo half-life of the drug, increase the local concentration of BCNU reaching glioma-invaded areas of normal brain, and avoid the concomitant toxicities of systemic drug application.

Polymer (prolifeprosan 20) wafers containing 3.85% (w/w) BCNU, which are also known by the brand name Gliadel®, are currently the only type of interstitial chemotherapy wafers licensed by U.S. and European agencies for treatment of de novo and recurrent malignant gliomas [Figure 1, A and B]. Wafers are placed directly into the resection cavity of a malignant glioma at the end of surgical resection of the tumour [Figure 1, B and C]. This may result in local BCNU levels approximately 100-fold of those obtained with systemic delivery. Gliadel® wafers have been shown to release BCNU in vivo over a period of approximately 5 days. When in continuous contact with interstitial fluid, wafers have been shown to degrade completely over a period of 6–8 weeks. Polymer degradation products are excreted as expired CO₂ or through the urine. BCNU degradation products are also excreted primarily through the urine.

No direct pharmacokinetic measurements have been made in humans after the implantation of Gliadel® wafers; however, drug distribution and clearance have been extensively studied in rodents and primates. In addition, degradation of the polymer matrix, the release kinetics of BCNU, and drug and polymer metabolism have been studied both in vitro and in vivo. Pharmacokinetic studies have demonstrated the capability of Gliadel® wafers to deliver high (mM) concentrations of BCNU within a few millimetres of the polymer implant and with a limited penetration distance. The limited interstitial spread of the drug is presumably due to the high transcapillary permeability of this lipophilic molecule. However, convective flow in the existing peritumoural oedema may augment diffusion.

In 1991, Brem et al. established a significant survival advantage of Gliadel® wafers over placebo in patients with recurrent malignant glioma. They conducted a randomised, placebo-controlled, prospective phase 3 clinical study to evaluate the effectiveness of Gliadel® wafers. Their multicentre study enrolled 222 patients with recurrent malignant brain tumours requiring re-operation. Cases were randomly assigned to surgically implanted wafers with or without BCNU. Median survival of the 110 patients who received Gliadel® wafers was 31 weeks compared with 23 weeks for the 112 patients who received placebo wafers (P = 0.006). Among GBM patients, 6-month survival in the treated group was 50% greater than in the placebo group. There were no clinically important adverse reactions related to the carmustine polymer, either in the brain or systemically.

A phase 1 trial was carried out in patients with newly diagnosed GBM. Twenty-two mostly elderly patients (mean age 60 years) with newly diagnosed malignant glioma (21 with GBM) were treated with Gliadel® wafers. Postoperatively, all patients received standard radiation therapy, but no additional chemotherapy in the first 6 months. The neurotoxicity of this regimen was equivalent to that occurring in other series of patients undergoing craniotomy and radiation. No significant bone marrow suppression occurred and there were no wound infections. Median survival of the whole group was 42 weeks, 8 patients survived 1 year, and 4 patients survived more than 18 months. The authors concluded that interstitial chemotherapy with Gliadel® wafers with subsequent radiation appears to be safe as an initial therapy for malignant glioma.

In 2004, Kleinberg et al. described, in a retrospective review, the clinical course, toxicity, and pathologic findings after Gliadel® wafer implantation for newly diagnosed malignant glioma. Forty-five consecutive patients, most of them with GBM, received Gliadel® wafer implants followed by radiotherapy and were available for follow-up. Postoperative infection, or the need for reoperation within 30 days was uncommon after Gliadel® wafer placement. Full-dose external radiotherapy was well tolerated after Gliadel® implantation; however, 13 patients developed neurological symptoms during radiotherapy, but responded to medication (dexamethasone and/or anticonvulsants). Fifteen of these 45 patients underwent re-operation or biopsy for a new local contrast-enhancing lesion. In 5 of these, histological analysis revealed necrosis or
post-treatment scars without active tumour. Median survival was 12.8 months for all GBM patients.\textsuperscript{29}

To corroborate the evidence from early clinical trials, a randomised controlled trial planned to include 100 cases was carried out in patients undergoing surgery for newly diagnosed gliomas, but was terminated prematurely after enrolment of 32 patients because the Gliadel\textsuperscript{8} implants became unavailable.\textsuperscript{20} The median overall survival time was 58.1 weeks for the active treatment group versus 39.9 weeks for the placebo group ($P = 0.012$). At the end of the study, 6 patients were still alive, 5 of whom belonged to the active treatment group. Results were, however, not corrected for the early termination and thus a possible bias may have been introduced in the statistical evaluation.\textsuperscript{30}

Westphal \textit{et al.} reported the results of a further multicentre phase 3 study investigating Gliadel\textsuperscript{8} wafer treatment in 240 patients with newly diagnosed GBM.\textsuperscript{25} Patients were randomised to receive either BCNU or placebo wafers at the time of primary surgical resection. All patients were given identical standard treatment consisting of surgical resection followed by fractionated external radiation. Exclusion criteria were recurrent tumour, prior brain radiotherapy, and multifocal disease. The primary clinical endpoint was overall survival. Median survival in the intent-to-treat (ITT) population was 13.9 months for the Gliadel\textsuperscript{8}-treated group, and 11.6 months for the placebo-treated group, with a 29% reduction in the risk of death in the treatment group. Time to decline in KPS and clinical parameters (e.g. vital signs, level of consciousness, neurological status), were also significantly improved in the treatment group compared to the placebo group. The adverse events (AE) were comparable for the two groups, except for cerebrospinal fluid (CSF) leak (5% in the Gliadel\textsuperscript{8} group versus 0.8% in the placebo group) and intracranial hypertension (9.1% in the Gliadel\textsuperscript{8} group versus 1.7% in the placebo group). This study is the largest clinical investigation of Gliadel\textsuperscript{8} activity to date.\textsuperscript{25}

In a later study, the authors reported the long-term follow-up results of the above patient group regarding survival benefits at 2 and 3 years after Gliadel\textsuperscript{8} implantation.\textsuperscript{26} Of the 59 patients available for long-term follow-up, 11 were alive at 36 months; 9 having received Gliadel\textsuperscript{8} and 2 placebo wafers. The median survival of patients treated with Gliadel\textsuperscript{8} was 13.8 months versus 11.6 months in placebo-treated patients ($P = 0.017$), with a hazard ratio of 0.73 ($P = 0.018$), representing a significant risk reduction of 27%. This survival advantage was maintained at 1, 2, and 3 years and was statistically significant ($P = 0.01$) at 3 years. Two of the 207 GBM patients remained alive at the end of the follow-up period, both in the Gliadel\textsuperscript{8} group. The authors concluded that malignant glioma patients treated with Gliadel\textsuperscript{8} at the time of initial surgery in combination with radiation therapy demonstrated a continuous and significant survival advantage at 2 and 3 years follow-up compared with placebo patients.\textsuperscript{26}

Additional studies were aimed at increasing the clinical effects of Gliadel\textsuperscript{8} treatment using different strategies. Olivi \textit{et al.} treated 44 adult patients with surgery and implantation of BCNU wafers.\textsuperscript{31} Six patients per dose level were studied using wafers with 6.5%, 10%, 14.5%, 20%, and 28% BCNU (weight/weight). Toxicity was assessed one month after wafer implantation. No dose-limiting toxicity was identified at the 6.5%, 10%, or 14.5% dose levels, although delayed wound healing, seizures, and brain oedema were noted. At the 20% dose, these side effects seemed more prominent, and 6 additional patients were treated at this dose and tolerated treatment well. Three of 4 patients receiving 28% BCNU wafers developed severe brain oedema and seizures. Nine additional patients received 20% wafers, confirming this as the maximum tolerated dose (MTD). Maximum BCNU plasma concentrations with the 20% wafers were 27 ng/ml. Overall median survival of the patients was 12 months. The authors concluded that 20% BCNU wafers are relatively well tolerated and result in minimal systemic BCNU exposure.\textsuperscript{31} Additional studies are however needed to establish the efficacy of high-dose BCNU wafers and a possible significant extension of survival due to the increased concentration of BCNU.

Beginning in 2004, concomitant temozolomide (TMZ) during fractionated brain irradiation (Stupp protocol) became the standard of care at most neurooncology centres in Europe and the USA.\textsuperscript{18} Patients with newly diagnosed GBM and Gliadel\textsuperscript{8} implantation received therefore concomitant TMZ and irradiation radiochemotherapy); however, it remained unstudied whether combining Gliadel\textsuperscript{8} and radiochemotherapy is safe or further improves survival in such patients. In 2010, McGirt \textit{et al.} reviewed retrospectively the initial experience with
Gliadel® and radiochemotherapy in GBM patients. Thirty-three patients were treated with the combined modalities and the median survival in the group was 20.7 months, with a 2-year survival rate of 36%. Six-month morbidity included surgical site infection in 1 case (3%), perioperative seizures in 2 cases (6%), deep-vein thrombus in 1 (3%), pulmonary embolism in 3 (9%), and cerebral oedema requiring intravenous dexamethasone administration in 1 case (3%). Myelosuppression required premature termination of TMZ in 7 patients (21%): (thrombocytopenia in 5, neutropenia in 2 cases). In patients ≤ 70 years of age, Gliadel® and radiochemotherapy were independently associated with improved median survival (21.3 versus 12.4 months, P = 0.005). Moreover, the combined modalities were not associated with an increase in postoperative morbidity in comparison with Gliadel® or radiation only. The authors concluded that radiochemotherapy can be safely administered to patients receiving Gliadel® wafers after resection of newly diagnosed GBM.

**Conclusions and Future Developments—Gliadel® Therapy**

Despite the convenience and relative simplicity of use of intraoperatively placed Gliadel® wafers, there are some factors limiting their widespread clinical application. Most importantly, there is only a modest short-term clinical benefit in most patients, while long-term survival is prolonged in a small subset of malignant glioma patients only. The side effects of Gliadel® are not negligible, and the high cost of treatment is an important issue, at least in most European Union (EU) countries. In the future, local release wafers will need to be optimised for increased anti-tumour activity, either by increasing the concentration and improving the release kinetics of well-known agents such as BCNU, or by employing novel agents with a more potent anti-tumour effect, e.g. mitoxantrone or IL-2.34 Combinations of local release wafers with standard adjuvant therapies such as radiochemotherapy or single-drug oral chemotherapy (e.g. with TMZ) may become a standard of care if more reliable clinical evidence is presented for increased survival and unchanged morbidity with these combinations.33

**Targeted Toxins (Immunotoxins)**

Targeted toxins represent a new class of anticancer agents providing high specificity for tumour cells selectively overexpressing some surface proteins. Currently used toxins are recombinant polypeptide molecules consisting...
of a tumour-selective ligand coupled to a highly potent peptide toxin, which is truncated to abolish native toxicity. The most frequently used and best researched ligands bind to tumour-associated molecules with receptor signalling properties, such as epidermal growth factor receptor (EGFR), transferrin receptor (TfR), interleukin-13 receptor (IL-13R), or interleukin-4 receptor (IL-4R). The toxin part of the molecule in all clinically used toxins is a polypeptide derived from bacteria (Corynebacterium diphtheriae, Pseudomonas aeruginosa), which has been modified by deleting the native targeting and internalisation chains of the polypeptide and replacing them with one of the above ligands [Figure 2].

The mechanism of action of targeted toxins may have important advantages over that of radiation and classic chemotherapeutic agents. Toxins are effective against radiation-resistant hypoxic tumour cells and far more potent than any chemotherapy drug. One single molecule of toxin is sufficient to cause tumour cell death independent of any malignancy-associated genetic alterations and/or mutations [Figure 3]. Multidrug resistance and apoptosis resistance are therefore not an issue with toxins; after receptor binding and internalisation, no tumour cell is able to survive the toxin part of the molecule. A few targeted toxins have advanced from the laboratory to the stage of phase I and II clinical studies, with two of these toxins reaching phase 3 trials.

**IL4-PE (NBI-3001)**

This chimeric recombinant fusion protein (also known as NBI-3001, Neurocrine Inc., S. Diego, CA) is composed of circularly permuted interleukin-4 (IL-4) and a truncated form of *P. aeruginosa* exotoxin (PE) A. PE is a 66 kD protein with three domains: Ia/Ib, II, and III. When domain Ia is removed, the resulting molecule (termed PE-40) retains its translocation function and elongation factor 2 (EF-2) inhibition properties, but is unable to bind to and kill human cells. Domain II is the site of proteolytic cleavage and is responsible for catalysing translocation of the toxin into the cytosol. Domain III, located at the C-terminus, possesses ADP-ribosylation activity which in turn leads to inactivation of EF-2 and to cell death. In the genetically engineered PE molecule PE38KDEL, amino acids (AA) 253–364 were linked to AA 381–608, which in turn were fused to KDEL (an endoplasmic reticulum retaining sequence) at position 609–612. To improve the binding of IL-4 toxin to the IL-4 receptor (IL4-R), a circularly permuted form of IL-4 was fused to the toxin. This new agent was termed IL-4(38-37)-PE38KDEL, or IL4-PE. IL4-PE was found to have a 16-fold higher affinity for binding to GBM cell lines than the native PE toxin, and was 3–30-fold more toxic.
Clinical Development of Experimental Therapies for Malignant Glioma

A multicentre, randomised, open-label phase 2 study with IL4-PE was carried out in patients with recurrent GBM to investigate continuous intratumoural infusion of the toxin followed by surgical resection of the tumour. The study was designed to evaluate the efficacy of IL4-PE, with a secondary objective to evaluate the safety and tolerability of the toxin. In the toxin group, patients received an intratumoural infusion of toxin at total doses of up to 90 µg and underwent surgical resection of the tumour between 2 and 7 days after the end of toxin infusion. Patients in the control group underwent tumour resection without prior toxin treatment. A total of 30 adult patients with unilateral, unifocal tumours with a volume <100 ml and a KPS ≤ 60 were enrolled. Recruitment was completed in 2003, but no final published results of the study are available yet. There are currently no phase 3 protocols using IL4-PE.

TP-38

TP-38 (Teva Pharmaceuticals, formerly IVAX Inc., Miami, FL, USA) is a recombinant chimeric protein composed of transforming growth factor α (TGF-α), an epidermal growth factor receptor (EGFR) ligand, and the genetically engineered form of PE described above (see IL4-PE).

The 170-180 kD transmembrane glycoprotein, epidermal growth factor (EGF) receptor, is one of four members of the erbB family of receptor tyrosine kinases, which consist of an extracellular domain that can bind ligands, a transmembrane domain and an intracellular tyrosine kinase domain. Binding of a ligand (EGF or TGF-α) to EGFR causes receptor dimerisation leading to tyrosine kinase activation. The resultant receptor autophosphorylation initiates signal-transduction cascades involved in cell proliferation and survival. After ligand binding, the whole receptor-ligand complex undergoes endocytosis and is translocated to the lysosomes, where the ligand dissociates from the receptor.

Human malignant gliomas and many other malignant tumours that metastasise to the brain express EGFR, and this is commonly associated with amplification and/or mutation of the EGFR gene during neoplastic transformation. Amplification and high expression of EGFR in gliomas may drive tumour growth and proliferation to a significant degree. By contrast, EGFR is expressed at very low levels, or is undetectable on normal human glial cells.
and neurons thus suggesting a potential therapeutic window. The ratio of EGFR expression in glioma versus normal control brain specimens has been shown to be as high as 300-fold.\(^6\)

Animal studies with TP-38 have been carried out in rodent and primate models. In tumour bearing mice, a total dose of 0.1 µg TP-38 at a concentration of 5 µg/ml was found to be safe and efficacious in prolonging survival. The MTD of intracerebral TP-38 in normal rats was 0.666 µg at a toxin concentration of 33.3 µg/ml. In non-human primates, it was demonstrated that an intracerebral bolus dose of 2 µg TP-38 at a concentration of 10 µg/ml is safe.\(^5\)

Sampson et al. investigated TP-38 in a phase 1 clinical trial.\(^5\) The primary objective of the study was to define MTD and dose limiting toxicity of TP-38 delivered by CED in patients with recurrent malignant glioma. A secondary objective was to detect the efficacy of the toxin. Twenty patients were enrolled in the study and doses were escalated from 25 to 100 ng/ml. TP-38 was infused by two stereotactically placed catheters at a flow rate of 0.4 ml/h for each catheter. A total volume of 40 ml was infused. TP-38 was tolerated well, and an MTD was not established. Non-specific toxicity was not found at any of the dose levels. The toxicity encountered was solely neurologic and mostly related either to infusion volume, recurrent tumour, or stereotactic catheter placement, but not directly to TP-38. Fifteen of the patients in this study died from progressive disease. When the study closed, 4 further patients had not had a recurrence of tumour and were 55, 56, 69, and 116 weeks from the time of TP-38 therapy. Overall median survival after TP-38 for all patients was 23 weeks, whereas for those without radiographic evidence of residual disease at the time of therapy, the median survival was 31.9 weeks. Overall, 3 of 15 patients with residual disease at the time of therapy have demonstrated radiographic responses.\(^5\)

A multicentre randomised open-label phase 2 study was conducted in adult patients with recurrent GBM.\(^5\) Patients were randomised to two dose levels of TP-38, 50 ng/ml or 100 ng/ml, and toxin was administered by CED via stereotactically placed catheters. Patients did not have to undergo tumour resection prior to treatment. The end points of the study were time to progression, progression-free survival, and overall survival. Three catheters were stereotactically placed in investigator-determined locations within the enhancing tumour area. The infusion rate was 200 µl/h per catheter. Each catheter delivered 13.4 ml over 67 h. The total volume infused was approximately 40 ml, and the total dose of TP-38 infused was 2 µg (50 ng/ml) or 4 µg (100 ng/ml). One patient had a complete response 48 weeks after infusion, and another showed a partial response stable over 60 weeks. Twenty-four patients remained stable. Post-infusion MRI scans in most patients showed unspecific treatment-related changes such as halo contrast enhancement around the infusion sites, which made assessment of response rather difficult. These changes usually resolved by 20 weeks post-treatment. There were no grade 3 and 4 toxicities related to TP-38, and all adverse effects of the treatment were reversible.\(^5\) A phase 3 clinical protocol employing TP-38 seems to be in development, but no further details are currently known.

**IL13-PE38**

IL13-PE38 (IL13-PE38QQR, also known as cintredekin besudotox, (NeoPharm Inc., Lake Forest, IL, USA) is a recombinant chimeric toxin consisting of human IL-13 fused to the mutated form of PE mentioned above [Figure 2]. The T-helper cell 2-derived immunoregulatory cytokine interleukin-13 (IL-13) inhibits the production of inflammatory cytokines in monocytes. The cell surface receptor for IL-13 (IL-13R) is expressed on many human cancer cells, including glioblastoma, AIDS-associated Kaposi’s sarcoma, ovarian carcinoma, renal cell carcinoma, but also fibroblast cell lines.\(^6\) The IL-13R structure varies according to cell type, existing in three different forms known as type I, type II and type III.\(^5\)

Subunit structure studies of the IL-13R complex in primary brain tumour cells established the IL-13Ra2 subunit as a tumour-specific protein.\(^5\) Immune cells, endothelial cells and normal glia and neurons generally express none or very low amounts of IL-13R.\(^6\)

IL13-PE38 was found to be highly selective and potent in killing human glioma cells in culture. The activity of IL13-PE38 is mediated via IL-13R, as its cytotoxicity is blocked by exposing cell lines to a 10–to 100-fold excess of human IL-13.\(^6\) Furthermore, IL-13R-negative cell lines or cell lines expressing low numbers of IL-13R, including human bone marrow-derived cells and PHA-activated T-cells, are not susceptible to IL13-PE38 mediated cytotoxicity.
were placed into the brain adjacent to the tumour resection cavity and on days 10 to 14, IL13-PE38 was given at 750 µl/h for 96 h at a fixed dose of 0.25 µg/ml (total dose 18 µg). In stage 2, intratumoural infusion was omitted and patients underwent resection of the tumour followed by a 96-h peritumoural infusion of IL13-PE38 at 0.5 µg/ml and 1.0 µg/ml. In stage 3, the duration of peritumoural infusion was increased from 5 to 7 days at a fixed dose of 0.5 µg/ml. Tumour necrosis up to 2.5 cm from the catheter tip was demonstrated radiologically in at least 5 patients with preoperative intratumoural toxin infusion. Tumour specimens from at least two patients after intratumoural injection of 0.5 µg/ml IL13-PE38 toxin revealed regional necrosis in an ovoid zone extending 1 to 2 cm from the catheter tip. Peritumoural post-resection MTD was defined as 0.5 µg/ml. Similar AEs occurred across all cohorts, most being neurological. The most frequent AE were headache (53%), hemiparesis (27%), sensory disturbance (20%), fatigue (17%), aphasia (13%), facial paresis (13%), abnormal gait (13%), convulsions (10%), hypeaesthesia (10%) and lymphopenia (10%). Prolonged individual patient survival has been observed after peritumoural therapy at concentrations of 0.25 µg/ml (total dose 18 µg) and above.39,66,67

A further multicentre phase 1/2 study investigated IL13-PE38 in adult recurrent supratentorial malignant glioma with confirmed radiographic evidence of recurrent or progressive tumour. The primary objective of the phase 1 portion of the study was to determine the optimum duration of infusion and drug concentration of IL13-PE38 delivered by CED via intratumoural catheters at 200 µl/h/catheter for 96 h (total 38.4 ml), in two courses 8 weeks apart. Escalation was planned through 9 levels ranging from 0.125 to 12.0 µg/ml (total dose 4.8 to 460.8 µg) in cohorts of 3 patients per level. Cohorts of 3 patients received 0.5, 1.0, 2.0 and 4.0 µg/ml. Concentrations of up to 2.0 µg/ml were safe and well tolerated. The AE reported across all dose ranges were mild and mainly neurological. The most frequent drug-related AE were headache (24%), hemiparesis (24%), brain oedema (14%), aphasia (10%), and ataxia (10%). In the phase 1 portion of the study, two histopathological and two radiographic responses were observed, with progression-free survival ranging from 3 to 88 weeks and overall survival of 147 weeks. No results have been published yet for the phase 2 portion of this study.63

Another phase 1 study investigated IL13–PE38 in adult supratentorial malignant glioma. In this four-stage study, the primary objectives were to determine effective dose of IL13-PE38 either prior to or post-resection of the tumour.64,65 In stage one, after biopsy and intratumoural catheter placement on day 1, IL13-PE38 was administered for 48 h at 400 µl/h on days 2 to 4 at escalating doses ranging from 0.25 to 2 µg/ml. The tumour was resected on day 8 and tissue was evaluated for necrosis adjacent to the catheter. Following resection, 2 or 3 catheters
(40%), seizures (40%), oedema (40%) and aphasia (30%). Progression-free survival ranged from 6 to 30 weeks and overall survival from 10 to 30 weeks.39,64

In all of the above studies, intratumoural infusion of IL13-PE38, with or without tumour resection in patients with recurrent or progressing malignant glioma, seemed to be well tolerated and did not result in any grade 3 and 4 AE, or in any AE in peripheral organs such as the liver, kidney and lungs. Neurological AE during and after toxin infusion were, however, encountered in a significant proportion of the treated patients. These included brain oedema, meningitis, seizures, headache, and symptoms of increased intracranial pressure. All these AE were temporary and mostly controllable by administration of steroids. In general, IL13-PE38 doses (0.5–2 µg/ml) showing biological activity (e.g. necrosis on MRI scans) in tumours were below the threshold of widespread neurotoxicity (4–12 µg/ml). Selecting of patients without incipient mass effect in the brain (due to tumour size and/or peritumoural oedema) seemed a particularly important factor for avoiding serious unwanted side effects. Some prolonged survival was observed in this selected population of patients.39,66,67

A multicentre phase 3 randomised, open label, active control, parallel assignment efficacy study (PRECISE) was carried out in order to determine whether overall survival duration, safety, and quality of life were improved for patients treated with IL13-PE38 compared to patients treated with Gliadel® wafers following surgical tumour removal in the treatment of first recurrence of GBM after initial surgery and external beam radiation therapy.43,68 Patients were randomised 2:1 to receive IL13-PE38 or Gliadel®. The IL13-PE38 toxin (0.5 µg/ml, total flow rate 0.75 ml/h) was administered over 96 hours via 2-4 intraparenchymal catheters placed after tumour resection. The primary endpoint was overall survival from the time of randomisation. Secondary and tertiary endpoints were safety and health-related quality-of-life assessments. From March 2004 to December 2005, 296 patients were enrolled at 52 centres. Median survival was 36.4 weeks (9.1 months) for the toxin group and 35.3 weeks (8.8 months) for the Gliadel® group (P = 0.476). For the efficacy evaluable population, the median survival was 45.3 weeks (11.3 months) for toxin and 39.8 weeks (10 months) for Gliadel® (P = 0.310). The AE profile was similar in both arms, except that pulmonary embolism was higher in the toxin arm (8% versus 1%, P = 0.014). This was the first randomised phase 3 evaluation of an agent administered via CED and the first with an active control group in GBM patients. There was no survival difference between IL13-PE38 toxin administered via CED and Gliadel® wafers placed at the time of surgery. Drug distribution was not assessed, but may be crucial for evaluating future CED-based therapeutics.43,68

### TransMID-107 (Tf-CRM107)

TransMID™ (known as TransMID-107, Tf-CRM107, or KSB-311; KS Biomedix Holdings plc, UK, now Xenova Group plc, UK, and Celtic Pharmaceutical Holdings PL, Bermuda) is a thioether conjugate of human transferrin (Tf) with a truncated natural mutant form of diphtheria toxin (DT) known as CRM-107, which lacks receptor binding.69

Native DT is produced by the bacterium C. diphtheriae and is composed of two disulphide-linked subunits. The A subunit catalyses ADP-ribosylation of EF-2, thereby stopping protein synthesis and killing the cell. The B subunit has two functions: binding to cell-surface receptors and translocation of the A subunit into the cytosol.70 In the natural mutant form of DT, designated CRM-107, two point mutations (phenylalanine at positions 390 and 525 of the DT sequence) reduce the binding 8,000-fold and its toxicity 10,000-fold, compared to native DT.71 However, conjugation of CRM-107 by thioether linkage to various new binding moieties was able to reconstitute the full toxicity of the mutant.72 By linking CRM-107 with Tf, a receptor-ligand complex was created that is rapidly internalised to an environment that facilitates toxin translocation.70

The Tf receptor (TfR) is a transmembrane glycoprotein that mediates cellular uptake of iron. TfR are overexpressed on rapidly dividing cells, such as hematopoietic and neoplastic cells, including glioma cells.73 TfR in normal brains are sparse and their expression is largely restricted to the luminal surface of brain capillaries.73 It is this relative difference in the density of TfR that TransMID™ uses to generate differential toxicity to highly TfR expressing neoplastic cells, while sparing low expressing normal brain cells.74 Low-density background expression of the respective
target receptor in normal tissue remains, however, a major concern with TransMID™ (and with all other targeted toxin based therapies).

TransMID™ has been thoroughly evaluated in cell culture25 and in vivo. The efficacy of locally administered TransMID™ against human glioma in nude mice was demonstrated by Laske et al. in 1994.76 These authors studied TransMID™ compared to 454A12-rRA, and administered toxins intratumourally in a subcutaneous model. Repeated intratumoural injections of 10 µg TransMID™, or 10 µg 454A12-rRA, were compared to equimolar doses of the untargeted native toxin CRM-107, 454A12 antibody, rRA, and phosphate-buffered saline. TransMID™ administration resulted in almost complete tumour regression (>95%), without evidence of recurrence by day 30. Unconjugated toxin components (CRM-107, 454A12, or rRA) caused significant, but less potent tumour growth inhibition than the conjugated toxin.76 In a more recent study in 2002, Engebraaten et al. compared the efficacy of TransMID™ with that of PE conjugated with the 425.3 antibody directed against EGFR.77 Mice with subcutaneous gliomas or rats with intracranial gliomas received different doses (1-10 µg) of TransMID™, or 425.3-PE, injected intratumourally in established tumours. Both toxins showed significant antitumour effects in subcutaneous tumours, but only 425.3-PE was effective in intracranial gliomas in rats. Intracerebral TransMID™ was toxic in doses above 10 ng, while intracerebral 425.3-PE was tolerated up to a dose of 4 µg per animal. The authors concluded that both toxins have promising efficacy in brain tumour models, but that 425.3-PE is better tolerated and has a more specific activity at higher doses.77

In 1997, Laske et al. treated 18 patients with recurrent malignant glioma with intratumoural high flow interstitial microinfusion of TransMID™ in a dose-escalating single arm phase 1 clinical trial.78 The drug was infused at a maximum flow rate of 4-10 µl/min at a toxin concentration of 0.1 µg/ml. Nine of the 15 evaluable patients responded to treatment by at least 50% reduction in tumour volume on MRI, including 2 complete responses. Reduction in tumour volume occurred no earlier than 1 month after completion of the first toxin infusion. In 4 patients, the response was not maximal until 6–14 months after the first treatment. Tumour response appeared to be concentration and dose dependent. Median survival after treatment in the responder group was 74 weeks, with 3 of the responders still alive at 102–142 weeks after the first treatment. Non-responders survived a median of 36 weeks. Intratumoural infusions of TransMID™ with total volumes of 5–180 ml were well tolerated. There were no treatment related deaths or life-threatening or irreversible toxicity. In this first clinical trial, the relationship between dose, drug concentration, and sustained neurotoxicity established a MTD of 26.8 µg (40 ml at 0.67 µg/ml). The trial results indicated that therapy with TransMID™ can reduce the size of malignant brain tumours refractory to conventional therapy without producing severe neurologic or systemic toxicity.78

An open-label, single-arm, multicentre phase 2 study investigated intratumoural CED infusion of TransMID in recurrent or progressive malignant glioma in adults.69 The primary study objective was evaluation of efficacy and safety of TransMID™, and the primary endpoint was a 50% reduction in tumour volume (measured by MRI) within 12 months after the second treatment. Patients received TransMID™ (0.67 µg/ml) at an escalating rate up to 200 µl/h per catheter for 4–5 days, until a total volume of 40 ml was delivered. Forty-four patients were enrolled in total and all received at least one TransMID™ infusion. Of the 34 patients evaluable for analysis, there were a total of 5 complete responders and 7 partial responders (total of 35% response), which was a statistically significant result. Median survival time for all 44 patients was 37 weeks. Infusions of TransMID™ within this clinical protocol resulted in symptomatic progressive brain oedema in 8 of 44 patients (14%). The results of this phase 2 clinical trial have confirmed the safety and tumour response data of the phase 1 trial.69

A multicentre randomised, open label, active control, parallel assignment, safety and efficacy phase 3 study (KSB311R/CII/001 trial) was launched in 2004 in order to compare TransMID™ with the best standard treatment available for patients with progressive and/or recurrent non-resectable GBM.79,80 Best standard treatment involved a chemotherapeutic regimen considered to be the best standard of care at the respective
distribution of the large molecules of immunotoxin in the microenvironment of the tumour and/or brain, as well as non-specific associated toxicity to glial and neuronal cells.\(^{19}\) With the introduction of MRI-based functional imaging and 3D volumetric techniques in routine clinical practice, it will be possible to determine quantitatively brain tumour volumes and the volume of infusate in the tumour and normal brain.\(^{81,82}\) Diffusion tensor imaging (DTI) is a new MRI imaging technique sensitive to directional movements of water molecules induced by tissue barriers.\(^{83}\) CED makes use of the extracellular space of the brain as a natural pathway for the widespread distribution of agents infused in an aqueous solution, and therefore DTI could be used for imaging of CED delivery without the need for contrast agents. Further key developments in MR imaging and MR-related computer software as well as infusion catheter design and computerised simulation and delivery modelling within the brain may provide much needed new resources to be combined with the biologically targeted toxins.\(^{19}\)

Conclusions and Future Developments—Immunotoxins

Targeted toxins have shown considerable promise in phase 1 and 2 clinical trials with recurrent malignant gliomas. There are however some major obstacles that need to be overcome before targeted toxins may enter the mainstream of brain tumour therapies. The most important of them are the heterogeneity of target receptor expression in malignant glioma cells, the non-uniform and poorly predictable distribution of the large molecules of immunotoxin in the microenvironment of the tumour and/or brain, as well as non-specific associated toxicity to glial and neuronal cells.\(^{19}\) With the introduction of MRI-based functional imaging and 3D volumetric techniques in routine clinical practice, it will be possible to determine quantitatively brain tumour volumes and the volume of infusate in the tumour and normal brain.\(^{81,82}\) Diffusion tensor imaging (DTI) is a new MRI imaging technique sensitive to directional movements of water molecules induced by tissue barriers.\(^{83}\) CED makes use of the extracellular space of the brain as a natural pathway for the widespread distribution of agents infused in an aqueous solution, and therefore DTI could be used for imaging of CED delivery without the need for contrast agents. Further key developments in MR imaging and MR-related computer software as well as infusion catheter design and computerised simulation and delivery modelling within the brain may provide much needed new resources to be combined with the biologically targeted toxins.\(^{19}\) Further improvements in the molecular diagnostics of malignant glioma with genetic profiling of individual patients and tumours should allow for selection of subgroups of cases with high expression of the target receptor, who are most likely to benefit from the administration of a targeted immunotoxin.

Clinical protocols have explored several points of presumed significance, such as the use
Clinical Development of Experimental Therapies for Malignant Glioma

Most of the viruses employed in clinical trials are derived from a retrovirus (RV), adenovirus (AV), or herpes simplex virus type 1 (HSV1) [Figure 4a and b]. Other viruses, such as the Newcastle disease virus (NDV) or reovirus, have been also employed in clinical trials, but to a much lesser extent.19,20,86-88

The effects of virus-mediated local therapy of malignant gliomas have been investigated only since the 1990s. The initially favoured and best explored gene therapy approach included the use of a replication-disabled, genetically modified virus vector capable of insertion of a toxicity-inducing gene into tumour cells.89-91 Most frequently, the inserted gene (transgene) rendered the infected tumour cells and their clonal progeny differentially sensitive to treatment with a pro-drug.91,92 The gene/vector system most widely utilised in initial clinical trials was the HSV thymidine kinase (HSV-tk) gene transferred by a replication-incompetent RV vector which was released in situ by fibroblast-derived virus-producing cells (VPC) [Figure 5].93

The most severe limitation of clinical gene therapy with non-replicating viruses was, however, their inability to achieve sufficiently high levels of gene expression in sufficiently large numbers of target tumour cells to result in a clinical benefit.19,94,95 A modified approach was therefore introduced in order to improve anti-tumour toxicity and virus distribution within the tumour mass and the surrounding normal brain. It employed conditionally replicating oncolytic viruses with a lytic life cycle instead of their non-replicating counterparts.85 Most of the viruses employed in clinical trials are derived from a retrovirus (RV), adenovirus (AV), or herpes simplex virus type 1 (HSV1) [Figure 4a and b]. Other viruses, such as the Newcastle disease virus (NDV) or reovirus, have been also employed in clinical trials, but to a much lesser extent.19,20,86-88

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**Figure 5:** Schematic representation of the mode of action of recombinant retrovirus (RV) vector-mediated suicide gene therapy in patients with malignant glioma. Up to 20 ml of a suspension of viable RV-producing cells (VPC) carrying the transgene coding for herpes simplex virus (HSV) thymidine kinase (HSV-tk) are manually injected into the walls of the tumour resection cavity at the end of the surgical removal of a human glioblastoma multiforme (GBM). RV vectors, which have been genetically engineered to become replication-deficient, are produced in high titres by the implanted VPC. GBM cells are highly mitotic and, after infection by RV, are able to produce most RV proteins, including HSV-tk. This enzyme converts the low-toxicity prodrug ganciclovir (GCV) into highly toxic metabolites, which block DNA replication during mitosis and render the tumour cells apoptotic. After repeated i.v. application of GCV to patients, all cells expressing HSV-tk are killed, including VPC. Cells not expressing HSV-tk may also be killed by the so-called "bystander effect" — transfer of toxic GCV metabolites from HSV-tk-expressing to HSV-tk-negative cells by direct cellular contact. Up to 10 non-expressing cells may be killed by one HSV-tk-expressing cell in cell culture.
areas of the tumours. Harsh et al. performed a gene-marking and neuropathological study in 5 patients with recurrent GBM who received multiple stereotactically guided intratumoural injections of RV-VPC during a single surgical session. This study showed an extremely low gene transduction efficiency of the RV-VPC used.

Finally, a large prospective randomised multicentre phase 3 clinical trial was carried out to allow definitive evaluation of RV-VPC treatment in patients with newly diagnosed GBM. A total of 248 patients with previously untreated GBM were randomised to two groups and treated using either standard surgical resection and radiotherapy (n = 124) or standard therapy plus adjuvant RV-VPC injected in the walls of the resection cavity at tumour surgery (n = 124). Systemic administration of GCV (5 mg/kg i.v. twice daily) was started two weeks after surgery and continued for another 2 weeks. Somewhat surprisingly, this study found no significant difference in the progression-free, median, or 12-month survival of both the standard and the gene-therapy groups, although the approach was proven exceptionally safe. Based on the results of this trial, local therapy of malignant glioma with RV-VPC expressing HSV-TK has been largely abandoned.

**NON-REPLICATING ADENOVIRUS**

Studies with AV vectors were conducted and published, mostly in the late 1990s. Trask et al. conducted a phase 1 study of AV-HSV-tk in 12 patients with recurrent malignant glioma. A single stereotactic intratumoural injection was used to deliver AV doses of 2x10^9-2x10^12 pfu. Adequate safety was reported with all doses except the highest, which caused significant CNS toxicity. Median survival in this study was only 4 months, however 3 patients showed long-term survival of 2 years or longer, which was interpreted as evidence for some local tumour control. Judy and Eck treated 13 patients with primary or recurrent malignant gliomas in a modified phase 1 study. Twelve patients received stereotactic intratumoural AV-HSV-tk injections and a week later underwent tumour mass resection with additional AV injections in the walls of the resection cavity. Total virus doses of 2x10^9-2x10^11 pfu were used. Transient side effects, such as increased intracranial pressure (ICP), were noted only at the highest dose level.

The median survival of all patients was 10 months, while 5 patients were alive at 1 year and 1 patient at 3 years after therapy. Germano et al. carried out a dose-escalating phase 1 study in 11 patients with recurrent GBM. AV-HSV-tk doses of 2.5x10^11-9x10^11 virus particles were administered intraoperatively to the walls of the resection cavity immediately after tumour resection. The median survival of treated patients was 59 weeks. No major toxicities occurred, and the few serious AEs were not related to AV administration. Smitt et al. conducted another phase 1 dose escalation study in 14 patients with recurrent malignant glioma. Patients received 4.6x10^8-4.6x10^11 virus particles injected manually in the walls of the tumour resection cavity at the end of tumour removal. Median overall survival was 4 months, however 4 patients survived for longer than 1 year following therapy. The treatment was safe and well tolerated.

Some groups were able to report significantly better clinical outcomes using the AV-HSV-tk approach. Sandmair et al. enrolled 21 patients with primary or recurrent malignant glioma in a comparative phase 1/2 study employing HSV-tk gene transfer by RV-VPC or by AV and comparing these with a third group receiving AV carrying only a marker gene, lacZ. The mean survival in the AV-HSV-tk group was significantly longer (15 months) than that of the other groups with mean survival of 7.4 months for RV-VPC and 8.3 months for AV-LacZ. No serious AE were reported in any of the groups.

Building on these promising results, Immonen et al. conducted a randomised prospective phase 2 study to evaluate further the efficacy and safety of AV-HSV-tk in patients with primary or recurrent malignant glioma. Seventeen of the 36 patients received intraoperative injections of AV-HSV-tk (3x10^10 pfu) into the walls of the surgically created resection cavity followed by GCV (5 mg/kg twice daily for 14 days), and 19 patients had tumour surgery without AV injections. Standard radiotherapy was carried out in all patients with previously untreated tumours. The mean survival of patients in the AV-HSV-tk group was significantly longer (70.6 weeks) than in the control group (39.0 weeks) (P = 0.0095). An additional post hoc subgroup analysis excluding patients with anaplastic astrocytoma showed that there still was
a significant survival benefit in GBM patients (55.3 versus 37.0 weeks, \( P = 0.0214 \)). The treatment was well tolerated and showed no major side effects.\(^{115}\)

A phase 3 randomised and standard care-controlled multicentre pivotal trial (also known as study 904) using the above AV-HSV-tk vector\(^{115}\) designated as sitimagene ceradenovec (Cerepro\(^{\text{r}}\), Arc Therapeutics Ltd., London, UK) has been carried out in patients with newly diagnosed malignant glioma.\(^{116}\) Patients were randomised to either standard care plus Cerepro\(^{\text{r}}\) or standard care alone. Standard care was surgery and radiotherapy or surgery and radiotherapy followed by temozolomide, resulting in 4 treatment groups. This allowed comparison of the efficacy of Cerepro\(^{\text{r}}\) and temozolomide in the same trial without denying patients the best possible standard care. The primary endpoint was survival, defined as time to death or re-intervention.\(^{116}\)

The overall primary endpoint analysis in the ITT population (n = 236) compared Cerepro\(^{\text{r}}\) with and without TMZ against controls with and without TMZ. It showed a 42 day improvement in median survival (310 days versus 268 days) in the two groups receiving Cerepro\(^{\text{r}}\). The improvement over standard care reached statistical significance \(( P < 0.032)\). At the primary endpoint, the group with Cerepro\(^{\text{r}}\) and TMZ showed an improvement of 68% in median survival time compared with standard care (surgery and radiotherapy) controls (350 days versus 208 days). Against the same controls, treatment with Cerepro\(^{\text{r}}\) alone showed an improved median survival trend approaching 50%, similar to those given treatment with TMZ alone after standard care (300 days and 307 days, respectively versus 208 days with standard care). Improvements in the combined Cerepro\(^{\text{r}}\) and TMZ treatment group (n = 58) and TMZ alone group (n = 76) were significant \(( P < 0.05)\). In the Cerepro\(^{\text{r}}\) alone treatment group (n = 61), the effect was not statistically significant \(( P < 0.065)\). Of the total 53 patients still to report an event, only 7 are in the surgery and radiotherapy control group and thus confidence intervals and statistical significance levels in all treatment groups might be expected to improve with time.

Whilst increases were observed in hemiparesis, aphasia and pyrexia following therapy, the serious AE reports for Cerepro\(^{\text{r}}\) were in line with those in the previous studies, indicating that the virus has an acceptable safety profile.\(^{116}\) The results of the study have not been published yet, and final data still need to be provided. Preliminary information in the public space however states that the trial may be statistically underpowered and that Cerepro\(^{\text{r}}\) has failed to show sufficient primary endpoint efficacy.\(^{117}\)

**ONCOLYTIC VIRUSES**

Genetically modified and conditionally replicating AV and HSV have been the most frequently used viruses in early clinical studies with oncolytic viruses.\(^{87,89,97,99}\) Four phase 1 trials in recurrent malignant glioma have used intracerebrally inoculated G207 - a conditionally replicating HSV with defects in both ICP6 and ICP34.5 genes, which has demonstrated anti-tumour efficacy in preclinical studies in glioma.\(^{89,118,119}\) Markert et al. in 2000 carried out a dose escalation study treating 21 patients with recurrent malignant glioma using stereotactic intratumoural injections of G207 (up to 3x10⁹ pfu total dose).\(^{89}\) MRI studies and neuropathological analysis suggested some anti-tumour activity of the treatment. The best responding 4 patients survived for a mean of 12.8 months, while the rest of the group had a mean survival of 6.2 months after therapy. Most importantly, this early trial showed that inoculation of a genetically engineered oncolytic HSV virus in the human brain is safe, despite the concerns about possible encephalitis well known from its wild type counterpart.\(^{89}\)

Rampling et al. (2000) conducted a first phase 1 dose escalation study using another selectively replicating HSV mutant with disrupted ICP34.5 genes known as HSV1716.\(^{120}\) Nine patients with recurrent malignant glioma used intracerebrally inoculated G207 - a conditionally replicating HSV with defects in both ICP6 and ICP34.5 genes, which has demonstrated anti-tumour efficacy in preclinical studies in glioma.\(^{89,118,119}\) Markert et al. in 2000 carried out a dose escalation study treating 21 patients with recurrent malignant glioma using stereotactic intratumoural injections of G207 (up to 3x10⁹ pfu total dose).\(^{89}\) MRI studies and neuropathological analysis suggested some anti-tumour activity of the treatment. The best responding 4 patients survived for a mean of 12.8 months, while the rest of the group had a mean survival of 6.2 months after therapy. Most importantly, this early trial showed that inoculation of a genetically engineered oncolytic HSV virus in the human brain is safe, despite the concerns about possible encephalitis well known from its wild type counterpart.\(^{89}\)
of HSV1716, allowing the virus to kill tumour cells over extended periods of time. Analysis of tumour explants showed viral replication for up to 9 days after initial injection, and the amount of recovered virus exceeded the input dose in some tumour samples. In addition, 1 patient was free of tumour progression at nearly 3 years, and 2 patients were alive and stable after almost 4 years.121 Harrow et al. (2004) followed 12 patients with newly diagnosed or recurrent malignant glioma treated in a previous study.122 Three long-time survivors with GBM were clinically stable at 15–22 months following surgery and virus injection. No long-term toxicity was reported.122

ONYX-015, a naturally occurring AV mutant with deletion in the viral E1B gene, has been previously used in clinical trials for head and neck cancer and gastro-intestinal tract malignancies.97,123,124 The first multicentre phase 1 study using ONYX-015 in patients with recurrent malignant glioma was published by Chiocca et al. in 2004.124 Twenty-four patients received intraoperative doses of ONYX-015 (1x10⁷–1x10¹⁰ pfu) injected manually in the walls of the surgical cavity immediately after tumour resection. The median survival time for all patients was 6.2 months. No definitive anti-tumour effect could be demonstrated, however none of the patients, at any virus dose, experienced serious AE and there was no dose-limiting neurological toxicity.124

Conclusions and Future Developments—Genetically Modified Viruses

The clinical efficacy of local therapy for malignant glioma with non-replicating viruses has been so far rather disappointing, possibly with the exception of the studies with AV-HSV-tk.115,116 No clinical studies using non-replicating virus vectors are currently enrolling patients.

Encouraging anti-tumour activity has been demonstrated in the some of the studies in malignant glioma treated with local injections of oncolytic AV and HSV.97–99,122 Systemic chemotherapy has been found to potentiate the anti-tumour effect of virus mediated oncolysis in other cancer types.123,125 Factors likely to be an issue with any type of oncolytic virus are the physical limitations of manual injection of virus into tumours or tumour-invaded surrounding brain, as well as intrinsic barriers to intra- and peritumoural spread in a glioma-harbouring brain, such as cysts, fibrotic membranes, and tumour necrosis.84,97

Therapy with oncolytic viruses seems to hold more promise in the few early clinical trials than the therapy with non-replicating virus vectors, although both approaches were already proven to be safe and lacking major side effects.96–99, 119,122,126 However, further major advancements in virus designs, application modalities, and understanding of the interactions of the host immune system with the virus are clearly needed before oncolytic virus therapy of malignant brain tumours may enter routine clinical use.

Final Conclusions and Future Developments of Drug Delivery Modalities to Brain Tumours

The therapeutic success of any tumour-targeting and locally delivered agent will depend not only on its ability to kill tumour cells selectively, but to a high degree also on the delivery mode and distribution throughout a tumour and the surrounding normal brain tissue.19,84 Diffusion of particles (e.g. viruses) or large molecules (e.g. recombinant toxins) in tissue is a rather inefficient way of distribution and will depend not only on the concentration of the compound, but also on its size, molecular weight, polarity, and its avidity for the target receptor.40,92,127 To circumvent this limitation, distribution of agents by CED, a much more efficient and fast mode for interstitial delivery by high-flow infusion, has been studied in several animal models.100,127 Local delivery of targeted toxins by CED seems to be the best approach to circumvent the limitations of the blood-brain barrier (BBB) and to increase therapeutic efficacy by high local concentrations of drug. All clinical trials with targeted toxins have adopted CED as the delivery mode of choice.19

Virus particles can also be delivered by CED, although this modality has been studied mainly in animal experiments,20,84,127,128 while all human clinical trials carried out so far have employed bolus injections of virus or carrier suspensions.91,94,101,124 Intratumoural stereotactically guided injections can provide adequate virus delivery only to spatially
limited areas of tumour, since the number of injection sites is limited for practical reasons by tumour size, length of surgery and increasing risk of haemorrhage.94 Direct intratumoural injections into the walls of the tumour resection cavity, although they can be performed under direct visual control and with multiple virus depots close to each other, have the same basic limitations as stereotactic procedures.95,101 Moreover, the depth of injection is limited to 10–15 mm from the visible resection border, which seems to be insufficient to reach tumour cells migrating away from the main mass.101

CED has been shown to improve significantly virus distribution in and around experimental brain tumours.127 Virus particles may be efficiently delivered by CED over large areas of tumour and surrounding brain and achieve widespread distribution and much larger coverage than with bolus injections.92,127,128 Since a large amount of clinical experience has become available in the clinical trials employing CED of targeted toxins, it seems a logical step that clinical protocols with CED of viruses should be implemented in future. Such trials may be able to combine the biological advantages of oncolytic viruses with the spatial distribution affordable by CED.

CONFLICT OF INTEREST

NGR has received research grants from Neurocrine Inc. (San Diego, CA) and IVAX Inc. (Miami, FL). The authors have no financial interests in any of the biotechnology companies mentioned in the review.

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Improving Road Safety through Deterrence-Based Initiatives
A review of research

Jeremy D Davey and James E Freeman

ABSTRACT: The efficacy of road safety countermeasures to deter motorists from engaging in illegal behaviours is extremely important when considering the personal and economic impact of road accidents on the community. In many countries, deterrence theory has remained a cornerstone of criminology and criminal justice policy, particularly within the field of road safety, as policy makers and enforcement agencies attempt to increase perceptions regarding the certainty, severity and swiftness of sanctions for those who engage in illegal motoring behaviours. Using the Australian experience (particularly the tremendous amount of research into drink driving), the current paper reviews the principles underpinning deterrence theory, the utilisation of the approach within some contemporary road safety initiatives (e.g., random breath testing) as well as highlighting some methods to enhance a deterrent effect. The paper also provides direction for future deterrence-based research, in particular, considering the powerful impact of non-legal sanctions, punishment avoidance as well as creating culturally embedded behavioural change.

Keywords: Deterrence; Road Safety Countermeasures; Sanctions; Non-legal Sanctions

Deterrence Theory
The importance of improving road safety within motorised countries is reflected in the wide array of countermeasures that are presently being employed to reduce the prevalence of engaging in unsafe driving behaviours, e.g., law enforcement, media campaigns, rehabilitation and education. Many of these countermeasures utilise deterrence theory as this theory is central to criminology and criminal justice policy.1,2,3 In regards to deterrence, the Classical Deterrence Theory remains the mostly widely understood model, and it proposes that individuals will avoid offending behaviour(s) if they fear the perceived consequences of the act.4 Two 18th century utilitarian philosophers, Bentham and Beccaria, are regarded as the founders of this theory which makes implicit assumptions regarding human behaviour. These are, namely, that law breaking is inversely related to the certainty, severity
and swiftness of punishment. This means that legal threats are most effective when possible offenders perceive a high likelihood of apprehension, and believe that the impending punishment will be both severe and swift.

**Certainty of Apprehension**

Within the Classic Deterrence Doctrine, a number of researchers have asserted that the most powerful deterrent effects on offending behaviour are produced by the perceived threat of the certainty of apprehension. Certainty in the present context refers to the perceived likelihood that an offender will be arrested and punished for their criminal act. In order for the "fear of punishment" to be effective, individuals must believe that the likelihood of apprehension for breaking the law is relatively high.

Evaluations regarding the certainty of apprehension have been extensively reviewed for a variety of different criminal acts (e.g., robbery, violent crimes, shop lifting, drug abuse), with a considerable body of research demonstrating a significant, although weak, negative relationship between certainty of arrest and crime rates. That is, individuals who perceive the chances of arrest as high are more deterred from committing an offence than individuals who believe that they are unlikely to be apprehended. As a result, road safety operations that increase the perceptions of apprehension certainty for engaging in illegal behaviours are likely to have a positive effect on deterring offenders.

**Severity of Sanctions**

The perceived severity of legal sanctions has also been considered to be extremely important when examining the deterrent effects of legal penalties on offending behaviour(s). The Classic Deterrence Doctrine proposes that individuals will be reluctant to commit an offence if they consider that the penalty for such an offence is severe. Not only have the deterrent effects of perceived severity of punishment not received the same level of attention as that of certainty, but also the results within the literature are conflicting.

A considerable body of early research demonstrated a weak negative relationship between perceived severity of sanctions and a range of illegal behaviours. That is, as perceptual severity increases, the likelihood of an individual committing that offence decreases; however, an opposing body of research demonstrates that perceptions regarding the severity of penalties do not have the salient deterrent impact that was once assumed. In fact, some researchers have reported a counter-intuitive relationship, with crime rates actually increasing with increases in the severity of the penalty. Nevertheless, it may be suggested that the greatest deterrent impact in regards to severity of sanctions will be found among those who have never committed an offence, rather than habitual offenders.

**Swiftness of Sanctions**

The third aspect of the Classic Deterrence Doctrine refers to the deterrent effect of celerity, as it is proposed that the application of punishments for illegal behaviour will be most salient when they are administered soon after the criminal act. This belief has direct links to models of learning and experimental psychology (e.g., conditioning), as it has been demonstrated that the time between stimulus and response is vital in regards to learning new behaviours. Likewise, it is recognised that for road safety, the swiftness of impending penalties is an important aspect for achieving deterrence. However, despite the link between the speed of the response and learned behaviour, the effects of the celerity of legal sanctions is by far the least studied of the three major deterrent mechanisms in the Classic Deterrence Doctrine. This is partly because penalties are rarely applied swiftly in the criminal justice system.

**Specific Versus General Deterrence**

While there are many different variations of deterrence, in the broadest sense there are two deterrence processes commonly known as specific and general deterrence. Specific deterrence is most commonly understood to be the process whereby an individual who has been apprehended and punished for a criminal act refrains from further offending behaviour for fear of incurring additional punishment. In contrast, general deterrence
occurs when an individual refrains from engaging in a criminal behaviour as a result of observing others being punished for the offending behaviour or they are warned of the impending penalties for committing an offence such as through media campaigns.3,4

In regards to specific deterrence, the application of legal sanctions following a conviction for an offence such as drink driving or speeding has a number of purposes including punishment, reform, retribution and possibly incapacitation.22 However, a primary goal of the sanctioning process is to deter offenders from repeating the same crime in the future, and thus, the penalty should be perceived as certain, severe and swift.3,22 Attempts to deter motoring offenders through the application of legal sanctions form a core component of current sentencing practices,22 and a growing body of research has demonstrated that sanctions have the capacity to reduce the likelihood of re-offending among a range of motoring groups for a range of offences including speeding,24,25 unlicensed driving,26,27 drink driving,3,28 etc.

In regards to general deterrence, a considerable body of evidence suggests that the threat of apprehension and subsequent legal sanctions, especially when supported by well-publicised media campaigns, can produce a deterrent effect, even if short, on offending behaviour.3,7,29 More specifically, campaigns to reinforce the consequences of an aberrant behaviour (such as drink driving in the Australian context), or increasing the perceived severity or certainty of penalties (as well as apprehension) have produced a beneficial effect on crashes and serious injury rates15 as well as actual perceptions of arrest certainty.3,29

Random Breath Testing as an Example of Targeting Cultural Change

In general, research has demonstrated that the utilisation of deterrence-based initiatives can create lasting attitudinal and behaviour change in regards to aberrant driving behaviours, such as speeding and drink driving. In fact, within Australia, deterrence-based countermeasures have been demonstrated to have the potential to create attitudinal and behavioural change even among established, entrenched and previously accepted cultural behaviours such as drink driving. In regards to the latter, arguably one of the best known examples of general deterrence working effectively is through the implementation of random breath testing (RBT). RBT was introduced into Australia in the 1980s and involves police officers randomly stopping motorists and analysing their breath samples, via a hand held device, to determine if they have consumed more alcohol than is legally permitted in order to operate a motor vehicle. While a number of factors have contributed to the reduction of drink driving in Australia over the past 30 years, studies evaluating the effectiveness of RBT have revealed this countermeasure to be one of the primary reasons why alcohol-related crashes have reduced in Australia.30,31 For example, a review of RBT in Queensland found that the introduction of the programme was associated with an 18% reduction in alcohol-related driver and rider fatalities.32 The general deterrent effect is achieved (in part) by the Queensland Police Service (QPS) conducting the equivalent of one (preliminary) breath test for every licensed driver per year. In the financial years 2001–2002 and 2002–2003, the QPS conducted over 2.6 million preliminary breath tests.33,34 This currently represents the highest rate of breath testing by any police jurisdiction in Australia,35 and demonstrates a high level of commitment by the QPS to the RBT program as well as to promote a general deterrent effect. This commitment to high testing levels has required high levels of resources including extra manpower, officer hours and equipment, to maintain. The QPS has also implemented improvements to RBT operations through the acquisition of state of the art breath testing equipment, booze buses (e.g., mobile testing units) and the implementation of coordinated intelligence efforts in relation to crash and offender hot spots.30,34

Although the apprehension of drink driving offenders is important, it has been argued that the main purpose of RBT is to deter the general driving population from drink driving.3 This also remains a central aim of other road safety initiatives, such as visible speed cameras on the side of the road to deter motorists from breaking the speed limit. Again in regards to drink driving (or other similar behaviours) the ideal general deterrence-based operation is one that is highly visible, sustained and widespread.3,24 However, these features should
remain central to all road safety countermeasures that aim to deter offending behaviours. In regards to RBT, it is also a communication tool, influencing community perceptions of the social unacceptability of drink driving. For example, the aim is not only to target the specific behaviour, but also the cultural climate in which that behaviour occurs and is supported. Community surveys conducted over the years have shown that since the introduction of RBT, there has been an increase in the number of people who disapprove of drink driving. As a result, deterrence-based initiatives have the potential to create lasting cultural and attitudinal change in behaviours that were once supported (or tolerated) within the community. For example, in regards to drink driving, while the behaviour was historically accepted for many decades in Australia, research has consistently demonstrated changes in community perceptions regarding the offence. Illustrating this, an earlier study by the Australian Transport Safety Bureau found that 54% of Australians believe that drink driving is a major cause of crashes. The same study also found that 97% of Australians support random breath testing enforcement by police.

In addition to RBT, a number of Australian states have commenced, using a similar method, to analyse randomly the oral fluid of motorists to determine if they have recently consumed illicit substances such as cannabis and amphetamines. Preliminary research is beginning to demonstrate that randomly testing motorists can also produce a general deterrent effect, although the practice should be complemented with a wide spread media campaign to increase the overall deterrent effect, e.g., increase motorists’ perceptions regarding the wide spread use of the technique as well as the increased likelihood of being detected. Taken together, a foundation of deterrence theory focuses on modifying road safety behaviour, and it can also be applied within a range of road safety concerns such as speeding, unlicensed driving, etc. as well as setting the agenda for cultural change. Within Australia, the tremendous amount of knowledge that has been obtained from focusing on deterring the drinking driver is now being re-directed towards other unsafe driving behaviours such as those mentioned above and which are more common in the Gulf States.

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**Extending Deterrence Theory: Non-Legal Sanctions**

Despite the prominence of the deterrence doctrine within road safety initiatives, a number of additional theories that focus on social, developmental, environmental and biological factors have been developed in an attempt to understand a range of criminal activities. As a result, a number of models have stemmed from, and expanded the scope of, the Classic Deterrence Doctrine. One significant direction of theoretical change has been to commence examining the deterrent effect that non-legal sanctions have on decisions to commit an offence, e.g., social control theory. This endeavour has resulted in an increase in the number of factors proposed to influence criminal behaviour, e.g., social disapproval, feelings of guilt, fear of physical injury. One of the reasons for this expansion was criticisms that traditional deterrence models did not account for the large array of non-legal factors that may influence an individuals’ decision regarding committing an offence, as it is recognised that penalties are not applied within a social vacuum. In fact, researchers have argued that road safety offences occur within a social context, and that there are a plethora of additional attitudinal and behavioural factors (e.g., morality, peer pressure, etc) that may produce a stronger impact on offending behaviour(s) than traditional legal sanctions.

As a result, a number of additional models have been developed that focus on rational choice and prospect theories, and thus suggest that both legal and non-legal sanctions affect a person’s decision to commit an unsafe driving behaviour. This re-orientation has resulted in an increase in the number of factors proposed to influence offending behaviour, such as peer/social sanctions, fear of being injured, moral attachment to the norm, and moral obligations to the law. As a result, such additional factors have now influenced associated educational campaigns designed to increase motorists’ attitudes regarding the importance of road safety. While a complete review of the many non-legal factors proposed to influence criminal and “at-risk” behaviour is beyond the scope of the current paper, some factors are briefly discussed below and may be relevant to societies with high
degrees of social pressure such as those in the Gulf area.

One non-legal sanction that has consistently been proposed to influence motorists’ driving behaviours has been the threat of injuring oneself or another motorist.\textsuperscript{3,44} This deterrent factor forms a central component of many road safety advertising campaigns that promotes the serious negative health consequences that may result from an offence such as drink driving, e.g., crashes and fatalities. A second non-legal sanction that has been hypothesised to affect criminal behaviour is moral commitment to the norm, such as whether individuals are willing to break the law. More broadly, both moral commitment to the norm and respect for the law have been identified as having an effect on the prevalence of criminal activities.\textsuperscript{3,10,42,43} As a result, increasing individuals’ awareness of social norms (such as not drink driving) has considerable merit to influence subsequent driving behaviours. Another non-legal factor involves the threat of social stigma resulting from informal sanctions. Given that deterrence is a psychological process that takes place within a larger social context of human activity,\textsuperscript{3} it has been hypothesised that social and cultural norms affect the prevalence of offending behaviours in a community.\textsuperscript{4,7,40} A considerable body of research has demonstrated that informal sanctions such as social disapproval or fear of social stigma produce a significant deterrent effect on a number of illegal acts such as shoplifting, violent behaviour, etc.\textsuperscript{4,15,17,41} In fact, some researchers have reported that the threat of informal sanctions produces a greater deterrent effect on offending behaviour than the threat of formal legal sanctions.\textsuperscript{12,44,45} As a result, the negative effect of social sanctions are also increasingly being included in campaigns designed to improve road safety.

Another prominent direction of theoretical development in regards to deterrence has been to consider the effect of avoiding punishment and exposure to others avoiding punishment, which has been proposed to have a major influence on subsequent offending behaviour. In 1993, Stafford and Warr proposed a reconceptualised model of deterrence that incorporates four categories of experiences that have been suggested to affect the deterrent process: a) direct experience of punishment; b) direct experience of punishment avoidance; c) indirect (vicarious) experience of punishment; and d) indirect (vicarious) experience with punishment avoidance. The model suggests that both general and specific deterrence have the potential to influence an individual’s decision to commit an illegal behaviour, and are thus compatible with contemporary learning theories through the acknowledgement that both experiential and vicarious experiences have a direct effect on learning and decision making.\textsuperscript{46} The model highlights the fact that the experience of punishment is not the only important factor to achieve deterrence, but also recognises that the process of punishment avoidance is likely to influence further offending behaviours.\textsuperscript{47} Preliminary research has suggested the model has considerable potential to shed light on why some individuals are not deterred by the threat of legal sanctions, particularly in regards to the problem of personally avoiding detection and punishment and/or observing others achieve similar outcomes. For example, preliminary research has demonstrated punishment avoidance to be negatively associated with perceptions of arrest certainty, and positively associated with illegal drug use in high school students.\textsuperscript{47} These findings highlight the need to implement road safety initiatives that maximise the probability of apprehending individuals who violate road rules.

**Directions for Future Research and Theoretical Limitations**

Despite the tremendous amount of research that has focused on the mechanisms and processes of deterrence over the past 30 years, researchers admit that the precise circumstances under which sanctions (or the threat of sanctions) are likely to influence or change a person’s behaviour are still not known.\textsuperscript{2,48} One limitation within the deterrence literature is the lack of research that has examined convicted offenders,\textsuperscript{6} particularly repeat offenders, and why they seem immune or impervious to the threat of legal sanctions.\textsuperscript{44} Specifically, research has yet to determine whether repeat offenders consider penalties to be “certain, severe and swift”, or why a considerable proportion continue to drink and drive despite incurring increasingly severe sanctions.\textsuperscript{3,15}

Another major limitation within the deterrence field is that the vast majority of deterrence research
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A review of research

has focused on college students and the general public. More specifically, current understanding regarding the mechanics of deterrence initiatives is heavily skewed towards programmes of research that have focused predominantly on younger populations. Thus, less is currently known about the general deterrent impact of possible future legal punishment on wider motoring populations’ actual offending behaviours. In part, this limitation stems from the difficulties associated with determining casual directions, eliminating competing explanations, and examining large groups of motorists’ self-reported attitudes and offending behaviours. What is commonly understood is that deterrence processes are generally unstable and fluctuate over time, which suggests that individuals’ perceptions of sanctions, and the impact that such sanctions have on their behaviours, are likely to change. Therefore, one of the primary concerns with deterrence theory is that deterrence is considered to be unstable and can change over time. There thus remains a continual need to investigate and refine the deterrent impact of current countermeasures on the motoring population.

Increasing Deterrent Effects

The various principles incorporated within deterrence theory have together been proven to increase road safety in a number of motorised countries (e.g., United States, Canada, Australia, etc.) and within a range of areas including speeding, unlicensed driving, drink driving, and drug driving. However, in order to maximise the greatest deterrent effect, it appears that policy makers and enforcement agencies need to maintain a balance between both the general and specific deterrent aspects of the theory. For example, in regards to speeding enforcement, the overall efficiency of the programme could be optimised by maintaining (and increasing over time) the high level of speed cameras and mobile operations as well as increasing the number of drivers detected. Importantly, in order to create and maintain a deterrent effect, policing operations should be highly visible, sustained and widespread. This ensures that all motorists, whether newly licensed or experienced, perceive a constant high risk of apprehension. If drivers do not regularly observe policing operations, they may become undeterred which may be then reinforced by successfully engaging in offending behaviours that remain undetected, e.g., punishment avoidance. Stemming from this, the effectiveness of any deterrence-based enforcement practice is heavily dependent upon increasing motorists’ perceptions regarding the risk of being apprehended for an offence, e.g., general deterrence. As a result, there is a need to utilise a variety of public education strategies to ensure motorists are aware of current efforts to apprehend offenders. One proven method is to conduct regular publicity campaigns that highlight sustained efforts to detect offenders through a variety of mediums including television advertising, radio, brochures, posters, etc. In general, research has begun to demonstrate that well-executed mass media campaigns (that are widely implemented, targeted and persuasive rather than fear eliciting) have the potential to reduce offending behaviours and/or culturally-embedded unsafe behaviours.

In summary, deterrence remains unstable and requires high levels of police resources and commitment in order to maintain it. As highlighted previously, it should also be noted that our current understanding of the mechanisms of deterrence is based heavily on studies that have focused on younger populations. In fact, the bulk of published deterrence-based studies are from a small number of highly industrialised countries (e.g., United States, Canada, Australia, etc), and thus deterrent forces are likely to fluctuate with the surrounding environment. In fact, it should be acknowledged that environmental modifications have the potential to create a greater level of behavioural change in some countries than deterrence-based initiatives. Nevertheless, in order to ‘maximise’ deterrent effects, enforcement operations should consider utilising targeted and intelligence-led enforcement methods to increase the likelihood of identifying and apprehending motorists engaging illegal behaviours. In regards to speeding, this might involve commencing mobile operations at high risk times in high risk locations where people are most likely to speed. This could be complemented with the use of crash and apprehension data which highlights where and when crashes or previous arrests have occurred. However, there are other enforcement methods
that have resulted in increased detection rates including the use of covert operations comprised of unmarked cars and plain-clothes police. This approach may prove particularly useful in rural areas and the greatest effects may be achieved through a mixture of overt and covert enforcement methods.

However, it is noted that any deterrence-based method employed in isolation does not offer a panacea for the problem of road accidents and fatalities, and thus researchers and policy makers also need to look beyond such principles to identify other methods both to increase motorists’ awareness of the importance of safe motoring as well as to create lasting behavioural change. In fact, there are a number of initiatives that are likely either to complement the general deterrent effect of law enforcement operations (e.g., use of publicity, media advocacy, changing community norms), or improve the management of convicted offenders, e.g., rehabilitation, licence actions, vehicle sanctions. Therefore, it is important that deterrence-based approaches are not considered in isolation, but rather, form part of a multi-modal approach (including education) to improve road safety and change entrenched “at-risk” driving behaviours. More generally, and when attempting to alter behaviour, there are two main pathways to ensure compliance: 1) the extrinsic pathway governed by systems and rules with rewards and punishments, and 2) the intrinsic pathway that establishes voluntary compliance via individual commitment to safety. While deterrence theory may be argued to be one of the key ingredients to improve road safety, it is noted that an excessive amount of extrinsic motivation in the form of policies and regulations may actual trigger further issues by reducing the intrinsic motivation of drivers to perform safety behaviours. As a result, establishing intrinsic commitment to road safety throughout the community can only assist in achieving more sustainable and reliable behavioural change.

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Advances in medicine and health care are influenced by technological achievements, information technology and evolving knowledge and discoveries. These, in turn, change the way in which we provide healthcare. The changes not only influence the practice within the various medical specialities, but they also influence the way those specialities interact with each other. The latter can not be more obvious than in supporting clinical services such as radiology and pathology.

Reasons for Changing Trends and Rising Challenges within Pathology

In addition to the changes outlined above, pathology practice has also been faced with its own changes. These have both necessitated considering new trends and also raised challenges in the way the service is delivered. Among these are: 1) increased demand for pathology services;² in diagnostic surgical pathology this has not only been in order to deal with the increased number of biopsies, but also to comply with guidelines for cancer case reporting³ and with the various recommendations of specimen handling and additional testing;⁴ 2) the need to improve turnaround time as a critical element in clinical management;⁵ 3) the need to comply with the statutory requirements of the various laboratory accreditation and quality assurance regulatory bodies; 4) the rising trend of subspecialisation within the clinical specialities;⁶ 5) the introduction of the principle of multidisciplinary team meetings for the management of cancer patients;⁷ 6) the decline in the number of autopsies; 7) the explosion in the number of rapidly evolving new techniques, and 8) advances in information technology and digital imaging.⁸,⁹
For these reasons, it was inevitable that the various aspects of the pathology service would need to respond to these challenges and to change the way that they deliver their services. Some of those challenges and the choices of solutions are discussed here as follows: subspecialisation in the diagnostic surgical pathology service and cytology; frozen sections; the autopsy; multidisciplinary team meetings; ancillary techniques; the pathology report and some other administrative duties. However, first, a brief reference to the pathology service users and their contribution in shaping the service is warranted.

Pathology Service Users

Any changes to the pathology service should ultimately take into consideration the needs of the service users. It is appropriate, therefore, to highlight first who are the main users of pathology services and how they contribute to the changing trends and the challenges that face the specialty. The main users of the pathology service include surgeons, oncologists, gynaecologists, hospital physicians, radiologists, general practitioners, students and various research groups.

Surgical specialties, including gynaecology, continue to be the major service users of the histopathology service and therefore remain the main drivers of the changes to service delivery and provision. It was the move of general surgery into various surgical subspecialties that originally drove the histopathology service to follow suit. As surgical techniques and approaches develop and progress, the histopathology service should be able to adapt and respond to rising needs and demands. This requires close interaction and better sustained communications between the pathologists and their various surgical colleagues. Equally, newly developed techniques and new knowledge within the various subspecialties of pathology need to be communicated to our surgical colleagues to explain how these new developments can contribute to the management and care of patients.

Radiologists are not strictly speaking service users, but they are increasingly providers of pathology material on behalf of other service users. In recent decades, they have contributed to important changes that happened within histopathology. On the one hand, advanced imaging techniques have improved significantly the accuracy of premortem diagnosis thus significantly decreasing the need for hospital autopsies and contributing to the decline in autopsy numbers. On the other hand, the accessibility by modern radiological techniques to various sites in the body and the provision of cytology or biopsy material from previously difficult or inaccessible locations has contributed to: 1) increasing the number of biopsies and consequently the pathologist’s workload; 2) better accuracy and adequacy of sampling of the targeted lesion; 3) understanding and unravelling of new pathological entities, and 4) better pathological staging of cancer cases and improved clinicopathological correlation.

The contribution of our oncologist colleagues in influencing the way we report cancer cases should also not be underestimated. They contributed significantly to the present format of cancer management and were instrumental in the establishment of multidisciplinary team meetings. Their input on the required contents in synoptic reporting and in minimal data sets as well as checklists and guidelines can not be overestimated.

The Diagnostic Surgical Pathology Service

In recent years, one of the most important developments that has changed the diagnostic surgical pathology service is the move towards subspecialisation in departments where the pathologists used to provide a general diagnostic service. This means that each pathologist now provides services only within one or very few subspecialties. For example, some pathologists report only on breast pathology, others specialise in gastrointestinal (GI) pathology or soft tissue diseases etc. Pathology subspecialisation has existed for a long time in some subspecialties such as paediatrics and neuropathology, but it has only recently spread into all other subspecialties, particularly within large teaching departments and big referral centres.

Subspecialisation in pathology was seen as the natural response to the move to subspecialisation in surgery and as a solution to the problem of increasing workload, whether due to increased numbers of biopsies or the increased reporting requirements for various cancer and non-cancer
It also seemed a natural progression in response to the overwhelming increase in our knowledge in the past three decades. Overall, and if it can be afforded, the advantages of subspecialisation outweigh those of the general diagnostic service.

The acknowledged benefits and advantages of subspecialisation include: 1) increased experience and skill of the pathologists at interpreting challenging cases within their own subspecialties, thus allowing timely and accurate diagnosis; 2) maximising clinical efficiency through enhanced teamwork and communications with the corresponding subspecialised clinical services; 3) optimisation of teaching; 4) promotion of research efforts in the various subspecialty areas, and 5) creation of an environment in which research can be successfully planned and performed.

However, subspecialisation has its demanding requirements and it can not be applied in all pathology laboratories. The main disadvantages include: 1) decreased staffing flexibility in comparison to laboratories which provide a wider general service; 2) increased operational overheads with every subspecialty operating as if it were a separate unit; 3) difficulties in measuring the equity of workload between staff of different subspecialty teams; 13,14,15 4) difficulties in evaluating the efficiency of the pathologists’ work due to weights and indicators varying from one subspecialty to another; 13,14,15 and 5) the need for more staffing which remains the biggest factor hindering the wider development of subspecialisation.

Subspecialisation: Choice or Necessity?

Subspecialisation remains largely a choice that is dictated by a variety of factors including the laboratory setting (service versus academic), specimen volume and specimen composition as well as level of staffing. For example, subspecialisation cannot be afforded by departments that are staffed by less than six consultant pathologists. Also, if they have a low number of biopsies or range of materials, the significant increase in costs and staff cannot be justified. On the other hand, subspecialisation should be seen ultimately as a necessity for large academic and teaching departments with heavy workloads. In this case, it will accommodate and improve research and optimise teaching obligations, in addition to the other benefits of subspecialisation.

The Fate of Cytology in Subspecialised Pathology Departments

Prior to the subspecialisation trend, cytology often constituted an independent department or a separate unit, within the pathology department while in a small department it was regarded as part of the general diagnostic service. As histopathology moved to subspecialisation, some institutions opted to integrating the various cytological specimens into the corresponding or appropriate subspeciality histopathology teams; for example, breast fine needle aspiration (FNA) was allocated to the breast team and so on. Cervical cytology was dealt as a separate issue in places where there were large screening programmes. Other places continued to have an independent cytology service that did not integrate into the subspecialty teams.

There are reasons that favour integrating cytology specimens into the corresponding histopathology subspecialty: 1) it allows the correlation of the histological and cytological findings, thus resulting in more accurate diagnoses and better patient care; 2) it increases experience and improves skill; 3) it enhances team work with the corresponding subspecialised clinical services, and 4) it optimises teaching and promotes research.

Equally, there are arguments against the need for such integration which call for cytology to remain independent. These include: 1) existing cytopathologists who are experienced across the whole of the cytology spectrum. With subspecialisation, a major part of their expertise would become redundant, lost or underutilised; 2) unlike histopathology specimens, there could be difficulties in assigning and allocating some materials: e.g. should ascetic fluid go to the GI team or to the gynaecology team? 3) the heavy cytology burden in some subspecialties in comparison to others, e.g. breast versus GI, and 4) the nature and amount of material where there are cervical cytology screening programmes. The reasons for and against integration of cytology seem to be equally strong. It therefore seems logical, for the time being at least, that the choice should depend on the circumstances of each establishment.
Intraoperative Consultation/Diagnosis (Frozen Sections)

Most pathologists accept that the frozen section procedure is an important and difficult procedure that requires experience, knowledge, the ability to make quick decisions under pressure and good judgement. It is often needed in order to confirm the extent of disease, the nature of a pathological process, the resection margins and the nature of a tissue, such as parathyroid. The procedure has its obvious limitations which are mainly time, sampling error, lack of consultation, lack of special stains/studies, inconsistency in the quality of the sections and the type of artefacts particularly ice crystal artefacts.

Changing trends in the practice of pathology have significantly contributed to the decline, relative or absolute, in the frequency of the pathologists' exposure to frozen section materials. The main trends responsible for this are subspecialisation and the availability of radiologically guided biopsies. The former have resulted in the number of available frozen sections being thinly distributed among a large number of trainee pathologists. The latter means that materials that were in the past only accessible through frozen sections became less dependent on this procedure. In either case, the result is that the frozen section procedure has become even more challenging for the less experienced pathologist. The problem is often further compounded by the requesting clinicians being unfamiliar with the difficulties and limitations of the procedure, when it comes to particular pathological processes such as follicular lesions of the thyroid. In today's practice, therefore, pre- and intraoperative consultation and communication between surgeons and pathologists are a necessity while the decision to have a frozen section for intraoperative diagnosis should ultimately be the surgeon's choice and decision.

The Autopsy

The availability of an autopsy service is a necessity both within the health and forensic services. The reasons for this include cases of unexplained, or suspected wrongful deaths in the case of forensic service. In the health service, autopsy is required in cases of death with no premortem diagnosis and for the purpose of auditing and correlating premortem diagnoses with those of the autopsy findings.

Various factors have contributed in the last three decades to the dramatic worldwide decline in the number of autopsies. These relate generally to the increasing public reluctance to give consent for an autopsy, exacerbated by the publicity given to certain infamous episodes. In addition and unfortunately, autopsy practice does not appeal nowadays to the majority of pathologists. They consider it disruptive to other increasing duties and a source of distraction for staff employed to meet the clinical needs of live patients. The decline has also been accelerated by advanced imaging and other diagnostic techniques that have significantly improved premortem diagnosis. The unpopularity and decline of the autopsy has confronted the specialty with the challenge of providing adequate training and experience for trainee pathologists.

There is a consensus that autopsy training is a necessity since, as outlined above, the autopsy service is a necessity. However, while it is essential to have proper autopsy training, it is not essential that all trainees in pathology should have such training. Making autopsy training an "elective" subspecialty will ensure that only genuinely interested trainees are able to get the proper experience from the declining pool of autopsies. Furthermore, centralisation of the service may be the solution in order to maximise the experience and exposure of those trainees to a larger number and wider spectrum of autopsy cases, improve the standard of service and cope with the problem of the declining number of pathologists interested in this particular subspecialty. It has also been suggested that the rigid rules and regulations that determine which cases should be considered for hospital autopsies need to be relaxed. Instead, requests for autopsy needs to be decided on a case by case basis and could include the few cases where the relatives of the deceased request an autopsy, even when it is deemed unnecessary by the treating physicians.

Multidisciplinary Team Meetings (MDTs)

To many pathologists, MDTs are looked upon as the “best thing” that happened to the speciality in recent times. Through participation in clinical
decision making, pathologists gain improved job satisfaction and have sense of being appreciated. MDTs have increased awareness among other health professions of the speciality of pathology and its important role in the management of cancer patients. They have also improved communication with our service users and other health professions leading to more accurate, meaningful and informative pathology results. For many, it is also regarded as part of continual professional development so that although it is a time consuming duty it is often welcomed. MDTs are undoubtedly here to stay as they have proven to be immensely useful both to the patients and to all parties involved in management and care of cancer patients. As MDTs are a relatively recent innovation, they need to be formally regulated to ascertain who should attend, the meeting venue, the technology and equipment required and the decision making method. MDTs should also be recognised as a significant component in the pathologist’s duties and job plan.

Old and New Ancillary Techniques

Special stains, enzyme histochemistry, virtual slide telepathology, confocal light microscopy, immunohistochemistry, flow cytometry, electron microscopy (with its various modalities including transmission electron microscopy [TEM], scanning electron microscopy [SEM], scanning transmission electron microscopy [STEM], low voltage electron microscopy [LVEM], reflective electron microscopy [REM]) and molecular pathology techniques are some of the techniques that pathologists utilise in their various activities and roles whether for diagnosis, prognosis, teaching and/or research. Ancillary techniques, including those which are new or technologically and scientifically highly advanced, are the means to achieve specific aims, be it for the diagnostic service or for research. In the drive for excellence, the focus on the aims can get distracted by the intriguing means to the point that the means become aims in themselves. We need always to remember to choose and apply techniques that answer our questions and address our needs rather than searching for irrelevant questions in order to adopt fascinating and attractive, yet irrelevant, new techniques.

In the recent past, several exciting and promising techniques did not match expectations. Others eventually occupied an important, yet significantly smaller, role than that which was originally expected. One example, more than two decades ago, was when silver staining for nucleolar organising regions was seen a promising tool as a proliferation marker; this topic generated an enormous number of publications. A second example is electron microscopy in the 1970s and 1980s which was seen as the "ultimate" means for identifying tumour differentiation, yet it has now largely been replaced by immunohistochemistry. On the other hand, it still makes an essential and major contribution to specific topics mainly in kidney and neuromuscular diseases.

We should also remember that the pace of technological advance far exceeds our ability to adopt or implement all newly emerging techniques. Before adopting them, we need to identify the problems they can address while recognising our limitations. Otherwise new technologies can have an adverse impact on our basic and essential functions. We should also seek opportunities in global collaboration as rapid email and transport communications make our world increasingly smaller.

The Pathology Report

Naturally perhaps, choices are limited when it comes to the style of the pathology report. For a long time, free text and summary style reports constituted the majority of pathology reports. They still remain the norm for reporting many non-neoplastic diseases. In the last two decades, with the increase in information requirements, synoptic reports have become fashionable particularly in reporting cancer cases. A mixture of both free text and synoptic style reports are also adopted by some, but they are often tedious and time consuming to compose. Digital images have also found their way into the pathology report in many centres worldwide, but their value and contribution is often questioned.

There is an overwhelming case for adopting a standardised synoptic report style especially for cancer cases. Their contents and designs are based on recommendations and guidelines issues
by international professional bodies.\textsuperscript{2,3} Most are simple and thorough in their contents, having the advantages of consistency, uniformity and being quicker to produce while avoiding oversight and typographic errors. They are also appealing to the service users who seem to recommend them. There does not seem to be a case against the synoptic report style. As such, it is reasonable to say that they should be regarded as a necessity for reporting cancer cases and at least a good choice for the reporting of non-neoplastic diseases.

Other Duties that Influence Today's Pathology Practice

The pathologists’ growing administrative duties have increased their work load and responsibilities in addition to the clinical duties referred to above. The former include the requirements to comply with various regulatory bodies for both the medical and non-medical aspects of the provision of the pathology laboratory service. Issues such as accreditation; external and internal quality assurances;\textsuperscript{28} continuing professional development activities; various performance indicators including appraisal and job planning; continuous internal and external audit activities and revalidation and participation in clinical governance activities are just some of the essential tasks expected of medical professionals nowadays. Regardless of whether we like them or not, believe in them or not, we have to carry them out. They are necessary because: 1) they justify our confidence in our practice; 2) they justify the confidence of our service users and managers; 3) they safeguard the standard of our practice, and 4) they ensure continuous pathology service improvement.

Conclusion - Maintaining the Momentum of Pathology Service Improvements

Finally, facing and responding to the challenges, as well as to the changing trends, are strategies through which pathology service improvements continue and are ensured. Certain approaches are fundamental within any organisation to maintain the momentum of ongoing improvement. These include: 1) effective communication, the significance of which cannot be overstated. This entails communication at all levels be it within the department, with other pathology departments and institutions or with service users and managers; 2) the strong support of service users and managers in order for the service to continue to thrive and improve; 3) taking radical decisions, if deemed necessary for improvements, for example, centralisation of some aspects of the service which could concentrate expertise and reduce costs; 4) participation in regulatory bodies and schemes; 5) continuous adaptation to and adoption of useful new technology and procedures, and 6) exploration of opportunities for collaboration. No organisation, regardless of its size, can survive in isolation with today’s rapidly changing pace of technological and medical advances.

References


Asthma Control in Oman
National Results within the Asthma Insights and Reality in the Gulf and the Near East (AIRGNE) Study

Nasser Al-Busaidi1 and Joan B Soriano 2

Abstract: Objectives: The Asthma Insights and Reality (AIR) study in the Gulf and Near East (one of a worldwide series of surveys conducted in adults and children to assess asthma control) was conducted in Oman to assess how closely asthma control meets international guidelines recommendations. Methods: From January 2007 to March 2008, asthmatics receiving treatment or currently suffering from asthma symptoms were interviewed among nationals randomly surveyed from the most populated urban areas in Oman (Muscat, Sohar and Nizwa). The standard AIR questionnaire was used to assess symptom severity, health care utilisation, limitation of activity and medication use. Results: From 201 asthmatic participants, 21% were under 16 years and 43% were female. Tobacco use was low in our asthmatics. Disparity in asthma perception was wide in Oman; while 57% of asthmatics perceived their asthma as well or completely controlled, actually 54% had poorly or not well controlled asthma. All recommendations for asthma control by the Global Initiative for Asthma were largely unmet, especially in child asthmatics, with 44% reporting night awakenings due to asthma during the previous 4 weeks and 47% exercise-induced asthma in the previous 12 months. Overall, 32.6% of children and 34.8% of adults reported absence due to asthma on the previous 12 months. Overall, 32.6% of children and 34.8% of adults reported absence due to asthma on the previous 12 months. Use of preventive inhaled corticosteroids was only 5%, one of the lowest even within the AIR Gulf and Near East study, producing an unacceptable ratio ICS/SABA (inhaled corticosteroid/short acting beta-agonist) of 0.054 in Ommani asthmatics. Conclusion: Asthma control in Oman falls far below the goals of current international guidelines therefore corrective strategies are needed.

Keywords: Asthma; Oman; Health survey; Asthma Prevention & Control; Adult; Child

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Accepted 10th Nov 10

Advances in Knowledge
1. Current international asthma initiatives, both for children and adults, recommend to measure the asthma burden worldwide. However,
Asthma Control in Oman: National Results within the Asthma Insights and Reality in the Gulf and the Near East (AIRGNE) Study

The Global Initiative for Asthma (GINA) guidelines, which were introduced in 1995, followed by many other local guidelines, all aimed at improving asthma patient care and ensuring better long-term control of the disease. Control was the cornerstone of the latest GINA update in 2006. Studies have shown that total asthma control is achievable in most patients. There exists, however, a wide gap between the goals of treatment as set out in the guidelines and actual real-life clinical practice outcomes. Recently, several Asthma Insights and Reality (AIR) surveys were conducted in various countries around the world, including the USA, Canada, the Asia-Pacific region, Western and Central-Eastern Europe, Japan, Latin America, Saudi Arabia, and most recently in the Gulf and the Near East. They all aimed to determine variations in asthma severity and control, both from the patient perspective and objectively, compared to what is recommended by the guidelines. Consistently, these surveys demonstrated a poor level of asthma control in all the above mentioned countries and regions, with local variations specific to each country.

Oman is in the Middle East at the northern edge of the Gulf with a population of about 2.6 million inhabitants. There is a paucity of statistics about asthma prevalence and asthma burden in Oman. By using the International Study of Asthma and Allergies in Childhood (ISAAC) methodology 2003, Al-Riyami et al. reported prevalence rates of diagnosed asthma in Oman of 20.7% in 13–14 year-olds, whereas in younger children (6–7 years) it was 10.5%.

The prevalence of severe asthma (sleep-disturbing wheeze and speech-limiting wheeze) and frequent symptoms in Omani schoolchildren (age between 6–7 years), compared with other ISAAC participating countries in the East Mediterranean region, was higher than in any other country in the study. Similarly, the prevalence of sleep-disturbing wheeze among Omani children was nearly four times that of Iran and almost double that of Malta. We also previously reported that more than 50% of our adult asthmatic patients felt that their asthma had a negative impact in their work, school or home duties. Nocturnal symptoms were common in our studied patients, only 44% reported having had no night-symptoms in the previous 4 weeks, while the rest felt their asthma often disturbed their sleep.

The GINA guidelines have been developed to promote standardised methods of diagnosis and treatment of asthma that now are generally accepted worldwide. Research published since the release of the GINA guidelines indicates that in many countries patients with asthma are unequally treated and that adherence to asthma treatment guidelines is poor.

All AIR studies aimed to assess the discrepancy between perceived symptoms and subjective assessments versus objective control and the burden of asthma in order to determine the implementation status of the goals and management recommendations advocated by GINA. They also have helped to shed light on the perceptions, knowledge and attitudes related to asthma at the local level in order to assist in future national policy development. Oman was one of the Gulf and Near East countries included in the AIR Gulf and Near East (AIRGNE) study, together with Jordan, Kuwait, Lebanon, and the UAE. The summary results have been published elsewhere, but the specific Omani data results warrant a closer look and are presented in this paper.

Methods

The AIRGNE survey was conducted between January 2007 and March 2008. The most populated urban areas in Oman were surveyed in AIRGNE, namely the capital area, Muscat/Seeb/Mutrah, with
was translated into Arabic and then translated back again; any discrepancies or inconsistencies discovered by this process were solved by consensus. In addition, the following items were included: self-completion of the asthma control test (ACT) questionnaire, various questions modified to reflect the local conditions and characteristics of asthma in Oman and some additional questions to reflect the latest GINA guidelines. It was administered with an English-Arabic side-by-side layout, available online from the International Journal of Tuberculosis and Lung Disease (IJTLD) website.

As per standard quality control procedures, all materials were piloted. There was also a personal briefing of all interviewers in each region, and each interviewer conducted two pilot interviews and reviewed the completed questionnaires with a supervisor before starting fieldwork. Completed questionnaires were checked locally and again centrally, and a random double check of interviews in all regions was conducted by telephone. Finally, all data were included in a database after independent double typing.

The frequency and severity of daytime and nighttime symptoms, exercise-induced symptoms and severe episodes, and total symptom frequency, were used to develop a symptom severity index similar to the GINA asthma severity scale. In addition, events such as hospitalisations and emergency care utilisation were documented, as well as the impact of days of school/work lost due to asthma. Patient demographic and asthma severity characteristics were compared using chi-squared analysis to identify factors that might account for differences in asthma management across the country, or analysis of variance for quantitative variables whenever required. Statistical comparisons within the country versus the published international AIR estimates were explored. All statistical tests were two-sided and comparisons with <5% probability of error were considered statistically significant.

**Results**

A total of 201 valid interviews with Omani asthmatics in the three participating cities were completed, of whom 115 (57%) were male. The age distribution was very young, with 21% of the sample being 5–15 years old and 33% 16–29
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Most participants had only completed primary (38%) or secondary (30%) education. Reported income was lower than $1,000 per year in 75% of surveyed participants, while reported smoking in adults was minimal [Table 1].

A total of 90 (57%) of adult asthmatic responders (n = 158) perceived their asthma as "well" or "completely" controlled. The actual figures show, on the contrary, that 54% of adult responders had "poorly" or "not well" controlled asthma. (P <0.05).

A total of 71% of participating Omani asthmatics reported day-symptoms during the previous 4 weeks. Similarly, 44% reported night awakenings due to asthma during the previous 4 weeks and 47% exercise-induced asthma in the previous 12 months, both were particularly frequent in child asthmatics [Table 3]. Exacerbations and use of health services were equally high, and limitations of daily activities due to asthma were widespread both in children and adult asthmatics. One in three asthmatics had never had their lung function tested and few owned a peak flow meter.

A total of 32.6% of children reported school absence due to asthma during the previous year, with a mean standard deviation (SD) of 6.1 (8.5) days. In adults 34.8% reported work absence due to asthma during the past year, with a mean SD of 9.9 (9.8) days [Table 4]. Use of health services was similarly high compared to other AIRGNE participating countries, with 30% of hospitalisations and 58% of emergency (unscheduled) medical visits.

Finally, current use of preventive inhaled corticosteroids was 5.0%, one of the lowest even within the AIRGNE study, with a mean of 14.6%. Most (92%) patients relied rather upon quick relief medications, producing an unacceptable ratio ICS/SABA (inhaled corticosteroid/short acting beta-agonist) of 0.054 [Table 5]. As mentioned above, there was a low prevalence of both ownership of a peak flow meter (25.4%) and ever having had a lung function test (35.0%) in the Omani asthmatics in this study.

### Discussion

AIRGNE-Oman was the first survey in the country to assess objectively the level of control and severity of asthma. It demonstrated that asthma management was poor in 2007–2008 compared to recommendations in published guidelines. As in

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**Table 1: Demographic characteristics of asthma patients.** Values correspond to numbers (percentages) of patients in the corresponding category except for age of adults and children at inclusion and age at diagnosis which are represented as means ± standard deviation (13.5 ± 6.7)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Population N = 201</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Interval age distribution</strong></td>
<td></td>
</tr>
<tr>
<td>5–15 years</td>
<td>43 (21%)</td>
</tr>
<tr>
<td>16–29 years</td>
<td>67 (33%)</td>
</tr>
<tr>
<td>30–49 years</td>
<td>71 (35%)</td>
</tr>
<tr>
<td>&gt; 50 years</td>
<td>20 (10%)</td>
</tr>
<tr>
<td>Age at diagnosis, mean;</td>
<td>13.5</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>115 (57%)</td>
</tr>
<tr>
<td>F</td>
<td>86 (43%)</td>
</tr>
<tr>
<td><strong>Level of education</strong></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>77 (38%)</td>
</tr>
<tr>
<td>Secondary</td>
<td>62 (30%)</td>
</tr>
<tr>
<td>University</td>
<td>62 (30%)</td>
</tr>
<tr>
<td><strong>Income</strong></td>
<td></td>
</tr>
<tr>
<td>&lt;1000 $</td>
<td>152 (75%)</td>
</tr>
<tr>
<td>1000–2000 $</td>
<td>36 (18%)</td>
</tr>
<tr>
<td>&gt;2000 $</td>
<td>13 (6%)</td>
</tr>
<tr>
<td><strong>Region</strong></td>
<td></td>
</tr>
<tr>
<td>Muscat</td>
<td>121 (60%)</td>
</tr>
<tr>
<td>Sohar</td>
<td>50 (25%)</td>
</tr>
<tr>
<td>Nizwa</td>
<td>30 (15%)</td>
</tr>
<tr>
<td><strong>Smoking habits in adults</strong></td>
<td></td>
</tr>
<tr>
<td>Never smoked</td>
<td>173 (96%)</td>
</tr>
<tr>
<td>Former smokers</td>
<td>7 (3%)</td>
</tr>
</tbody>
</table>

---

**Table 2: Comparison between objective and subjective evaluation of asthma control**

<table>
<thead>
<tr>
<th>Subjective asthma control§</th>
<th>Objective asthma control*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poorly and not well controlled</td>
<td>68 (43%)</td>
<td>86 (54%)</td>
</tr>
<tr>
<td>Well/ completely controlled</td>
<td>90 (57%)</td>
<td>72 (46%)</td>
</tr>
</tbody>
</table>

Note: § = Adult responders only (n=158); * = objective evaluation of asthma control relies on the asthma control test (ACT). An ACT of 5 to 19 corresponds to a poorly and or not well controlled asthma, and an ACT of 20 to 25 corresponds to a well controlled asthma for adult responders only (n=158).
all previously published international AIR studies, asthma is poorly managed in Oman with the performance far below the recommended goals of any guidelines. This was obvious when the actual GINA recommendations of control were compared to the AIRGNE-Oman findings [Table 2], clearly showing that guideline-based control was not achieved at the time of our study.

In the European and Asia Pacific AIR studies approximately half of the adult patients reported daytime symptoms. The overall figure in the AIRGNE study was 68%, and it was equally high in Oman (71%) in the present study. These results are close to the findings of Rawas et al. as they found nearly 60% of all current wheezers reported at least one of the symptoms indicating severe or uncontrolled asthma. Night awakenings were also frequent in the AIRGNE-Oman study (44%), and this finding is compatible with the study of Al-Riyami et al. where the prevalence of sleep-disturbing wheeze in Oman was nearly four times that of Iran (3.5% versus 0.9%) and more than double that of Malta (3.5% versus 1.5%). It was even higher than that of Australia (3.5% versus 2.8%), a country with the highest prevalence rate of wheeze among all ISAAC participating countries, being more than three times that of Oman.

The frequency of hospitalisation in Oman in the previous twelve months was also high, reaching 30%. Emergency department visits were high in Oman, as in other AIRGNE countries, the figures being 58% and 51% respectively. These figures were much higher than those in the study conducted by Al Rawas et al. Of the asthmatic patients in his study, who were attending asthma specialty clinics, only 31.9% had visited the emergency department and 15.0% patients had been hospitalised at least once during the previous year.

<table>
<thead>
<tr>
<th>GINA definition for control of asthma</th>
<th>AIRO findings</th>
<th>Adults (%)</th>
<th>Children (%)</th>
<th>All (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimal (ideally no) chronic symptoms, including nocturnal symptoms</td>
<td>Asthma symptoms</td>
<td>73</td>
<td>81</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>During day (past 4 weeks)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Night waking (past 4 weeks)</td>
<td>45</td>
<td>60</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Exercise-induced asthma (past 12 months)</td>
<td>51</td>
<td>60</td>
<td>47</td>
</tr>
<tr>
<td>Minimal exacerbation</td>
<td>Sudden severe episodes in past 12 months</td>
<td>95</td>
<td>84</td>
<td>93</td>
</tr>
<tr>
<td>No emergency visit for asthma</td>
<td>Hospitalisation (past 12 months)</td>
<td>35</td>
<td>42</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Emergency department visit (past 12 months)</td>
<td>18</td>
<td>36</td>
<td>21</td>
</tr>
<tr>
<td>Minimal need for short-acting β2-agonists</td>
<td>Current use of quick-relief bronchodilators</td>
<td>-</td>
<td>-</td>
<td>85</td>
</tr>
<tr>
<td>No limitation on activities, including exercise</td>
<td>Asthma restricts</td>
<td>38</td>
<td>77</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>Sports and recreation</td>
<td>35</td>
<td>70</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>Normal physical activity</td>
<td>18</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Choice of jobs/careers</td>
<td>32</td>
<td>42</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Social activities</td>
<td>22</td>
<td>56</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Sleeping</td>
<td>21</td>
<td>49</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Lifestyle</td>
<td>20</td>
<td>49</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Household chores</td>
<td>17</td>
<td>35</td>
<td>25</td>
</tr>
</tbody>
</table>

**Legend:** PEF = peak expiratory flow.
Asthma Control in Oman
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On the other hand, school absence in children was significantly less frequent in Oman than in other AIRGNE countries (32.6% versus 51%; \( P < 0.05 \), Chi 2 \( p \) statistic when compared to AIRGNE results.) Another positive finding among adult asthmatics in AIRGNE-Oman study was the very low prevalence of smoking, with only 3% of respondents reporting either a current or previous smoking habit. This is probably one of the lowest figures recorded worldwide,\(^{13}\) and indeed an achievement to be sustained in the future.

The current use of asthma medications in Oman is disappointing. According to the findings in this study, only 5% of asthmatics were using inhaled corticosteroids compared to 14.6% in other AIRGNE countries (\( P < 0.05 \)). On the other hand, the use of rescue medication was strikingly high, with 92% of asthma patients reporting daily use of them compared to 55.5% in other AIRGNE countries. Interestingly, these findings totally differ from Al Rawas et al. where 92% of asthma patients attending asthma specialty clinics used inhaled corticosteroids.\(^{12}\) The discrepancy is likely due to the fact that patients in asthma specialty clinics are seen by chest specialists who are aware that steroid inhalers are the cornerstone of asthma treatment; it is also possibly due to the fact that these patients have more severe asthma.

Most patients overestimated their level of control and underestimated their disease severity, as there was a disparity in the patient subjective versus objective asthma severity perception. While 90 (57%) of asthmatics perceived their asthma as well or completely controlled, actually 54% had poorly or not well controlled asthma as objectively identified by an ACT score of 5 to 19 (\( P < 0.05 \)). The frequency of lung function tests was generally low in Oman, being at similar levels to other AIRGNE countries, as only 35% reported their lungs ever tested, and only 25% owned a peak flow meter.

Overall, when comparing the Omani results with the AIRGNE average, the management of asthma in Oman was worse in terms of reporting a higher use of rescue medications and very low uses of inhaled corticosteroids, as well as unacceptably frequent visits to emergency departments.

### Table 4: Evaluation of asthma burden in the past year in Oman by comparison to Asthma Insights and Reality in the Gulf and Near East (AIRGNE) study results

<table>
<thead>
<tr>
<th></th>
<th>Oman (( N = 201 ))</th>
<th>AIRGNE (( N = 1,000 ))</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>School absence in children, %</td>
<td>32.6</td>
<td>51.7</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Mean number of days (SD)</td>
<td>6.1 (8.5)</td>
<td>7.9 (9.6)</td>
<td>0.420</td>
</tr>
<tr>
<td>Work absence in adults, %</td>
<td>34.8</td>
<td>29.7</td>
<td>0.054</td>
</tr>
<tr>
<td>Mean number of days (SD)</td>
<td>9.9 (9.8)</td>
<td>7.3 (8.1)</td>
<td>0.054</td>
</tr>
</tbody>
</table>

### Table 5: Current use of preventive inhaled corticosteroids and quick relief medications, and evaluation of lung function in Oman by comparison to Asthma Insights and Reality in the Gulf and Near East (AIRGNE) study results

<table>
<thead>
<tr>
<th></th>
<th>Oman (( N = 201 ))</th>
<th>AIRGNE (( N = 1,000 ))</th>
<th>( P ) value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Use of ICS, %</td>
<td>5.0</td>
<td>14.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Use of quick relief, %</td>
<td>92.0</td>
<td>55.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ratio ICS/SABA</td>
<td>0.054</td>
<td>0.26</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Own a peak flow meter, %</td>
<td>25.4</td>
<td>17.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ever had a lung function test, %</td>
<td>35.0</td>
<td>32.7</td>
<td>0.746</td>
</tr>
</tbody>
</table>

Legend: ICS= inhaled corticosteroids; SABA= short acting beta-agonist.
There are some potential limitations of this survey. First, sampling was not performed according to Random Digit Dialing (RDD) as in most other AIR surveys. In countries where telephone ownership levels approach 100% and comprehensive databases are available, RDD can approximate a representative random sample of the population. However, RDD was not considered appropriate in Oman, and overall in the GNE, because of the low penetration of telephone coverage.

Second, there are problems associated with the term asthma in our country, therefore many doctors avoid using this term, and use instead the term allergy, with an intention to making it milder and more acceptable to patients themselves or to their parents. Perhaps third, the sample size of 201, while being considerable enough, gives some subgroup analyses (by young children or in severe asthma) reduced statistical power. Therefore, more studies are needed to monitor all trends and assess current interventions.

Conclusion

The AIR study in Oman highlights the gap between the recommended long-term asthma management guidelines and the reality in Oman. International guidelines recommend treating inflammation and not symptoms, but the trend of poor inhaled corticosteroid utilisation among Omani patients with persistent asthma suggests undertreatment. This implies an immediate need to improve communication and awareness among patients and physicians, specifically to reinforce the use of anti-inflammatory medications. Underestimation of the severity of asthma and overestimation of asthma control by both patients and physicians are important factors contributing to poor asthma control.

CONFLICT OF INTEREST

The AIRGNE survey was sponsored by GlaxoSmithKline. All authors had access to the database and discussed and drafted this report independently from the sponsor.

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6. Rickard KA, Stemple DA. Asthma survey demonstrates that the goals of the NHLBI have not been accomplished. J Allergy Clin Immunol 1999; 103:S171.


Vitamin D Status in Pregnant Omanis
A disturbingly high proportion of patients with low vitamin D stores

Moza Al Kalbani,1 *Omayma Elshafie,2 Mohammed Rawahi,2 Ali Al-Mamari,2 Abdullah Al-Zakwani,3 Nicholas Woodhouse4

ABSTRACT: Objectives: The objective of this study was to determine the vitamin D status of pregnant Omanis by measurement of their circulating 25 hydroxy vitamin D levels. Methods: Blood samples were obtained from a cohort of 103 consecutive healthy pregnant Omanis at the Armed Forces Hospital, Muscat, on their first antenatal visit. The study took place in May, June and July 2010. Results: Vitamin D deficiency was present in 34 (33%) of patients (25OHD3 <25 nmol/L), ‘at risk’ levels were found in 67 (65%) patients (25OHD3 25–50 nmol/L); two patients (1.9%) had values between 50 and 75 nmol/L, and no patients in the optimal range >75 nmol/L. Conclusion: If confirmed, these findings indicate the need for vitamin D replacement during pregnancy and lactation. Although not evidence based we recommend at least 1000 IU of cholecalciferol, (vitamin D3) daily.

Keywords: Pregnancy; Oman; 25 hydroxyvitamin D3 (25OHD3); Vitamin D deficiency

D eiciency of vitamin D is common worldwide1 including the Gulf states.2,3 The latter is surprising as sunlight is abundant in the Middle East. More than 90% of our vitamin D is provided by sunlight4 and it is therefore obvious that those persons affected in Arabian countries have little sunlight exposure and a diet deficient in vitamin D.

The role of vitamin D in normal physiology is complex and wide ranging. It has important immune modulating effects protecting against infection,4,5,6 autoimmune disorders7 and certain cancers, in addition to its well documented effects on the prevention of osteoporosis, fractures, falls in the elderly4,5 and impaired cognitive function.8 As we continually see patients with vitamin D deficiency in our clinics, it became important to establish whether or not vitamin stores (25OHD) are normal in a healthy Omani population. For this reason, we chose patients in their first and second pregnancy.
trimester of pregnancy as pregnancy and lactation are associated with profound alterations in calcium absorption and skeletal remodelling necessitating increased utilisation of vitamin D. Our findings are reported below.

**Methods**

Blood samples were obtained from a cohort of 103 consecutive healthy Omani patients at their first antenatal visit, usually in the first, but sometimes the second trimester. They were then assayed for serum calcium (Ca), phosphate (Phos), and serum alkaline phosphatase (ALP), which were measured by spectrophotometry, (COBAS Integra 800, Roche Diagnostics, Indianapolis, USA) on the same day. Serum samples for parathyroid hormone (PTH) were measured by immunochemiluminescence (Access 2, Beckman Coulter, Inc., CA, USA), and 25(OH)D₃ by the LB211 gamma counter (Berthold GmBH & Co. KG, Bad Wildbad, Germany), having been centrifuged and deep frozen at −40ºC. PTH and 25(OH)D₃ were then measured on the same day at the end of the study.

Statistical analysis was performed to determine the relationship between the level of serum Ca, Phos, ALP and PTH versus the level of serum 25(OH)D. We used a correlation test to calculate the significance of these relationships. As this showed no significant linear correlation, a polynomial trend curve was employed. The calculations were made for all groups of patients.

Fully informed consent was obtained from each patient to extract the blood needed for the above procedures in addition to that required for routine antenatal screening. The study was approved by the Armed Forces Hospital authorities.

**Results**

Serum 25OHD₃ levels were deficient (<25 nmol/L) in 34 patients, between 25 and 50 nmol/L in 67 patients (at risk) and two patients had values between 50 and 75 nmol/L. There was no significant linear correlation between 25(OH)D₃ and serum Ca, Phos or ALP levels or parity. However, a significant relationship between PTH and 25(OH)D₃ was observed which differed between the ‘deficient’ and the ‘at risk’ groups. This was confirmed using a polynomial curve, which had a significant correlation value of 0.55 [Figure 1].

**Discussion**

This is the first study to report vitamin D status in normal pregnant Omanis. The results are alarming: 34% of these apparently healthy women were vitamin D deficient and a further 64% ‘at risk’ at a time when there is a critical need for calcium metabolism to be normal. A pregnant woman must provide 25 to 30 gm of calcium to support the developing foetal skeleton. Much of this demand
occurs in the third trimester when the foetal skeleton undergoes mineralisation. This demand is compensated for by an increased absorption of calcium from the gut induced by rising levels of PTH and the active metabolite of vitamin D, 1,25 dihydroxy vitamin D (1,25(OH)2D). At this point 25(OH)D will be utilised to make more 1,25(OH)2D and those patients with low stores will be at considerable risk for the development of vitamin D deficiency and osteoporosis in old age.

To compound this problem, many Omani women have 6 or more children and breast feed for up to 2 years. During lactation, hyperabsorption of calcium does not occur and remineralisation of the maternal skeleton only starts after weaning when PTH, and 1,25(OH)2D levels rise, calcium absorption increases and urinary calcium levels fall, a process that may persist for months. Obviously, adequate stores of 25(OH)D are vital during this period, otherwise skeleton remineralisation will be seriously impaired and possibly result in the development of osteoporosis in old age.4,5

Interestingly, none of the patients in the 25(OH)D3 deficiency group were symptomatic and serum PTH and ALP levels were still within the normal range. However there was a significant increase of the percentage rise in PTH in the face of a declining 25(OH)D level suggesting the early stages of a biological response to vitamin D deficiency in the “at risk” group. In the deficiency group the reverse response was seen. Our 25(OH)D3 immunoassay results have been carefully checked and are correct. It is possible therefore that an overt rise of PTH and ALP levels has been suppressed by an oral intake of calcium in excess of 1 gm daily as this will delay the development of secondary hyperparathyroidism.12,13 Other factors such as body mass index may also be relevant but this was not examined here.

In addition to its musculo-skeletal actions, vitamin D deficiency is reportedly associated with the development of certain cancers, the metabolic syndromes and infections, as well as type 1 and type 2 diabetes,4,5 disorders which are common in Oman.

Our results confirm that vitamin D3 stores are low even in Omani of reproductive age. These findings are similar to those reported in Saudi Arabians more than 25 years ago and more recently in the UAE and Qatar.12 It therefore seems sensible to advocate vitamin D supplementation for all pregnant women in the Middle East. At the present time, there are no clear cut recommendations as to the dose, but we recommend at least 1000 IU of vitamin D3 a day which should be continued throughout lactation. Until more is known about the daily calcium intake of Omanis, it would be prudent to advocate calcium supplementation as well.15

### Conclusion

This study shows that vitamin D3 scores are low in pregnant Omani. Further studies are required to confirm these findings. Until then, we recommend supplementation with vitamin D3 (cholecalciferol) for all pregnant and lactating mothers.

### CONFLICT OF INTEREST

The authors reported no conflict of interest.

### References


Abstract: Objectives: Sunlight exposure has a vital role in vitamin D synthesis. Although vitamin D deficiency has been well documented in temperate zones, studies have been scarce in tropical countries where the population is well covered and for various reasons avoids sun exposure. The objective of this study was to investigate serum 25-hydroxyvitamin D [25(OH)D] levels and its relationship to biochemical bone profile, exposure to sunlight and vitamin D intake amongst Omani women of childbearing age. Methods: 41 apparently healthy women working at the Royal Hospital, Muscat, Oman and aged 18–45 years, with mean ± SD of 29 ± 6 years, were included in this study conducted in December 2006. They completed a questionnaire regarding the duration of sun exposure, food intake and type of clothing worn. Blood samples were collected from them and analysed for serum 25(OH)D, calcium, phosphate, alkaline phosphatase and parathyroid hormone levels. Results: All the women had a 25(OH)D level <50 nmol/L as the cut-off for deficiency. 25(OH)D levels were strongly correlated with the lack of sun exposure (r = 0.672, P < 0.001) and a significant correlation was also found between 25(OH)D level and food intake (r = 0.482, P < 0.01). Conclusion: Subclinical 25(OH)D deficiency may be prevalent amongst Omani women. Risk factors such as poor sunlight exposure should be addressed in women of childbearing age and, if increased sunlight exposure is not possible, oral supplementation should be considered to avoid all the consequence and complications of vitamin D deficiency. Keywords: Vitamin D deficiency; 25-hydroxyvitamin D; Women; Sunlight; Oman
Vitamins D is produced endogenously by the exposure of skin to sunlight, and absorbed from food containing or supplemented with vitamin D. When it penetrates the skin, ultraviolet light is converted from 7-dehydrocholesterol to vitamin D₃. Vitamin D from the skin or diet is metabolised in the liver to 25-hydroxyvitamin D [25(OH)D], then further metabolism occurs in the kidney to form the active form 1,25-dihydroxyvitamin D [1,25(OH)₂D]. The renal production of 1,25(OH)₂D is tightly regulated by plasma parathyroid hormone levels as well as serum calcium and phosphate levels. Deficiency of vitamin D results in rickets in children and osteomalacia, muscle weakness and increased risk of fractures in adults.

The discovery that most tissues and cells in the body have vitamin D receptors and some of them have the enzymatic capability to convert vitamin D to its active form, 1,25(OH)₂D, may explain the important role of vitamin D in skeletal and non-skeletal health. Vitamin D also plays a role in decreasing the risk of chronic conditions such as diabetes, cancer, and autoimmune, infectious and cardiovascular diseases.

Vitamin D deficiency is a well-recognised epidemic problem worldwide. Given the significant role of sunlight in vitamin D synthesis, it is quite logical to suggest a low prevalence of vitamin D deficiency in tropical countries. However, studies carried out in the last two decades have shown a high prevalence of vitamin D deficiency in many tropical countries such as Turkey, Iran, Saudi Arabia, United Arab Emirates, Kuwait, Bangladesh and Tunisia.

Vitamin D status has so far been poorly documented in the Omani population. The present study aimed to investigate vitamin D levels among Omani females of childbearing age where a sufficient level is important to prevent neonatal hypocalcaemia and impaired bone growth. This study also investigated the prevalence of vitamin D deficiency among Omani women and its relationship to sun exposure, food intake and type of clothing.

Methods

Forty-one apparently healthy women aged 18–45 years with (mean ± standard deviation (SD), 29 ± 6 years), working in various departments of the Royal Hospital, Muscat, Oman, volunteered for this study. Exclusion criteria included known hepatic, renal or gastrointestinal disease, pregnancy, lactation and medication influencing bone metabolism such as calcium or vitamin D supplements. The study was approved by the Research Ethical Committee at the Royal Hospital. Informed consent was taken from all subjects who also were asked to complete a questionnaire before blood collection. The study was conducted in December 2006 (winter-autumn in Oman) where the temperature ranges from 15 to 28 ºC.

All subjects completed a questionnaire on medical history, exposure to sunlight (estimated in minutes per week), skin type, sunscreen usage and type of clothing (i.e. exposure of hands and face). The questionnaire also estimated their dietary calcium and vitamin D intake, covering the commonest food preparations consumed by Omani. Data on frequency of consumption (daily, weekly, monthly) as well as quantities consumed were collected and analysed.

The relation of vitamin D levels, sun exposure, dietary intake and application of sun screen were assessed. The sun exposure was calculated from the questionnaire and expressed in minutes per week. The dietary intake was also calculated using different parameters. In the questionnaire, the frequency of food consumption was quantified per day, per week and per month. The amount of food, weight of the product, vitamin content in the product, total vitamin D intake in that product and finally the total vitamin D consumed were also calculated. Then, vitamin D amounts per product were calculated from different sources (UK tables, Danish tables, and product ingredient information and the subjects’ estimations for home cooked food).

Blood specimens were collected in Greiner Vacuette tubes (Greiner Bio-One Gmbh, Baden-Württemburg, Germany) from each subject for the Application to Patient Care

1. This study highlights important public health issues such as the need for adequate maternal vitamin D levels in pregnancy/lactation and the promotion of outdoor activities.
measurement of the following analytes: serum total calcium, phosphate, albumin, alkaline phosphatase (ALP), parathyroid hormone (PTH) and 25(OH)D. For PTH analysis, serum samples were separated within 30 minutes following blood collection and kept frozen until analysis. Serum total calcium, phosphate, ALP and albumin were measured by Synchron LX20 PRO (Beckman Coulter, Inc., CA, USA); PTH was measured by Access 2 immunoassay (Beckman Coulter, Inc., CA, USA) at the Clinical Biochemistry Laboratory in the Royal Hospital. Frozen samples unprotected from light were sent to Melbourne Pathology, Australia for the measurements of 25(OH)D using the LIAISON assay (DiaSorin, Inc., Minnesota, USA). This is a direct competitive chemiluminescent immunoassay for the quantitative measurement of total 25(OH)D. Using Biostat software, Spearman’s rank correlation coefficient was calculated for the correlation between serum 25(OH)D levels; sun exposure and dietary intake.\\n
Results

This study investigated vitamin D levels among childbearing Omani women and the prevalence of vitamin D deficiency and its relationship to sun exposure, food intake and type of clothing. The mean ± SD of age was 29 ± 6 years, 25(OH)D levels were 25 ± 6 nmol/L, vitamin D intake was 9 ± 4 ug/d and sun exposure was 70 ± 60 min/week. In all the subjects (100%), vitamin D levels were <50 nmol/L. The mean serum concentration of 25(OH)D in the subjects was 25nmol/L (results ranged from 12 to 37 nmol/L). In 20 women (49%), 25(OH)D levels were ≥25 nmol/L. In the remaining 21 women (51%) the level was <25 nmol/L. All subjects had vitamin D deficiency which varied from mild, to moderate and even severe deficiency using the recommended cut off <50 nmol/L as a low 25(OH)D.\\n
All except one subject had serum PTH, serum calcium and phosphate within the reference interval. No significant correlation between serum 25(OH)D levels and calcium levels or serum phosphate level was noted in the subjects investigated. The results of different biochemical bone markers are presented in Table 1.

The most significant correlation of the 25(OH)D level was with sun exposure, as all the subjects covered their whole body except the face and hands (r = 0.672, P <0.001) compared to the correlation with food intake (r = 0.482, P <0.01). The correlation between 25(OH)D and age was weak and not statistically significant (r = 0.211, P >0.05). Sun screen was used by 15 subjects (36%) and was only applied on the face and not on regular basis and no correlation was found between application of sun screen and 25(OH)D levels (r = -0.02, P >0.05).

As sun exposure had the strongest correlation, two cut offs were defined for sun exposure time to assess the relation between 25(OH)D and sun exposure time [Table 2]. With sun exposure at <60 min/week, the mean 25(OH)D and PTH were 27 nmol/L and 4.14 pmol/L respectively, while with sun exposure ≥ 60 min/week, the mean 25(OH)D and PTH were 29.5 nmol/L and 2.92 pmol/L respectively. The mean of serum calcium and phosphate was comparable in both subgroups [Table 3].

Discussion

This study reveals that 25(OH)D deficiency is likely to be very common among Omani adult women given the prevalence of 100% in the studied group. This may seem an unexpected result as Oman is one of the sunniest countries in the world where people would be expected to have adequate sun exposure. Although vitamin D deficiency is defined as serum 25(OH)D level <50nmol/L, an epidemiological survey-based recommended cut-off, this may not necessarily reflect the desirable level among Omani

Table 1: Biochemical bone markers in the study group (N = 41)

<table>
<thead>
<tr>
<th>Biochemical markers</th>
<th>Mean ± SD</th>
<th>Median (Range)</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total calcium (nmol/L)</td>
<td>2.31 ± 0.07</td>
<td>2.31 (2.18–2.43)</td>
<td>2.1–2.6</td>
</tr>
<tr>
<td>Phosphate (mmol/L)</td>
<td>1.23 ± 0.12</td>
<td>1.26 (0.92–1.57)</td>
<td>0.7–1.4</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>51.59 ± 13.63</td>
<td>48 (30–98)</td>
<td>30–125</td>
</tr>
<tr>
<td>PTH (pmol/L)</td>
<td>3.81 ± 2.06</td>
<td>3.6 (1.4–11.7)</td>
<td>1.6–9.3</td>
</tr>
<tr>
<td>25(OH)D (nmol/L)</td>
<td>27.61 ± 5.35</td>
<td>28 (19–37)</td>
<td>75–250</td>
</tr>
</tbody>
</table>

Legend: ALP = alkaline phosphatase; PTH = parathyroid hormone; 25(OH)D = serum 25-hydroxyvitamin D.
women.

The low vitamin D level may be due to several factors including direct and indirect avoidance of sunlight exposure due to cultural and social reasons (concern about appearance, unwillingness to change skin colour, the burning effect of the sun, and the risk of hot weather related sickness). Most of the women included in the study were working full-time so they were indoors for most of the day-time and, like the majority of Omanis, they anyway prefer only to be outdoors after sunset. Most of the women included in the study were asymptomatic; they did not suffer from bone pain, myalgia or tiredness and their biochemical markers were within the normal range.

It appears that the way people dress is not the only reason for the low vitamin D status. Islam et al. for example, showed that women in Bangladesh, regardless of their lifestyle and clothing, were at risk of developing vitamin D deficiency and that both veiled and unveiled women can have vitamin D deficiency.11

In this study, many women were aware about the need for a healthy diet containing high calcium intake and some were also aware of the importance of vitamin D for health. Hence, they were trying to compensate for this demand by having adequate calcium and vitamin D intake either by increasing the intake of fish and egg or using fortified food like margarine and milk which they know to be rich in these constituents. However, although in the study the adequate vitamin D intake was defined to be 5ug/day (200 IU/day) for those aged <50 year,17,20 surprisingly only 6 subjects (15%) had less than 5ug/day. The discrepancy may be due to coexisting vitamin D deficiency, malabsorption, overestimation of vitamin D content in the food consumed, (when using the UK and Danish tables), lack of information on labels of fortified food and wrong estimations for home cooked foods. The latter could have led to inter-individual variations in estimating vitamin D content for home cooked food. Hence, there is a concern about the subjects’ reliability and accuracy in estimating their food intake.

Although a significant correlation was observed between 25(OH)D and sun exposure, none of the subjects had enough sun exposure. The amount vitamin D produced by the human skin is proportional to the surface area of skin exposure to sunlight. All the subjects were known to be covered (except the face and hands), but at least 15–20% of body surface needs to be exposed to sun to provide the minimal erythemal dose of ultraviolet light that is sufficient for the first step in vitamin D synthesis (conversion of cholecalciferol from its precursors).17

The amount of sunlight exposure that is needed also varies depending on skin type, time of day, altitude and season. Since, for religious and cultural reasons, it is hard for Omani women to increase the body surface area that is exposed to sun to provide the minimal erythemal dose of ultraviolet light that is sufficient for the first step in vitamin D synthesis (conversion of cholecalciferol from its precursors). Special consideration is required for pregnant and lactating women in order avoid the perinatal and post-natal complications associated with low maternal vitamin D.

### Conclusion

A high prevalence of subclinical vitamin D

<table>
<thead>
<tr>
<th>Biochemical bone markers</th>
<th>Sun exposure</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≥60 min/week</td>
<td>&lt;60min/week</td>
</tr>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Median (range)</td>
</tr>
<tr>
<td>Total calcium (mmol/L)</td>
<td>2.32 ± 0.007</td>
<td>2.33 (2.19–2.42)</td>
</tr>
<tr>
<td>Phosphate (mmol/L)</td>
<td>1.20 ± 0.10</td>
<td>1.23 (1.01–1.31)</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>39.18 ± 9.11</td>
<td>48 (38–69)</td>
</tr>
<tr>
<td>PTH (pmol/L)</td>
<td>2.92 ± 1.05</td>
<td>2.60 (1.7–4.8)</td>
</tr>
<tr>
<td>25(OH)D (nmol/L)</td>
<td>28.7 ± 5.33</td>
<td>29.50 (20–37)</td>
</tr>
</tbody>
</table>

Legend: ALP = alkaline phosphatase; PTH = parathyroid hormone; 25(OH)D = serum 25-hydroxyvitamin D.
deficiency amongst Omani females of childbearing age has been observed in this population. Sunlight is major contributor to 25(OH)D levels, but their exposure still appears to be insufficient. If the subjects can not improve their sunlight exposure, they should take oral vitamin D supplements. Further studies may be recommended to determine the incidence of osteomalacia and osteoporosis in the Omani population and assess vitamin D status in patients with chronic diseases.

ACKNOWLEDGEMENTS

The author would like to thank all women who volunteered to take part in this study. She would also like to thank Dr Ken Sikaris (Melbourne Pathology, Australia), Dr Waad-Allah Mula-Abed (Royal Hospital, Oman), and the staff of the clinical biochemistry laboratories at both Melbourne Pathology, Australia, and the Royal Hospital, Oman.

CONFLICT OF INTEREST

The author reported no conflict of interest.

References

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Table 3. Biochemical bone markers according to 25 (OH) D levels. Data are presented as mean ± standard deviation (median)

<table>
<thead>
<tr>
<th>Biochemical bone markers</th>
<th>25(OH)D</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total calcium (mmol/L)</td>
<td>&lt;25 nmol/L: 2.31 ± 0.06 (2.30)</td>
<td>≥ 25 nmol/L: 2.31 ± 0.06 (2.32)</td>
</tr>
<tr>
<td>Phosphate (mmol/L)</td>
<td>1.22 ± 0.11 (1.22)</td>
<td>1.24 ± 0.15 (1.28) (0.92–1.57)</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>48.2 ± 12.75 (45)</td>
<td>54.15 ± 12.73 (51)</td>
</tr>
<tr>
<td>PTH (pmol/L)</td>
<td>4.38 ± 2.52 (4.30)</td>
<td>3.68 ± 1.83 (3.10)</td>
</tr>
<tr>
<td>25(OH)D (nmol/L)</td>
<td>19.47 ± 1.77 (19)</td>
<td>30.35 ± 3.48 (30)</td>
</tr>
</tbody>
</table>

Legend: ALP= alkaline phosphatase; PTH= parathyroid hormone.


Abstract: Objectives: The use of complementary and alternative medicine (CAM) for diabetes mellitus is becoming increasingly popular; however, little is known about the prevalence of CAM use in patients with diabetes mellitus in Oman. The objectives of this study were to estimate the prevalence of use of CAM among diabetic patients in Muscat region, Oman, and to determine the types of CAM used as well as to identify the demographic features influencing the use of CAM.

Methods: The study was performed from May to August 2009 on diabetic patients from 4 health centres in Muscat region. A total of 146 patients were interviewed. Information was obtained on demographics, and the prevalence and pattern of use of CAM.

Results: Sixty two (42%) of the participants used CAM for the treatment of diabetes. Thirty (48%) were satisfied about its use and 27 (43%) intend to use it again. The only types of CAM used by participants in this study were herbs (n = 49, 79%), and/or food supplements (n = 7, 11%). Family and friends (n = 47/62, 76%) and/or traditional healers (n = 19, 31%) were the main source of information on CAM in the treatment of diabetes. There was no significant correlation between demographic characteristics and the use of CAM for diabetes.

Conclusion: CAM is used widely for diabetes in Muscat region, Oman. Patients have strong faith in CAM in terms of effectiveness. Doctors should recognise this and be prepared to talk more freely with patients about its use and potential side effects.

Keywords: Complementary medicine; Alternative medicine; Complementary and alternative medicine (CAM); Diabetes mellitus (DM); Oman

Advances in Knowledge
1. This study is the first in Oman to explore the use of complementary and alternative medicine (CAM) in patients with diabetes. It is the key for further research in this field with the hope of improving management of people with diabetes.

2. This study has shown how common the use of CAM is in Oman.

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Man has a long history of using traditional and herbal remedies; however, few studies have been conducted in the country.1,2,3 These remedies are supplied by traditional healers, or self-obtained, and are administered in the management of a wide variety of both acute and chronic conditions. Despite the extensive development of the health care system in Oman and the availability and accessibility of free health facilities and drugs, traditional medicine is still widely used.

Traditional medicine, or complementary and/or alternative medicine (CAM) refers to “health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being.”4 CAM has maintained its popularity in all regions of the developing world and its use is rapidly spreading in industrialised countries.5 For instance, in Africa, 80% of the population uses traditional medicine to help meet their health care needs.6 In Europe, North America and other industrialised regions, over 50% of the population have used CAM at least once in their lives.7

The use of CAM among diabetics is common. A recent review of 18 studies from 9 countries showed that the prevalence of CAM use among patients with diabetes varied from 17% to 72.8%. Most of the studies reviewed were conducted in developed countries and the majority of papers were derived from the USA and Australia.8 In developing countries, such as Saudi Arabia, 17.4% of patients with diabetes in Riyadh9 and 30% in Mecca10 used some forms of herbs. In the United Arab Emirates, 76% of patients with diabetes had previously used herbs and 38% were currently using some forms of CAM.11 In Bahrain, 63% of patients with diabetes had used CAM within the previous 12 months.12

Of all chronic diseases, diabetes mellitus has emerged as a major and growing health problem in Oman. The Oman National Health Survey of Diabetics, conducted in 1991, showed that the prevalence of diabetes is 9.75%, while the follow-up survey in the year 2000 showed an increase in the prevalence to 11.6% among adults over 20 years of age.13-15 The prevalence estimate of diabetes mellitus in Oman by the International Diabetes Federation (IDF) in 2010 is 13.4% and is expected to increase in the next 25 years.16

This makes diabetes the second most common cause of morbidity in males and females above the age of 45 years and the fourth highest cause of death.17 To our knowledge, no studies have yet emerged from Oman on the use of CAM by patients with diabetes. Therefore, this study was conducted to determine the prevalence of CAM use among patients with diabetes mellitus in the Muscat region and to reveal the types of CAM most commonly used as well as the factors contributing to their use in terms of demographic characteristics and disease features.

Methods

A multi-centric, cross sectional study was conducted over a 4-month period from May to August 2009 in four health centres in the Muscat region namely Ruwi, Wadi Al-Kabir, South Mawaleh, and Al-Khoudh. Each of these health centres serves a population of approximately 500 patients with diabetes. The diabetic clinics in these health centres are well developed in terms of availability of a diabetic register, appointment system, trained family physicians and a wide range of modern...
Complementary and Alternative Medicine Use among Adults with Diabetes in Muscat Region, Oman

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pharmacological anti-diabetic medications.

The target population of this study was patients with diabetes mellitus attending primary health care facilities. All patients (N = 146) attending the diabetes or general clinics in these health centres during the study period were enrolled in the study. The following people were excluded: patients who did not speak Arabic or English; those with dementia or learning difficulties; those with no time to complete the questionnaire; people who visited the health centres for purposes other than patient care, and those who refused to give consent.

Face-to-face interviews were conducted by trained family medicine residents using a pre-coded and pre-tested questionnaire that was developed by the authors based on a pilot study and previous similar studies to assess the use of CAM by patients with diabetes.9-12 The types of CAM used were defined based on the US National Center for Complementary and Alternative Medicine (NCCAM) categorisation of CAM.18 The questionnaire was translated into Arabic and the interview was conducted in Arabic. Inquiry was made into demographic characteristics and type and duration of diabetes. In addition, records were reviewed to determine the presence of complications and co-morbidities, treatment, and the status of blood glucose levels during the three months prior to the study. A glycosylated haemoglobin level of <7% was taken to reflect a controlled blood glucose level.

Participants were also asked whether they had used CAM in general, and in particular for the treatment of diabetes. Among users of CAM, detailed information was obtained on: CAM use during the previous year; current use; type and frequency of use; source of information about CAM; extent of satisfaction and side effects (if any); use with prescribed medication/s; whether information on CAM use had been given to the treating physician, and intention of using CAM again. Finally, interviewees were asked to give their opinion on the safety and efficacy of CAM compared to modern medicine.

Descriptive statistics were used to describe the data. For categorical variables, frequencies and percentages were reported. Differences between groups (CAM status, yes/no) were analysed using Pearson’s χ² tests (or Fisher’s exact tests for cells less than 5). For continuous variables, mean and standard deviation were used to present the data while analysis was performed using the student’s t-test. An a priori two-tailed level of significance was set at the 0.05 level. Statistical analyses were conducted using STATA, Version 11.0 (STATA Corporation, College Station, TX, USA).

Informed consent was obtained from all participants before the interview. Ethical approval for the study was granted in 2009 by the Medical Research & Ethics Committee of the College of Medicine & Health Sciences at Sultan Qaboos University, Oman.

Results

This study included a total of 146 patients with diabetes mellitus type 1 (n = 6, 4%) and type 2 (n = 140, 96%). The majority of patients (n = 136, 93%) were Omani nationals. More than half of these patients were women (n = 84, 58%), and in the age group 46 to 65 years (n = 82, 56%) [Table 1].

The duration of diabetes ranged from 2 months to 40 years (mean 8.48 ± 6.5 years). Nearly a third of the patients (n = 56, 38%) reported the presence of one or more complications including retinopathy (n = 21, 14%), ischaemic heart diseases (n = 18, 12%), nephropathy (n = 17, 12%), neuropathy (n = 11, 8%), and transient ischaemic attacks and strokes (n = 4, 3%). Co-morbidities were reported by 105 (72%) of the patients including hypertension (n = 76, 52%), dyslipidemia (n = 17, 12%), gastrointestinal diseases (n = 14, 10%), joint diseases (n = 13, 9%) and mood disorders (n = 44, 30%). A review of the records for these patient for the 3 months prior to the study revealed that 109 (75%) of the patients had uncontrolled blood glucose levels.

Table 1 reveals that users and non-users of CAM for the treatment of diabetes are comparable in regard to their demographic and clinical characteristics. The ever use of CAM was reported by 76 (52%) of patients. A total of 42% (n = 62) of those who had ever used CAM had used it specifically for the treatment of diabetes. Just over a quarter of these patients (n = 41, 28%) had used CAM within the last 12 months and 28 (19%) were current users. The main types of CAM used were herbal remedies (n = 49/62, 79%) and/or food supplements (n = 7/62, 11%). Most herbal remedies were in mixed/compounded forms and more than half of the patients (n = 36/62, 58%) had used...
several types of herbal remedies. “Harmel” (*Rhazya stricta*) (n = 6/62, 10%), fenugreek (*Trigonella foenum*, Arabic “helba”) (n = 5/62, 8%) and black seeds (*Nigella sativa*) (n = 4/62, 6%) were the most commonly used.

Among those who used CAM for the treatment of diabetes, 48% (n = 30) expressed satisfaction with its use and 44% (n = 27) stated that they intend to use it again. In addition, 23% (n = 14) perceived CAM as more effective than modern medicine and 27% (n = 17) as safer. Interestingly, 45% (n = 28) of the participants were taking herbal remedies concurrently with their Western anti-diabetic medications and only 13% (n = 8) of them had informed their treating physicians about CAM use. The main source of information on CAM for the treatment of diabetes was family and friends (n = 47, 76%) and/or traditional healers (n = 19, 31%). The self-reported rate for adverse events associated with CAM use was 13% (n = 8).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total frequency (N = 146)</th>
<th>Use of complementary and alternative medicine in diabetic patients</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Yes n = 62 (42%)</td>
<td>No n = 84 (58 %)</td>
</tr>
<tr>
<td>Age, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21–34</td>
<td>8 (5%)</td>
<td>2 (3%)</td>
<td>6 (7%)</td>
</tr>
<tr>
<td>35–45</td>
<td>32 (22%)</td>
<td>16 (26%)</td>
<td>16 (19%)</td>
</tr>
<tr>
<td>46–55</td>
<td>38 (26%)</td>
<td>18 (29%)</td>
<td>20 (24%)</td>
</tr>
<tr>
<td>56–65</td>
<td>44 (30%)</td>
<td>18 (29%)</td>
<td>26 (31%)</td>
</tr>
<tr>
<td>66–88</td>
<td>24 (16%)</td>
<td>8 (13%)</td>
<td>16 (19%)</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>84 (58%)</td>
<td>32 (52%)</td>
<td>52 (62%)</td>
</tr>
<tr>
<td>Married</td>
<td>123 (84%)</td>
<td>52 (84%)</td>
<td>71 (84%)</td>
</tr>
<tr>
<td>Education, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>63 (43%)</td>
<td>23 (37%)</td>
<td>40 (48%)</td>
</tr>
<tr>
<td>School</td>
<td>58 (40%)</td>
<td>30 (48%)</td>
<td>28 (33%)</td>
</tr>
<tr>
<td>Post-school</td>
<td>25 (17%)</td>
<td>9 (15%)</td>
<td>16 (19%)</td>
</tr>
<tr>
<td>Nationality, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Omani</td>
<td>136 (93%)</td>
<td>60 (97%)</td>
<td>76 (90%)</td>
</tr>
<tr>
<td>Diabetes mellitus (DM) type, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM Type 2</td>
<td>140 (96%)</td>
<td>60 (97%)</td>
<td>80 (95%)</td>
</tr>
<tr>
<td>Duration of DM, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5 years</td>
<td>42 (29%)</td>
<td>21 (34%)</td>
<td>21 (25%)</td>
</tr>
<tr>
<td>5-7 years</td>
<td>34 (23%)</td>
<td>9 (15%)</td>
<td>25 (30%)</td>
</tr>
<tr>
<td>8-10 years</td>
<td>37 (25%)</td>
<td>17 (27%)</td>
<td>20 (24%)</td>
</tr>
<tr>
<td>&gt;10 years</td>
<td>33 (23%)</td>
<td>15 (24%)</td>
<td>18 (21%)</td>
</tr>
<tr>
<td>Control of blood glucose (&lt;7% HbA1c), n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controlled</td>
<td>37 (25%)</td>
<td>14 (23%)</td>
<td>23 (27%)</td>
</tr>
<tr>
<td>Complications* present, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>56 (38%)</td>
<td>22 (35%)</td>
<td>34 (40.48)</td>
</tr>
<tr>
<td>Concurrent diseases present, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>105 (72%)</td>
<td>47 (76%)</td>
<td>58 (69%)</td>
</tr>
</tbody>
</table>

*Complications include retinopathy, nephropathy, neuropathy, ischaemic heart diseases, transient ischaemic attacks and stroke.*
Discussion

The present study has shown that 42% of the diabetic patients had used CAM in the treatment of diabetes and 28% had used CAM in the previous 12 months. This is comparable with other similar studies in the Gulf States such as Saudi Arabia (30%), the United Arab Emirates (38%), and Bahrain (63%) as well as in the USA (73%), and India (68%), but it is higher than in Australia (24%) and the UK (17%). This variation can be due to the use of different CAM definitions and the different timeframes of the studies as well as to different cultures. In our study, the types of CAM used were defined based on the NCCAM categorisation of CAM.

There was no significant association between the use of CAM and the socio-demographic or clinical characteristics of the participants. Furthermore, the use of CAM was not associated with either blood glucose level, glycosylated haemoglobin A1C control or the presence of complications or concurrent illnesses. This could be due to the small sample size and the fact that the participants were health centre patients. This is particularly important as patients in the community may be using CAM more frequently and the way they use it may be different. The common types of CAM used were herbal remedies and food supplements. The composition of most of these local remedies is not known as they are passed on from generation to generation and purchased ready made from the traditional healers or market (souk) outlets. Most of the patients had listed a variety of herbal mixtures and a few specified the name of the herbs used like harmel, fenugreek, and black seeds. The number of people taking herbal and modern medicines at the same time was high. The potential for adverse interactions with modern medicines is therefore very likely. This issue needs to be recognised by physicians to ensure that patients are not putting themselves in unnecessary danger. An appreciable number of people reported adverse effects while taking herbal products; however, it is almost impossible to establish causality because most of them took mixed herbs and the survey relied mainly on their recall.

Participants relied heavily on family and friends for advice on CAM use. Health education on this matter should be simultaneously provided to patients and their families. Moreover, education is recommended to inform their doctors about its use since the majority of patients did not inform their doctors, while simultaneously almost half expressed satisfaction with CAM use and intended to use it again. Patients may be worried regarding the negative attitude of doctors towards the use of CAM so they do not inform their doctors about it. Therefore, a more positive attitude from doctors may encourage patients to talk more freely regarding their use of CAM.

Participants had a strong faith in herbal medicines with regard to their effectiveness and safety. Nearly 25% of respondents indicated that CAM is more effective and safer than modern medicine in treating diabetes. This result will have important health implications if confirmed on a larger sample. Over-reliance on ineffective herbal remedies could lead to people either refraining from using or delaying the use of more effective modern medicine. This study also shows that CAM users have higher expectations of CAM than they do of conventional therapies. Therefore, physicians should recognise their patients’ underlying desire for improving their health status and be able to advise patients on the use of CAM.

Faith in CAM use is probably part of national heritage. These products have been used for generations with no apparent harm. This view is reinforced by family and friends who are the main influences on respondents’ decisions to use CAM. This, however, is not culturally unique as other studies in the region have shown that family and friends are intensely involved in decision making regarding the use of herbal medicine. Many researches have studied the anti-diabetic effects of some herbs and plants. However, the safety and efficacy of these herbal treatments are still to be determined. Patients may be putting themselves at risk by the use of these treatments. Some herbal products contain powerful substances that can be toxic either alone or in combination with other medications. The most important risk is that CAM is used as a true alternative to conventional treatments for serious medical conditions. In addition, there is no control over the quality of these products, which can be easily purchased in special outlets in the market (souk). Herbal remedies are widely considered to be inexpensive, but this is often not the case. This specific aspect of CAM use needs to be studied in a proper clinical setting.
With the increasing importance of CAM in modern health care, medical and nursing education should include information about complementary practices. Physicians will be increasingly expected to address issues related to CAM use, but may not be able to become knowledgeable about all CAM practices. However, they can apply the principles of evidence-based medicine to CAM as to any area of health care. Physicians can search the published medical literature and evaluate the applicability of CAM for specific patient problems.

The study has several limitations. The real percentage of CAM use in the treatment of diabetes might be found to be higher than that reported if the duration of the study were extended, and especially if extended to the community level. This is particularly important as patients in the community may be using clinical services less frequently and the way they use CAM may be different. This study was conducted in only one region and the results may not be generalisable to diabetic patients in other regions of Oman or to the entire country.

In addition, this study did not investigate the objective effectiveness of CAM on diabetes, such as showing that patients’ blood glucose was not controlled on their conventional therapy, but became controlled when CAM was added or substituted. Further research will be required involving many regions and to obtain data on any health benefits achieved through CAM usage.

Conclusion
This study found out that many diabetic patients used CAM and most of those patients used herbal remedies. Family and friends played a significant role as sources of CAM information. Many patients did not inform their doctors about CAM use, taking them simultaneously with anti-diabetic medications, and had the intention to use them again. Doctors should recognise that CAM is widely used by diabetic patients and should appreciate that these medicines can cause adverse effects. Doctors should therefore be prepared to question their patients and try to encourage them to talk about their use of CAM as it may affect the outcome and the management of their disease.

CONFLICT OF INTEREST
The authors reported no conflict of interest.

ACKNOWLEDGMENT
The authors are grateful to Dr Randa Yousif, for her constructive review and comments.

References
Indicators of Renal Glomerular and Tubular Functions in Patients with Beta-Thalassaemia Major
A cross sectional study at the Royal Hospital, Oman

*Waad-Allah S Mula-Abed,1 Huda S Al-Hashmi,1 Muhanna N Al-Muslahi2

Abstract: Objectives: There are limited data concerning the assessment of renal function in beta-thalassaemia major, with no study of such involvement in Omani patients. The objective of this study was to establish the pattern of renal glomerular and tubular function using traditional and specific laboratory tests in patients with beta-thalassaemia major. Methods: This cross-sectional study, from January–July 2008, included 30 patients of the Thalassaemia Clinic at the Royal Hospital, Oman, with transfusion-dependent homozygous beta-thalassaemia major. They included 15 males and 15 females, aged 16-32 years with mean ± standard deviation of 21.23 ± 3.42 years. The medical records were reviewed and renal function states assessed as follows: serum creatinine, estimated glomerular filtration rate (eGFR); urea; phosphate, fractional excretion of filtered sodium (FENa); urine albumin: creatinine index; urine 62-microglobulin:creatinine index; tubular reabsorption of phosphate (TRP), and tubular maximum phosphate reabsorption (TmP) GFR. Results: All patients had eGFR >90 ml/min/1.73m2; serum creatinine <90 μmol/L; serum urea <6.0 mmol/L, and urine albumin:creatinine <2.5 μg/mmol. Only 2 (6.7%) patients had FENa >1% and 3 (10.0%) patients had urine 62-microglobulin: creatinine >22 μg/mmol. All patients had TRP >0.85, of whom seven (23.3%) patients had values within the range of 0.85-0.95 and 23 (76.7%) had values

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Haeimoglobinopathies are a common medical problem worldwide including in Oman. A recent prospective neonatal screening programme in two major cities of Oman, which included consecutive cord blood samples from a total of 7,837 neonates, revealed that the overall incidence of alpha-thalassaemia (alpha-thal) was 48.5%. Beta-globin-related abnormalities accounted for 9.5% of the samples (4.8% sickle cell trait, 2.6% beta-thalassaemia trait, 0.9% haemoglobin (Hb) E trait, 0.8% Hb D trait, 0.08% Hb C trait, 0.3% sickle cell disease and 0.08% homozygous beta-thalassaemia). Also, data from an earlier community-based survey of the most common genetic blood disorders among Omani children reported a prevalence rate of 2% for beta-thalassaemia trait, 0.07% for beta-thalassaemia major, 6% for sickle cell trait and 0.2% for sickle cell disease. Comparable data has also been reported from Gulf Cooperation Council states and other neighbouring countries.

Beta-thalassaemia major is an inherited monogenic disorder that was first described in 1925 by Cooley and Lee. It is caused by a mutation at the β-globin gene locus resulting in persistence of α-globin chain that is precipitated within the erythroid precursors in the bone marrow associated with severe dyserythropoietic anaemia. The combination of early diagnosis, improvement in monitoring complications and advances in supportive therapy has enabled patients with thalassaemia major to have improved life expectancy. The cornerstone of management is life-long blood transfusion with frequent iron chelation therapy to minimise the deleterious effect of chronic iron deposition and its accumulation in tissues. Despite this, these patients are prone to long-term organ dysfunction particularly in their cardiovascular, hepato-biliary, endocrine and skeletal systems. The renal system, particularly the kidneys, also undergoes pathophysiological changes and may be affected by the burden of iron and the chelation therapy. This is the first study of the assessment of laboratory-based renal function tests in patients with beta-thalassaemia major in Oman.

The objective of this study was to evaluate the degree of renal dysfunction, both glomerular and tubular, using traditional and specific laboratory tests of renal function in adult Omani patients with transfusion-dependent homozygous beta-thalassaemia major at the Royal Hospital, Oman.

>0.95. Also, all patients had TmP/GFR >1.0 mmol/L, of whom only one (3.3%) patient had TmP/GFR of 1.0–1.5, and 29 (96.7%) patients had TmP/GFR >1.5 mmol/L. Finally, 24 (80%) patients had serum phosphate >1.4 mmol/L. Linear regression revealed a highly significant correlation between serum phosphate and TmP/GFR (r = 0.904, P < 0.001). Conclusion: Renal function, glomerular and tubular, appears to be well preserved in beta-thalassaemia major. Almost all renal function indicators were within the recommended ranges. Raised TmP/GFR and TRP were noted in the majority of patients, reflecting an up-trend in serum phosphate and therefore increasing renal phosphate reabsorption.

**Keywords:** Beta-thalassaemia; Glomerular; Tubular; Renal function; Oman

**ADVANCES IN KNOWLEDGE**

1. The cornerstone in the management of patients with beta-thalassaemia major is life-long blood transfusion with frequent iron chelation therapy. Despite this, these patients are prone to long-term organ dysfunction particularly in their cardiovascular, hepato-biliary, endocrine and skeletal systems. The renal system, particularly the kidneys, also undergoes pathophysiological changes and may be affected by the burden of iron and the chelation therapy. This is the first study of the assessment of laboratory-based renal function tests in patients with beta-thalassaemia major in Oman.

2. Glomerular tests are usually within the reference ranges, while alteration in certain tubular tests (such as raised TRP and TmP/GFR) may reflect an upward trend in serum phosphate and, as a consequence, in increasing renal phosphate reabsorption. Otherwise, renal function appeared to be well preserved with no overt renal disease.

**APPLICATION TO PATIENT CARE**

1. This study helps health care professionals to select and interpret renal function tests in patients with beta-thalassaemia major using glomerular indicators (eGFR, serum creatinine, urine microalbumin) and tubular indicators (FENa, β2-microglobulinuria, TRP and TmP/GFR).
Methods

This cross-sectional study was conducted during a six months period (1 January to 31 July 2008) and covered adult Omani patients with transfusion-dependent homozygous beta-thalassaemia major registered with the Thalassaemia Clinic at the Royal Hospital, Oman. This is one of a number of thalassaemia clinics in Oman which together deal patients with this disease. The study included 30 patients (15 male, 15 female), aged 16–32 years who were seen at the clinic at 3 monthly intervals. The diagnosis of homozygous thalassaemia was based on the characteristic haematological criteria (peripheral blood evaluation and haemoglobinopathy screening) at presentation or screening from the early years of life.

The study protocol was a naturalistic observation, an integral part of routine clinical procedure through reviewing the medical records of these thalassaemic patients from the hospital computer records including the haematologists’ clinical review as well as results of laboratory investigations. The clinical haematologists are regularly consulted on the management of these patients which includes supervision of blood transfusion and chelation therapy, as well as monitoring of organ dysfunction due to predicted iron deposition in tissues. The patients had been regularly transfused every three weeks since the early years of life with packed red blood cells, and were regularly taking iron chelators such as desferrioxamine (40 mg/kg body weight) as subcutaneous infusions 5 days per week, and deferiprone (75 mg/kg body weight) tablets daily.

For the laboratory investigations, blood samples were collected in the fasting state, and early morning urine specimens were provided from all the patients during their visits to the clinic. In addition to complete blood count and serum ferritin, different biochemical function profiles are regularly done to screen for any possible dysfunction. Evaluation of renal function is usually performed, together with other core function profile tests. The biochemical tests included: serum creatinine, and creatinine-based estimated glomerular filtration rate (eGFR); urea; phosphate as well as fractional excretion of filtered sodium (FENa); urine albumin: creatinine index (for microalbuminuria); urine β2-microglobulin: creatinine index (for β2-microglobulinuria); tubular reabsorption of phosphate (TRP), and tubular maximum phosphate reabsorption/GFR (TmP/GFR). All these parameters were measured in the Clinical Biochemistry Laboratory at the Royal Hospital. Creatinine was analysed by kinetic Jaffe reaction; urea and sodium by ion-selective electrodes; phosphate by molybdenum blue, and albumin by the immunoturbidimetric method (all from Synchron LX20, Beckman Coulter, Inc, CA, USA), and β2-microglobulin by chemiluminescent microparticle immunoassay method on AXSym (Abbott, USA).12 Urinary ratios of microalbumin and β2-microglobulin in relation to urine creatinine, as well as FENa were calculated. The eGFR was calculated by the Modification of Diet in Renal Disease (MDRD) equation13 and TRP as well as TmP/GFR were calculated as recommended by Payne.14 The reference ranges for the concerned biochemical parameters were as follows: serum creatinine (female 40–90 µmol/L; male 50–100 µmol/L); eGFR (>90 ml/min/1.73m²); serum urea (3.0–6.0 mmol/L); serum phosphate (0.7–1.4 mmol/L); FENa (<1%); urine albumin: creatinine ratio (female < 3.5 mg/mmol, male < 2.5 mg/mmol); urine β2 microglobulin: creatinine ratio (<22 µg/mmol); TRP (0.85–0.95), and TmP/GFR (1.0–1.5 mmol/L).

The results were analysed and numerical data presented as mean ± standard deviation (SD) and range. ANOVA (analysis of variance) was used to compare the differences in the means of results between the different groups. Pearson’s correlation coefficient was also used to compare between certain parameters where appropriate, which include serum phosphate and TmP/GFR. The statistical significance was assigned at \( P < 0.05 \).15

Results

Thirty homozygous β-thalassaemia patients (15 male, 15 female), aged 16–32 years, mean 21.23 years, SD of ± 3.42, were evaluated in this study. The recommended cut-off for the studied renal function tests include: eGFR >90 ml/ min/1.73m²; serum creatinine <90 µmol/L (in females) and <100 µmol (in males); serum urea <6.0 mmol/L; serum phosphate <1.40 mmol/L; FENa <1.0 %; urine albumin: creatinine <3.5 mg/mmol (in females) and <2.5 mg/mmol (in males); urine β2micglobulin: creatinine
The results of the renal function tests, glomerular and tubular, are shown in Table 1. For eGFR, and due to the possible effect of imprecision and analytical bias in creatinine assays at concentrations within the reference range, it has been recommended to report eGFR values higher than 90 ml/min/1.73m^2 as >90 ml/min/1.73m^2 with no numerical value. Hence, statistical data was not applied for eGFR results when calculated eGFR >90 ml/min/1.73m^2. All thirty patients had eGFR >90 ml/min/1.73m^2; serum creatinine <90 µmol/L (all group median 43, range 23–73 µmol/L); serum urea <6.0 mmol/L (median 3.3, range 1.3–5.6 mmol/L), and urine albumin:creatinine <2.5 mg/mmol (median 0.5, range 0.1–1.7 mg/mmol). Only two (6.7%) patients had FENa >1% (median 0.30, range 0.06–1.35%), and three (10%) patients had urine ß2-microglobulin:creatinine >22 µg/mmol (median 7.1, range 1.14–60.4 µg/mmol).

Concerning renal phosphate handling, all patients had TRP >0.85 (all group median 0.97, range 0.91–0.99), and TmP/GFR >1.0 mmol/L (median 2.01, range 1.15–4.48 mmol/L). Further characterisation of the results revealed that seven (23.3%) patients had TRP within the recommended reference range of 0.85–0.95 and 23 (76.7%) patients had values >0.95, with no patient having TRP <0.85. For TmP/GFR, only one (3.3%) patient had TmP/GFR within the reference range of 1.0–1.5 mmol/L, and 29 (96.7%) patients had raised TmP/GFR >1.5 mmol/L; 13 (43.3%) patients had values 1.51–2.00 mmol/L, nine (30%) had values 2.01–2.50 mmol/L, five (16.7%) had values 2.5–3.0 mmol/L and two (6.7%) had values >3.0 mmol/L; no patient had TmP/GFR <1.0 mmol/L. Also, six (20%) and 24 (80%) patients had serum phosphate ≤1.4 and >1.4 mmol/L respectively (median 1.66, range 1.10–3.14 mmol/L). A linear regression analysis revealed a highly significant correlation between serum phosphate and TmP/GFR (r = 0.904, P <0.001) [Figure 1].

In order to compare the parameters of phosphate handling, the results were also assessed in relation to the endocrine complications that were previously reported and reviewed in these patients. The patients were grouped as those with ≥ one endocrinopathy, n = 22; hypogonadism alone, n = 12; hypogonadism with diabetes mellitus, n = 7; with primary hypoparathyroidism, n = 1; with hypoparathyroidism and diabetes mellitus, n = 1; and with hypoparathyroidism and hypothyroidism, n = 1, and those with no endocrinopathy, n = 8. A significant difference (P <0.005) was noted in TmP/GFR (2.456 ± 0.69 versus 1.818 ± 0.255 mmol/L) and in serum phosphate <22 µg/mmol; TRP 0.85–0.95, and TmP/GFR 1.0–1.5 mmol/L.

Table 1: Indicators of renal glomerular and tubular function tests in 30 patients with homozygous beta-thalassaemia.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD of Results</th>
<th>No. and (%) of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal Values</td>
<td>Abnormal Values*</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73 m^2)</td>
<td>&gt; 90</td>
<td>-</td>
</tr>
<tr>
<td>Serum creatinine (µmol/L)</td>
<td>44.4 ± 10</td>
<td>-</td>
</tr>
<tr>
<td>Serum urea (mmol/L)</td>
<td>3.4 ± 1.0</td>
<td>-</td>
</tr>
<tr>
<td>Serum phosphate (mmol/L)</td>
<td>1.33 ± 0.12</td>
<td>1.91 ± 0.39</td>
</tr>
<tr>
<td>Urine albumin:creatinine (mg/mmol)</td>
<td>0.69 ± 0.5</td>
<td>-</td>
</tr>
<tr>
<td>FENa (%) **</td>
<td>0.35 ± 0.22</td>
<td>1.24 ± 0.14</td>
</tr>
<tr>
<td>Urine ß2.microglobulin:creatinine (µg/mmol) **</td>
<td>7.1 ± 4.9</td>
<td>39.3 ± 18.3</td>
</tr>
<tr>
<td>TRP ***</td>
<td>0.96 ± 0.02</td>
<td>-</td>
</tr>
<tr>
<td>TmP/GFR *** (mmol/L)</td>
<td>2.28 ± 0.646</td>
<td>-</td>
</tr>
</tbody>
</table>

Notes

* All patients marked as (-) had values within the reference ranges with no patient exceeded the cut-off for the renal function test
** Only 2 patients had high results for FENa and 3 patients had high results for urine ß2-microglobulin:creatinine; statistical data are not presented as the number is low
*** Abnormal values indicate high values for all parameters, except for TRP and TmP/GFR which are indicated by low values

Legend: eGFR = estimated glomerular filtration rate; FENa = fractional excretion of filtered sodium; TRP = tubular reabsorption of phosphate; TmP/GFR = tubular maximum phosphate reabsorption/gglomerular filtration rate.

<ref>Indian Medical Journal, February 2011, Volume 11, Issue 1</ref>
phosphate (1.928 ± 0.42 versus 1.46 ± 0.18 mmol/L); however, no significant difference was noted in TRP (0.962 ± 0.021 versus 0.960 ± 0.025) in patients with and without endocrinopathy respectively. The three patients with primary hypoparathyroidism had the highest TmP/GFR values (4.48, 3.36, and 2.32 mmol/L). No significant difference was noted in serum ferritin between these two groups (6480 ± 3418.6 versus 5647 ± 3663 µg/L).

Discussion

Patients with beta-thalassaemia major are prone to metabolic complications, including different organ dysfunction which can occur as single or multiple involvements. Although the actual mechanism is not definitive, the most likely explanation is related to anaemia and iron overload, in addition to lipid peroxidation, oxidative stress and free radical release.18

In this study, all 30 beta-thalassaemia major patients had normal glomerular renal function tests as indicated by their normal levels of eGFR >90 ml/min/1.73m²; serum creatinine <90 µmol/L; serum urea <6.0 mmol/L and urine albumin: creatinine <2.5 mg/mmol. Despite the increased iron deposition and the consequence of its possible oxidative stress on the renal parenchyma of these patients, many workers have also reported normal serum creatinine and creatinine clearance in patients with beta-thalassaemia major.19 The absence of microalbuminuria in our patients is another reflector of intact renal glomerular function. Microalbuminuria is an early indicator of nephropathy in patients at risk of developing kidney involvement, such as those with underlying chronic diseases including diabetes mellitus, hypertension or cardiovascular disease.21

In this study, almost all patients also had normal tubular renal function tests. This is indicated by the normal levels of FENa and urine β2-microglobulin: creatinine, with only two (6.7%) patients having slightly raised FENa >1% and three (10%) patients with raised urine β2-microglobulin: creatinine >22 µg/mmol; serum β2-microglobulin was not measured in these three patients. Also, no patient had TRP <0.85 or TmP/GFR <1.0 mmol/L, which exclude the possibility of a renal tubular phosphate leak. Hence, all our patients had normal renal indicators for handling microalbumin and phosphate, with the vast majority of them having normal renal indicators for handling sodium and β2-microglobulin. These are amongst the important tubular functions tests, which have almost excluded a significant impairment in tubular renal function in our patients’ series. Gosling reported an estimate that when there is complete failure of renal tubular absorption, there will be an approximately 1,800-fold normal increase in β2-microglobulin loss and 20-fold normal increase in urine albumin loss.22 In comparison with other studies, Aldudak et al. reported no significant

**Figure 1:** Correlation between serum phosphate and tubular maximum phosphate reabsorption/glomerular filtration rate (TmP/GFR) in 30 homozygous beta-thalassaemia major patients.
Indicators of Renal Glomerular and Tubular Functions in Patients with Beta-Thalassaemia Major

A cross-sectional study at the Royal Hospital, Oman

Difference in creatinine clearance and FENa between thalassaemics and healthy controls. Kalman et al. also reported no significant difference in many tubular function indicators that include fractional excretion of sodium, magnesium, uric acid as well as calcium, protein, glucose, urine β-2-microglobulin, N-acetyl-β-D-glucosaminidase (NAG) levels and TRP in children with beta-thalassaemia major and thalassaemia intermedia. However, there are reports by other researchers who observed impairment in renal function when using other sensitive renal tubular function tests. Smolkin et al. reported normal creatinine clearance, FENa and TRP; however, urine NAG excretion was significantly raised in beta-thalassaemics, as also reported by Aldudak et al. and Sumboonnnanonda et al., who observed increase in both urinary NAG and malondialdehyde (MDA) excretion. MDA is an indicator of lipid peroxidation. Hence anaemia and increased oxidative stress, possibly iron induced, may play a role in precipitating some degree of tubular dysfunction, which may be mild, and so can not be detected when using other tubular function indicators.

In our study, 96.7% patients had TmP/GFR of >1.5 mmol/L and 76.7% patients had TRP values >0.95, indicating enhanced renal tubular phosphate reabsorption. This may be due to the raised phosphate level, due to the accelerated turnover of erythrocytes from the excess haemolysis in beta-thalassaemia. This is supported by the observation that 80% of our thalassaemics had raised serum phosphate and the significant correlation (r = 0.904) observed between serum phosphate and TmP/GFR levels. This compares with other studies where some researchers revealed no change while others observed some degree of altered TRP. However, no data is available in the literature for comparing TmP/GFR as a renal tubular index in beta-thalassaemic patients, although this test is more representative of renal tubular handling of phosphate than TRP alone, but there is limited awareness about its importance.

Also, in this study significantly higher mean TmP/GFR and serum phosphate values were observed in beta-thalassaemics with endocrinopathies compared with those with no endocrinopathy. Hypogonadism, the most frequent endocrine disorder that was observed in 22 (73.3%) thalassaemics in our series, appears to be the most likely underlying aetiology. The upward trend in TmP/GFR in these patients may be due to the decline in sex hormones (estrogens in women and androgens in men) as a consequence of impaired gonadal function resulting in a decline in the stimulating effect of sex hormones on calcitonin secretion, and accelerated bone resorption through inhibiting the formation of osteoclasts. Multiple pathways have been described for the inhibitory effect of estrogens and androgens on osteoclasts with calcitonin receptors and sex hormones have been demonstrated at multiple steps in the osteoclast lineage. The highest TmP/GFR values in our patients were observed in three beta-thalassaemics who had primary hypoparathyroidism in addition to hypogonadism. While the main function of the parathyroid hormone (PTH) is calcium homeostasis, it is also an important determinant of phosphate reabsorption, with TmP/GFR being increased in hypoparathyroidism and decreased in hyperparathyroidism. Normal or raised TmP/GFR may offer an advantage, as low values are associated with hypercalciuria, nephrocalcinosis and tubular proteinuria. This is presumably a result of direct tubular dysfunction, although it is controversial and may be difficult to prove. However, regardless of the underlying mechanism, in patients with renal tubular dysfunction whether due to the effect of metabolites, drugs or toxins, the response of low TmP/GFR to therapy may assist in monitoring these patients.

Conclusion

Renal function, glomerular as well as tubular, appears to be well preserved in beta-thalassaemic adult patients. In this study, almost all renal indicators that include eGFR, serum creatinine and urea as well as FENa, urine microalbumin, and β2-microglobulin were within the recommended
reference ranges. Raised TRP and TmP/GFR values were noted in the majority of thalassaemics, studied reflecting an upward trend in serum phosphate and its consequence in increasing renal reabsorption of phosphate. Despite the impairment in certain laboratory-based renal tubular indicators in some patients, especially those reported in the reviewed literature, these beta-thalassaemics had no overt renal disease, and so it remains questionable whether such functional abnormalities would have any long-term effect.

CONFLICT OF INTEREST
The authors reported no conflict of interest.

References


Hepatitis B Vaccine Coverage and the Immune Response in Children under ten years old in Sana’a, Yemen

Hassan A Al-Shamahy,1 Samira H Hanash,2 Iqbal A Rabbad,3 Nameem M Al-Madhaji,1 Samarih M Naser1

Objective: The study was undertaken, first, to determine the coverage rate of hepatitis B (HB) vaccine and second to evaluate the immune response to HB vaccine among children under 10 years old by measuring the level of circulating anti-HB surface antigen (anti-HBs) antibodies after immunisation with three doses.

Methods: First, 840 children were randomly selected from 4 randomly selected sites in Sana’a city to study the coverage rate of the vaccine; of these, 504 children vaccinated against HBV prior to the study, were tested (56% males and 44% females). Sera were tested for anti-HBs antibodies by ELISA quantitative technique. Each individual's data was collected in a pre-designed questionnaire including: vaccination date, sex, and age at the time of the study.

Results: The coverage rate of HB vaccine was only 69.9%, being slightly higher among male children (72.1%) than female children (66.8%). A total of 276 (54.8%) of the 504 children responded to the vaccine with anti-HBs antibody level ≥ 10 mIU/ml, while 228 (45.2%) of the 504 children had non-protective anti-HBs antibodies levels (<10IU/ml). Children of ages 3–5 years had the highest protective rate (63.6%), and the lowest protective rate was in the 9–10 years age group.

Conclusion: This study revealed a low coverage rate of HB vaccine and a low protective rate against HBV infection. A considerable proportion of vaccinated children should be considered for either revaccination or booster doses. There is also the need to complete HBV vaccine coverage among the child population in Sana’a, Yemen.

Keywords: Hepatitis B; Vaccine; Children; Yemen; Sana’a

Abstract: Objectives: The study was undertaken, first, to determine the coverage rate of hepatitis B (HB) vaccine and second to evaluate the immune response to HB vaccine among children under 10 years old by measuring the level of circulating anti-HB surface antigen (anti-HBs) antibodies after immunisation with three doses. Methods: First, 840 children were randomly selected from 4 randomly selected sites in Sana’a city to study the coverage rate of the vaccine; of these, 504 children vaccinated against HBV prior to the study, were tested (56% males and 44% females). Sera were tested for anti-HBs antibodies by ELISA quantitative technique. Each individual's data was collected in a pre-designed questionnaire including: vaccination date, sex, and age at the time of the study. Results: The coverage rate of HB vaccine was only 69.9%, being slightly higher among male children (72.1%) than female children (66.8%). A total of 276 (54.8%) of the 504 children responded to the vaccine with anti-HBs antibody level ≥ 10 mIU/ml, while 228 (45.2%) of the 504 children had non-protective anti-HBs antibodies levels (<10IU/ml). Children of ages 3–5 years had the highest protective rate (63.6%), and the lowest protective rate was in the 9–10 years age group. Conclusion: This study revealed a low coverage rate of HB vaccine and a low protective rate against HBV infection. A considerable proportion of vaccinated children should be considered for either revaccination or booster doses. There is also the need to complete HBV vaccine coverage among the child population in Sana’a, Yemen.
Hepatitis B (HB) is a human disease caused by a virus that attacks the liver. The hepatitis B virus (HBV) can cause lifelong infection, cirrhosis of the liver, liver cancer, liver failure and death. HB infection is one of the world’s most common and serious infectious diseases. It is estimated that more than one third of the world’s population has been infected with HBV. About 5% of the population are chronic carriers of HBV, and nearly 25% of all carriers develop serious liver diseases such as chronic hepatitis, cirrhosis and primary heptocellular carcinoma (HCC). HBV infection causes more than one million deaths every year.

For long-term protection against HBV, there are two types of vaccines: plasma-derived HB surface antigen (HBsAg) vaccine, and yeast-derived HBsAg vaccine. HB immunisation, using either type of vaccine, has been shown to eliminate HBV transmission and prevent HBV-related chronic liver disease. HBV vaccine can be routinely given to children and individuals at risk, along with other commonly used vaccines in a variety of schedules that results in excellent immunogenicity and do not interfere with the immunogenicity of other vaccines. The sero-conversion rate for vaccination is influenced by a number of factors, the most important ones being age and sex. Rates in excess of 95% are seen in young women, whereas the rate may drop to 80% in older men. Immunosuppressed patients, smokers, and obese individuals show even lower rates.

According to the Yemeni National Infectious Viral Hepatitis Control Programme, Yemen was recognised as HBV-endemic area. In 1998, the World Health Organization (WHO) recommended the inclusion of HB vaccine in the national immunisation programme of Yemen, particularly among neonates, where vertical transmission is common, regardless of the prevalence of HBsAg. In Sana’a city, the HB antigen carrier rate is classified as intermediate, rather than high. The incidence of acute HBV has declined dramatically in the past decade since the start of the vaccination programme, especially among young people, although it may still take several decades until the effect of vaccination is translated into reduced transmission and morbidity.

The aims of this study were first to determine the coverage rate of HBV among children and second to evaluate the immune response to HBV vaccine among vaccinated children with the three doses of HBV vaccine by measuring the level of circulating anti-HB surface antibodies.

Methods

This cross sectional study was carried out from January to March 2010. The study proposal was approved by the Department of Medical Microbiology, Faculty of Medicine & Health Sciences, Sana’a University, Yemen. First, 840 children, aged <1–10 years, (470 males and 370 females) were randomly selected by a systematic random sampling of every fifth child attending randomly selected health centres or from the lists of randomly selected primary schools in Sana’a city. The histories of all 540 children who had received three doses of HBV vaccine were investigated. A total of 81.9% of them had received yeast-derived vaccine and 18.1% plasma-derived vaccin; 62.2% of the children had been vaccinated at a dose interval of 0, 1, 2 months and 37.8% at a dose interval of 0, 1, 6 months. The immune response to the HBV vaccine of these 504 vaccinated children was then evaluated. A consent form was completed by the parents for each participant.

A full history was taken from each individual studied and the findings recorded in a pre-designed questionnaire. The data collected included name, age at the time of the study, sex, residence, and vaccination date according to the last dose of HBV vaccine, number of doses, intervals between the three doses and type of vaccine. A full history of vaccinations against other diseases was also taken.

Four millilitres of whole blood were collected from each subject. Then the sera were separated and frozen at -20 °C until tested. Anti-HBs antibodies were determined by an enzyme-linked immunosorbent assay (ELISA) using a
commercially available kit (Biokit, S.A., Barcelona, Spain). Bio-ELISA anti-HB is a direct immunoenzymatic method of the “sandwich” type in which the samples to be analysed are incubated in wells of micro-plate that are coated with highly purified HB antigens.

In order to differentiate naturally occurring immunity to HBV infection, total anti-HB core antibodies were measured; all individuals with anti-HB core antibody positive were excluded from the evaluation of HBV vaccine group.

The data and results were analysed by using EPI-Info Version 6, Centers for Disease Control (CDC, Atlanta, GA, USA).

**Results**

The coverage rate of HBV vaccine was only 69.9%, slightly higher among male children (72.1%) than females (66.8%) [Table 1].

Table 2 shows the immune response to HBV vaccine by quantifying anti-HBs antibody levels among males and females. A total of 276 (54.8%) of the 504 children responded to the vaccine with anti-HBs antibody levels ≥10 mIU/ml, while 228 (45.2%) had non-protective anti-HBs antibodies level (<10 IU/ml). The average protective rate in both sexes was 54.8%, but higher among males (57.4%) than females (51.4%). There was no statistically significant variation between both sexes. Table 3 shows the protective rate among different age groups, the rate being higher in younger compared to older age groups. Children aged 3–5 years had the highest protective rate (63.6%); the lowest protective rate was in the 9–10 year old age group (32.4%) [Table 3].

Table 4 shows the protection by yearly intervals after primary immunisation against HBV. Children immunised one year or less prior to the study had a higher protective rate (66.7%), than those immunised 4 years prior (44.2%).

In this study, different HBV-markers were obtained from the whole 504 group of vaccinated children. It was found that the frequency of HBsAg positivity among these children was 1.8%.

**Table 1:** The coverage rate of hepatitis B vaccine (HBV) among randomly selected male and female children under ten years old in Sana’a, Yemen

<table>
<thead>
<tr>
<th>Status</th>
<th>Male (n = 470)</th>
<th>Female (n = 370)</th>
<th>Total (n = 840)</th>
<th>X2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinated for HBV</td>
<td>339 72.1</td>
<td>247 66.8</td>
<td>586 69.9</td>
<td>2.83</td>
<td>0.09</td>
</tr>
<tr>
<td>Non-vaccinated for HBV</td>
<td>131 27.9</td>
<td>123 33.2</td>
<td>254 30.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>470 56</td>
<td>370 44</td>
<td>810 100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2:** The immune response to hepatitis B vaccine by quantifying anti-HBs antibody levels and protective and non-protective levels among males and females

<table>
<thead>
<tr>
<th>Antibody levels</th>
<th>Male</th>
<th>%</th>
<th>Female</th>
<th>%</th>
<th>Total</th>
<th>%</th>
<th>X2</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-responders (&lt;10 mIU/ml)</td>
<td>120</td>
<td>42.6</td>
<td>108</td>
<td>48.6</td>
<td>228</td>
<td>54.1</td>
<td>1.8</td>
<td>0.17</td>
</tr>
<tr>
<td>Low-responders (10–100 mIU/ml)</td>
<td>87</td>
<td>30.9</td>
<td>45</td>
<td>20.3</td>
<td>132</td>
<td>26.2</td>
<td>7.2</td>
<td>0.007</td>
</tr>
<tr>
<td>Adequate responders (&gt;100–1000 mIU/ml)</td>
<td>45</td>
<td>16</td>
<td>45</td>
<td>20.3</td>
<td>90</td>
<td>17.6</td>
<td>1.6</td>
<td>0.2</td>
</tr>
<tr>
<td>High-responders (&gt;1000 mIU/ml)</td>
<td>30</td>
<td>10.6</td>
<td>24</td>
<td>10.8</td>
<td>54</td>
<td>10.7</td>
<td>0.0</td>
<td>0.95</td>
</tr>
<tr>
<td>Immune response</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protective anti-HBs</td>
<td>162</td>
<td>57.4</td>
<td>114</td>
<td>51.4</td>
<td>276</td>
<td>54.8</td>
<td>1.9</td>
<td>0.17</td>
</tr>
<tr>
<td>Non-protective anti-HBs</td>
<td>120</td>
<td>42.6</td>
<td>108</td>
<td>48.6</td>
<td>228</td>
<td>45.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes: *X2≥3.84, P <0.05 (significant); Protective anti-HBs ≥10 mIU/ml; Non-protective anti-HBs <10 mIU/ml.
Discussion

Since the 1980s, there has been an increasing body of information on viral hepatitis in Yemen, which is a major public health problem affecting thousands of people throughout the country. Viral hepatitis is a major cause of morbidity and mortality in humans in Yemen, both from acute infection and its chronic sequelae which include hepatitis B and hepatitis C infection, chronic hepatitis cirrhosis and primary liver cancer. The endemic rate of hepatitis B virus infection is considered high in Yemen, where the prevalence of the positive (HBsAg) ranges from 8–20%, and up to 50% of the general population have serological evidence of previous HBV infection.

Yemen introduced a universal immunisation programme against HBV for infants and high risk groups in early 2000, but feedback on the coverage rate of vaccination and its efficacy in the community have been ignored for a long period. In addition, there has been inadequate information on the prevalence and risk determinants of viral hepatitis as well as on vaccination coverage rate among children in Yemen. This study was carried out in response to this information gap.

One of the aims of this study was to determine the coverage rate of HBV vaccine among children. The study findings showed that the vaccination coverage rate was 69.9%. This result is lower than findings in other HBV endemic countries, where HBV vaccine coverage rates among children range from 90–98%.

Also the study findings showed that only 54.8% of all vaccinated individuals were regarded as protected (≥10 mIU/ml), and that the protective rate of HBs antibody was higher in males (57.4%), than in females (51.3%). Different findings were reported elsewhere among children, where a high protective anti-HB response rate was found among vaccinated children (97.4%), and the rate for females was also higher than that for males. This difference in the findings could be attributed to a different response in the primary course of vaccination, different age groups, to the different degrees of exposure to natural boosters, or to differences in nutritional status and socioeconomic factors, race factors, or the type of vaccines used.

Concerning the rest of the study group, 45.2% developed a low antibody level (<10 mIU/ml), indicating a poor anti-HBs response after receiving

Table 3: Immune response among vaccinated children in different age groups

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Protected (%)</th>
<th>Non-protected (%)</th>
<th>X2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–2 years (n = 72)</td>
<td>39 (54.2)</td>
<td>33 (45.8)</td>
<td>0.01</td>
<td>0.91</td>
</tr>
<tr>
<td>3–5 years (n = 165)</td>
<td>105 (63.6)</td>
<td>60 (36.4)</td>
<td>7.8</td>
<td>0.005</td>
</tr>
<tr>
<td>6–8 years (n = 165)</td>
<td>99 (60)</td>
<td>66 (40)</td>
<td>2.7</td>
<td>0.09</td>
</tr>
<tr>
<td>9–10 years (n = 102)</td>
<td>33 (32.4)</td>
<td>69 (67.6)</td>
<td>25.9</td>
<td>0.0000004</td>
</tr>
<tr>
<td>Total</td>
<td>276 (54.8)</td>
<td>228 (45.2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes: Protected (anti-HBs ≥10 mIU/ml); Non-protected (anti-HBs <10 mIU/ml).

Table 4: Protected and non-protected vaccinated individuals according to period since vaccination

<table>
<thead>
<tr>
<th>Year intervals</th>
<th>Protected (n = 276)</th>
<th>Non-protected (n = 228)</th>
<th>X2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 year or less prior to study (n = 72)</td>
<td>48 (66.7)</td>
<td>24 (33.3)</td>
<td>4.8</td>
<td>0.02</td>
</tr>
<tr>
<td>2 years prior to study (n = 125)</td>
<td>78 (62.4)</td>
<td>47 (37.6)</td>
<td>3.9</td>
<td>0.04</td>
</tr>
<tr>
<td>3 years prior to study (n = 119)</td>
<td>69 (57.9)</td>
<td>50 (42.1)</td>
<td>0.65</td>
<td>0.41</td>
</tr>
<tr>
<td>4 years prior to study (n = 86)</td>
<td>38 (44.2)</td>
<td>48 (55.8)</td>
<td>4.7</td>
<td>0.03</td>
</tr>
<tr>
<td>5 years or more prior to study (n = 102)</td>
<td>48 (47.1)</td>
<td>54 (52.9)</td>
<td>3.1</td>
<td>0.08</td>
</tr>
<tr>
<td>Total no. = 504</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes: Protected ≥10 mIU/ml; Non protected <10 mIU/ml.
a full course of vaccine, as shown in Table 2. It can be deduced from this finding either that these vaccinated individuals were hypo-responsive to the immunisation and that their antibodies may have waned rapidly over time, or that the vaccine was of poor quality. Even in these instances, loss of antibodies does not necessarily imply loss of protection. Considering that anti-HBs may disappear in a substantial proportion of vaccinees after initially successful vaccination, a booster dose of vaccine, following the administration of the primary course, is recommended by most national bodies. However, the results of long-term follow-up studies, together with assessment of the role of immunological memory among vaccinees, now call into question the necessity of providing booster doses following successful course of primary immunisation. Other studies showed that protection is still maintained among vaccinees, even in HBV-endemic countries, despite waning or undetectable anti-HBs levels.

In this study, different HBV-markers were obtained from all the vaccinated children studied. However, due to the lack of serological data, either before or after vaccination, it was impossible to conclude whether these children were already infected at the time of vaccination or had been infected subsequently. In the present study, it was found that the frequency of HBsAg positivity among the whole group of children was 1.8%, which was lower than the rate of the non-vaccinated children (2.8%) in Sana’a city in 2001. This indicates the efficacy of HBV vaccine in preventing chronic carriage of infection. In a long-term follow-up study (over c. 16 years) on HB vaccine immune efficacy in China, the positive rate of HBsAg for children born after the introduction of the immunisation programme was much lower than those of the background group before vaccination. Also our result were similar to that found in Egypt among children, where HBsAg positivity was 0.8% in vaccinated children compared to 2.2% for non-vaccinated children. No clinically overt hepatitis has been reported so far among the studied vaccinated individuals. This was similar to findings reported elsewhere.

Host factors, such as age, may influence the immune response to the vaccine. Increasing age was shown to be correlated with a decreasing level of protection rate [Table 3]. The response rate of anti-HBs declined from 63.6% in the 3–5 years age group to 32.4% in the 9–10 years age group. Similar findings were reported from Saudi Arabia, showing that being in the >10 years age group correlated with a decreasing protection rate. In another study conducted in European countries, the main age for children who had non-protected levels against HBV was 9.5 years, while the main age for those who responded and had protected levels of antibodies was 5.7 years.

In this study, there was a difference in protection rate at the various annual intervals (1–5 years) since vaccination [Table 4]. Zhou et al. reported that protective levels of antibodies decrease with time, however, they can remain sufficient in healthy individuals for at least 10 years after primary immunisation.

## Conclusion

This study revealed a low coverage rate of HBV vaccine, and a low protective rate against HBV infection. A considerable proportion of vaccinated children should be considered for either revaccination or booster doses due to a non-existent, inadequate, or low response. An effort to complete HBV vaccine coverage to 100% among the Yemeni child population is recommended, especially among newborns.

## Conflict of Interest

The authors reported no conflict of interest.

## References


In Silico Design of Novel Anticoagulant Peptides targeting Blood Coagulation Factor VIIa

Manal S Q Al-Amri, Khalid Alrasadi, Riad Bayoumi, Yajnavalka Banerjee

ABSTRACT: Objectives: The coagulation cascade initiated during vascular injury prevents bleeding. Unwanted clot formation is however detrimental and requires the use of anticoagulants for prophylaxis and treatment. Anticoagulants targeting a specific step or an enzyme in the clotting process are most preferred as they minimise disadvantageous side-effects. A principal step in the discovery of novel anticoagulants encompasses the in silico design of potential leads. This study depicts the in silico design of peptide anticoagulants targeting coagulation factor VIIa. Methods: Applying the proline bracket rule and using various bioinformatics tools: the basic alignment search tool (BLAST) of National Center for Biotechnology Information; the T-coffee module provided by European Molecular Biology Laboratory-European Bioinformatics Institute, and several modules available on the ExPASy server, we designed five bivalent chimeric anticoagulant peptides targeting factor VIIa, using factor VIIa inhibitors – hemextin A from Hemachatus haemachatus (African Ringhals cobra) venom and factor VIIa exosite-inhibitor peptide as templates. Six peptides were derived from hemextin A, which were concomitantly fused with factor VIIa exosite-inhibitor peptide intermediated by a polyalanine spacer, and analysed for structural stability using the SWISS-MODEL software developed at the Swiss Institute of Bioinformatics and WebLab ViewerPro (Version 4.2). Results: Twelve chimeric peptides were obtained; only five exhibited stable structures in silico. Conclusion: The five peptides obtained are probable anticoagulant leads that should be further evaluated using suitable in vitro and in vivo assays. Further, this study shows how simple web-based modules can be used for the rational design of probable leads targeting specific physiological molecular targets.

Keywords: Anticoagulant; Factor VIIa; In silico drug design

Advances in Knowledge
1. Five potential bivalent chimeric peptide inhibitors of coagulation factor VIIa have been designed, which should be assessed using in vitro and in vivo assays.
Blood coagulation is a physiological response to vascular injury in which zymogens of serine proteases present in the plasma milieu are sequentially activated culminating in the formation of the fibrin clot. Coagulation factor VIIa (FVIIa), along with cofactor tissue-factor (TF), act as the prima ballerinas to initiate the coagulation cascade. The extrinsic pathway is responsible for initiating the clotting process whereas the intrinsic pathway propagates the process of coagulation. A comprehensive description of both these pathways is summarised in Figure 1.

Although coagulation reactions are pivotal for appropriate homeostasis, unwanted clot formation can mediate debilitating affects leading to thrombosis and associated disorders. Anticoagulants are pivotal for the prevention and treatment of thromboembolic disorders, and ~0.7% of the Western population receives oral anticoagulant treatment. Coumarins and heparin are the most well known clinically used anticoagulants. Coumarins inhibit the activity of all vitamin K-dependent proteins, including procoagulants (thrombin, FXa, FIXa, and FVIIa) and anticoagulants (activated protein C and protein S). Heparin mediates its anticoagulant activity by enhancing the inhibition of thrombin and FXa by antithrombin III. The non-specific mode of action of these anticoagulants accounts for their therapeutic limitations in maintaining a balance between thrombosis and haemostasis. These limitations have provided the impetus for the development of new anticoagulants that target specific coagulation enzymes or a particular step in the clotting process. Because of its relatively low concentrations in blood (10 nM) and its pivotal role in the initiation of the coagulation reactions, FVII/FVIIa is an attractive drug target for the development of novel and specific anticoagulant agents.

So far, only two proteins that specifically inhibit the TF-FVIIa complex have been evaluated in clinical trials, viz. tissue factor pathway inhibitor (TFPI) and nematode anticoagulant peptide c2 (NAPc2). TFPI is an endogenous inhibitor of this complex, whereas NAPc2 is an exogenous inhibitor isolated from canine hookworm (Ancylostoma caninum). Of these, only NAPc2 has future viability as a probable anticoagulant lead. In a phase II study, NAPc2 showed promise in preventing venous thromboembolism after elective knee replacement surgery. Because of the lack of natural inhibitors that specifically interfere with FVIIa activity, a number of artificial inhibitors have been designed and developed. They include proteins that block the association of TF and FVIIa, such as antibodies against TF and FVIIa, TFAA (a TF mutant with reduced cofactor function for FX), FFR-VIIa (inactivated form of FVIIa with 5-fold higher affinity for TF compared with native FVIIa), and peptides derived from TF and FVIIa. In addition, two series of peptide exosite inhibitors were selected from phage display libraries for their ability to bind to the TF-FVIIa complex. They bind to two distinct exosites on the serine protease domain of FVIIa and exhibit sterical and allosteric inhibition. Although both peptide classes are potent and selective inhibitors of the TF-FVIIa complex, they fail to inhibit 100% activity even at saturating concentrations. This is overcome either by the fusion of the two peptides, or by using a protease switch with substrate phage. A number of synthetic compounds have also been designed as active-site inhibitors of FVIIa as well as the TF-FVIIa complex. A number of naphthylamidines have recently been reported to have FVIIa inhibitory activity. They were synthesised by the coupling of amidinobenzaldehyde analogs to a polystyrene resin. However, apart from inhibiting FVIIa activity, these synthetic compounds nonspecifically inhibit the activity of other blood coagulation serine proteases.

In the past, we reported the identification and
Manal S Q Al-Amri, Khalid Alrasadi, Riad Bayoumi and Yajnavalka Banerjee

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in-depth characterisation of a novel FVIIa inhibitor, christened hemextin AB complex, from the venom of the African Ringhals cobra.26-29 This synergistic anticoagulant complex consists of two proteins, hemextin A (HA) and hemextin B (HB), both of which belong to the three-finger family of snake venom proteins [Figure 2]. Of the two subunits, HA individually possesses anticoagulant activity whereas HB is inactive. Unlike NAPc2, HA of hemextin AB complex does not use coagulation factor Xa as a scaffold for FVIIa inhibitory activity positing a very unusual mechanism of anticoagulation.29 Thus HA provides us with a novel and intriguing anticoagulant lead that can be further developed into an anticoagulant.

In this study, we report the design of a short chimeric anticoagulant mimetic (with probable FVIIa inhibitory function) using peptides derived from HA (by applying the proline bracket rule) and fusing them with an already characterised peptide FVIIa-exosite inhibitor,20 using simple web-based bioinformatics. Only five of the 12 designed peptides exhibited structural stability and warrant assessment with regards to anticoagulant activity. This study therefore not only provided a foundation in the quest for the rational design of anticoagulants.
specifically targeting FVIIa, but also depicted a scheme that can be utilised in the rational design of drug leads using web-based bioinformatics.

**Methods**

The complete sequence of HA protein had been determined earlier using Edman degradation protein chemistry, and is depicted below (using the single letter amino acid code):

```
LKCHNKLVPFLSKTCPEGKNLCYKMTLMKPK1PKI1KRGCTDACPKSSLLVVKVVCCNKDKCN
```

The sequence of the 26-mer FVIIa-exosite inhibitor peptide was obtained from the literature, and is as follows: EEVEVLCTWTWETCGERGVEELWEWR.

The various software employed in this study are freely available on the Internet and do not require special access to any paid database or server. Most of these modules are Windows based, however some operate at an optimum on a LINUX platform.

Sequence alignment involving HA involved a detailed search of homologous sequences from the National Center for Biotechnology Information protein database, following which the basic alignment search tool (BLAST) was employed. The sequence alignment of HA was carried out by looking at the position of the cys residues using the online T-coffee module provided by the European Molecular Biology Laboratory-European Bioinformatics Institute. In order to look at the various specifics of the amino acid sequence, appropriate modules available on the ExPASy server were used. In order to model...
the three-dimensional structures of the HA and chimeric peptides, the sequences of the proteins were submitted to the SWISS-MODEL developed by the Protein Structure Bioinformatics group at the Swiss Institute of Bioinformatics (SIB) and the Biozentrum at the University of Basel.37

The raw data obtained were then analysed using the WebLab ViewerPro (Version 4.2) (Accelrys, Inc. San Diego, CA, USA). The surface charge distribution of the modeled as well solved structures obtained from the protein data bank were analysed using the Molecular Graphics Laboratory Tools (MGLT) package obtained from The Scripps Research Institute (TSRI) molecular modeling server (TSRI, La Jolla, CA, USA). The Python Molecular Viewer (PMV) of the MGLT package was used predominantly in this study.38,39

**Results**

Our finding on the alignment of HA, was that HA belongs to the three-finger family of snake venom proteins [Figure 2A] and not to the family of snake venom serine protease inhibitors which are Kunitz type inhibitors with a very different cys bonding pattern. Proteins belonging to this group exhibit a characteristic β-sheet structure [Figure 2B]. In order to design short peptides rationally from HA, we used the proline bracket rule.40,41 In this rule, unique physicochemical properties of proline, the most commonly found imino acid in proteins, are used for the prediction of active sites. The distinct properties of proline residues are due to the bulky pyrrolidine ring and the lack of protons important for hydrogen bond formation in the α-helix and β sheet. Proline residues are the common structural elements found in the flanking segments of protein-protein interaction sites and they enhance the protein-protein interactions. The proline residues most likely reduce the number of possible conformations due to their ability to constrain the conformation of the neighbouring residues. The short segments of 3-7 residues flanked by proline residues have been identified as the potential protein-protein interaction sites directly from the amino acid sequence of the protein. A case in point is that incorporation of proline residues on either or both sides of the interaction site of an antiplatelet peptide (peptide that prevents platelet aggregation), IARGDMNA, enhances the antiplatelet activity to approximately the same extent (1.5- to 2.5-fold). The incorporation of proline residues on both sides enhances the activity by 7- to 13-fold. This enhancement of the biological activity of the peptide is probably due to a reduction in the number of possible conformations of the peptide, without introducing the rigidity that would accompany cyclization.40 Therefore we hypothesised that proline residues will be present on the flanking regions of the probable interaction/active sites. Based on this premise, we designed the six short peptides from HA [Figure 3]. We also kept two amino residues beside each proline residue on each side to provide structural stability to the peptide.

![Figure 3: Peptides derived from HA. Six short peptides were derived from HA using the proline bracket rule. We kept two amino acids on the flanking region of each proline residue in order to provide structural stability to the peptide.](image-url)
designed peptide.

For the modelling of the HA 3D-structure, the sequence alignment showed that HA has conserved cys residues with other members of the three-finger toxin (3-FTX) family, which indicated that HA is a 3-FTX. Earlier studies involving circular dichroism spectroscopy also have supported this finding. In order to confirm further that HA has a similar architecture to three-finger proteins, we modelled the HA. As observed in Figure 4 A, it has a characteristic fold of a three-finger toxin, with anti-parallel β-sheets. Further, we mapped the surface charge distribution of HA using PyMOL molecular viewer (PMV) [Figure 4B]. HA shows a predominance of blue or positively charged patches, from which it can be concluded that HA is a basic protein and therefore will have a higher propensity of binding to negatively charged regions on its corresponding target molecule. This observation was further confirmed by the calculating the theoretical PI for HA, which was found to be 9.34, confirming that the protein is of basic nature.

In order to predict the binding sites of HA on FVIIa, the structure of FVIIa was modelled using the X-ray crystallographic structure of TF-FVIIa as a template (PDB ID 1DAN). As shown in Figure 5, FVIIa consists of two chains, the heavy and the light chains. The heavy chain consists of the protease domain of the enzyme, whereas the light chain consists of two epidermal growth-factor-like domains and a γ-carboxyglutamic acid domain. HA does not bind to the light chain of FVIIa (data not shown) and therefore its binding site is located on the heavy chain. The heavy chain of FVIIa consists of a patch of negatively charged residues (highlighted in yellow in Figure 5) and HA, being predominantly basic in nature, will have its binding/interaction site localised to this acidic patch. Therefore, peptides derived from HA are supposedly going to bind to this acidic or negatively charged patch on FVIIa.

As to the design of chimeric peptides, since peptides derived from HA will bind to the acidic patch on the FVIIa molecule, we fused these HA derived peptides with another peptide that binds to the basic patch on the heavy chain. This peptide has already been characterised with regards to the structure-function details of the peptide. The binding site of the peptide on the FVIIa heavy chain is shown in Figure 6 A and was determined using X-ray crystallography (PDB ID 1JBU). The peptide is inherently highly acidic in nature and binds to a predominantly positively charged region on the FVIIa heavy chain, as shown in the surface potential plot in Figure 6 B. HA derived peptides were fused with this FVIIa exosite-inhibitory peptide. A spacer consisting of polyalanine residues was introduced between the HA derived and FVIIa exosite-inhibitory peptides in order to maintain...
the distance between the acidic and the basic patches. The designed chimeric peptides are shown in Table 1.

The structural stability of the chimeras was assessed by modelling their structure in a way similar to that used for HA. Only five of the 12 chimeric proteins were found to be structurally stable [Figure 7A-E], whereas the 3D-structure modelling of the other peptides failed, thus indicating their structural instability.

Discussion

In the present study, we designed bivalent anticoagulant peptides targeting coagulation FVIIa, the clotting factor responsible for the initiation of the clotting reactions, employing simple web-based bioinformatics. For our study, we used two different proteins as templates: HA, a FVIIa inhibitor isolated and characterised from the venom of *Hemachatus haemachatus* (African Ringhals cobra), and factor VIIa exosite-inhibitor peptide which is a synthetic peptide identified in phage-display libraries.

The complete sequence alignment of HA revealed that it belongs to the three-finger family of snake venom proteins. This family of proteins is found only in the venoms of elapids (cobras, kraits and mambas) and hydrophids (sea snakes) and not those of vipers and crotalids (rattlesnakes). They contain four or five disulphide bridges, of which four are conserved in all members. Accordingly, all proteins of this family show a similar pattern of protein folding: three β-stranded loops extending from a central core containing the four conserved disulphide bridges [Figure 2]. Despite the overall similarity in structure, these polypeptides differ from each other in their biological activities. However, because of their well folded and compact structure, proteins belonging to this family are attractive as drug leads (if the parent protein is pharmacologically active) or are used as molecular scaffolds in the peptide based drug design. For our study one of the principal reasons for choosing HA was its well-folded and compact structure.

The idea of designing a bivalent inhibitor for a coagulation serine protease was first successfully implemented in case of thrombin, where two peptides acetyl-(d)F-P-R-P-Q-S-H-N-D-G-D-F-E-E-I-P-E-Y-L-Q (binding to the catalytic active site) and (d)F-P-R-P-G-G-G-G-N-G-D-F-E-E-I-P-E-Y-L (binding to the exosite of thrombin) were covalently linked, and the resultant peptide mimicked the activity of hirudin and had higher inhibitory potency than the individual
In Silico Design of Novel Anticoagulant Peptides targeting Blood Coagulation Factor VIIa

Some bivalent peptides, although having weak binding properties, have been derived from the activation sequences of thrombin-activated receptors, also referred to as protease-activated receptors (PARs). These peptides carry the LDPR sequence and binding motifs targeting the fibrinogen-recognition exosite of thrombin.45,46

The essence of designing such inhibitors is to use peptides which are structurally stable and do not have overlapping binding sites.

In our study, we targeted coagulation FVIIa, since this serine protease is responsible for the initiation of the clotting cascade. Therefore, inhibiting the activity of this protein will inhibit the cascade in its initial stages. Also the levels of this protein in blood is low in comparison to some of the other coagulation serine proteases, necessitating the use of low levels of the designed anticoagulant, a key factor involved in the success of any drug.

Based on the surface charge calculations [Figure 4 B], it is pertinent that HA is a basic protein and therefore peptides derived from it will bind to predominantly negatively charged regions on its target molecule. Also, previous studies have shown that HA is a specific inhibitor of FVIIa.29 Based on this premise we identified the regions of FVIIa which have a high density of negatively charged clusters. Such clusters exist in both the heavy and light chains of the FVIIa molecule. However, these clusters in the light chain are not accessible to HA since they are involved in co-factor (TF binding) as revealed in the crystallographic structure solved by Banner et al.42 and later confirmed by us (data not shown). Therefore, we localised the negatively charged cluster on the heavy chain of the protein using the solved structure of TF--FVIIa (PDB ID 1DAN) as the model [Figure 5, highlighted in yellow].

In order to derive peptides from HA we used the proline bracket rule [Figure 3] (see above with regards to the details). In our next step, we needed to select an FVIIa-inhibitory peptide whose binding site will not overlap with HA derived peptides. After a literature search we found a peptide (EEWEVLCWTWETCERGEGVEELWEWR) which is predominantly negatively charged (PI = 3.98) as the ideal candidate to be used for the design of the chimera. Firstly, because of its inherent acidic nature, it will be involved in binding to a patch of residues which are positively charged and secondly [Figure 6 A and B], the presence of a disulfide bond in the peptide guarantees its having a well defined fold. Since HA derived peptides are positively charged and might interact with the FVIIa-inhibitory peptide, we introduced a spacer of nine alanine residues in between them. The designed chimeras were then assessed for structural stability using the appropriate tools mentioned in the Methods section. Only five of the designed 12 chimeras were found to be structurally viable.
One of the intriguing highlights of the study was that the designed structurally viable peptides had the positively and negatively charged residues separated as observed in Figure 7.

Also, we designed various combinations of chimeras where the HA derived peptides were placed at the amino-terminal as well as the carboxy-terminal ends. Switching of ends was found to affect the stability of the designed chimera. As shown in Table 1, for combination 3, the presence of the FVIIa-inhibitory peptide on the carboxy-terminal end leads to structural instability of the designed chimera, whereas its presence on the amino-terminal does not affect its structural viability.

These putative bivalent anticoagulants still need to be evaluated in various in vitro and in vivo assays before they can be assessed in clinical trials. One of the advantages of the current strategy is that it can be successfully applied without the involvement of large financial commitment by researchers with limited knowledge of bioinformatics. However, the designed chimeric anticoagulant, although structurally viable, might lose its activity since the fold it attains might not favour its binding to its specific interaction sites.

**Conclusion**

Table: 1. Designed Chimeric Peptides and Result of Structural modeling

<table>
<thead>
<tr>
<th>Hemextin A (peptide)</th>
<th>Terminal link</th>
<th>Chimera (HA p + Lazarus p)</th>
<th>Structure Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. A- LVPFLSKTAPEG</td>
<td>COOH-terminal</td>
<td>LVPFLSKTAPEG + EEEVLCTWETCERGVEEELWEWR</td>
<td>No recognisable fold was obtained</td>
</tr>
<tr>
<td>B- LVPFLSKTAPEG</td>
<td>NH2-terminal</td>
<td>EEEVLCTWETCERGVEEELWEWR + LVPFLSKTAPEG</td>
<td>No recognisable fold was obtained</td>
</tr>
<tr>
<td>2. A- TCPEGKNLCYKMTMLKMPKI</td>
<td>COOH-terminal</td>
<td>TCPEGKNLCYKMTMLKMPKI + EEEVLCTWETCERGVEEELWEWR</td>
<td>No recognisable fold was obtained</td>
</tr>
<tr>
<td>B- TCPEGKNLCYKMTMLKMPKI</td>
<td>NH2-terminal</td>
<td>EEEVLCTWETCERGVEEELWEWR + TCPEGKNLCYKMTMLKMPKI</td>
<td>No recognisable fold was obtained</td>
</tr>
<tr>
<td>3. A- KIPIKRGCTDACPKS</td>
<td>COOH-terminal</td>
<td>KIPIKRGCTDACPKS + EEEVLCTWETCERGVEEELWEWR</td>
<td>Distinct fold was observe wafer modeling</td>
</tr>
<tr>
<td>B- KIPIKRGCTDACPKS</td>
<td>NH2-terminal</td>
<td>EEEVLCTWETCERGVEEELWEWR + KIPIKRGCTDACPKS</td>
<td>Distinct fold was observe wafer modeling</td>
</tr>
<tr>
<td>4. A- TCPEGKNLCYKMTMLKMCKIPIK</td>
<td>COOH-terminal</td>
<td>TCPEGKNLCYKMTMLKMCKIPIK + EEEVLCTWETCERGVEEELWEWR</td>
<td>Distinct fold was observe wafer modeling</td>
</tr>
<tr>
<td>B- TCPEGKNLCYKMTMLKMCKIPIK</td>
<td>NH2-terminal</td>
<td>EEEVLCTWETCERGVEEELWEWR + TCPEGKNLCYKMTMLKMCKIPIK</td>
<td>Distinct fold was observe wafer modeling</td>
</tr>
<tr>
<td>5. A- LVPFLSKTCCEGKNLCYKMTMLKMPKI</td>
<td>COOH-terminal</td>
<td>LVPFLSKTCCEGKNLCYKMTMLKMPKI + TCCEGKNLCYKMTMLKMPKI</td>
<td>Distinct fold was observe wafer modeling</td>
</tr>
<tr>
<td>B- LVPFLSKTCCEGKNLCYKMTMLKMPKI</td>
<td>NH2-terminal</td>
<td>EEEVLCTWETCERGVEEELWEWR + LVPFLSKTCCEGKNLCYKMTMLKMPKI</td>
<td>Distinct fold was observe wafer modeling</td>
</tr>
<tr>
<td>6. A- KMPKICIKRGCTDACPKS</td>
<td>COOH-terminal</td>
<td>KMPKICIKRGCTDACPKS + EEEVLCTWETCERGVEEELWEWR</td>
<td>No recognisable fold was obtained</td>
</tr>
<tr>
<td>B- KMPKICIKRGCTDACPKS</td>
<td>NH2-terminal</td>
<td>EEEVLCTWETCERGVEEELWEWR + KMPKICIKRGCTDACPKS</td>
<td>No recognisable fold was obtained</td>
</tr>
</tbody>
</table>

Notes: + denotes the nine alanine spacer; HA p denotes the peptide fragment derived from hemextin A; Lazarus p denotes the peptide designed at Genentech CA, USA by Dr Bob Lazarus and his colleagues.
In summary, we designed bivalent inhibitors for FVIIa using simple bioinformatics. The five structurally viable inhibitors will be assessed for functional potency using appropriate in vitro and in vivo assays/experiments in the near future. Although a great deal of effort was involved in the analysis of data and literature search during the study, it was relatively inexpensive since all the tools used were freely available on the Internet. Further, a similar strategy can be used for designing peptide based drugs for other physiological target molecules. Thus this study introduces a novel paradigm in strategy design for drug-leads.

CONFLICT OF INTEREST
The authors reported no conflict of interest.

References
15. Vlasuk GP, Rote WE. Inhibition of factor VIIa/tissue


The introduction of highly active anti-retroviral therapy (HAART) has markedly decreased the rates of opportunistic infections, the progression to AIDS, and the overall mortality for HIV-infected patients. In some patients, however, the effective immune reconstitution that follows HAART can result in an exuberant inflammatory response leading to paradoxical clinical deterioration. The presence of IRIS does not mean that HAART has failed. In fact it shows that the treatment is effective.

In this report, we describe five patients at Sultan Qaboos University Hospital, Oman, who developed IRIS shortly after the initiation of HAART. In addition, a literature review on the epidemiology, aetiology, risk factors, pathogenesis, diagnosis and management of infection-related IRIS is also included in this report.

Case One

A 23 year-old male was diagnosed in 2002 with HIV-1 infection when he presented with Cryptococcus neoformans menigitis. At diagnosis, his CD4 count was 58cells/mm³ and the HIV-1 viral load 110,000 ribonucleic acid (RNA) copies/ml. He received amphotericin B 1mg/kg intravenously (IV) daily for the first 2 weeks, then fluconazole 400 mg PO (per os, i.e. orally) daily for 8 weeks followed by maintenance therapy. Cotrimoxazole was started for pneumocystis pneumonia (PCP) prophylaxis. HAART (zidovudine, lamivudine, indinavir) was started in March 2003, but was later stopped due to poor compliance. In January 2007, he was again restarted on HAART (zidovudine, lamivudine, lopinavir/ritonavir). His pre-HAART CD4 count was 35cells/mm³ and HIV-1 viral load was 159,000
RNA copies/ml. Seven weeks later, he presented with fever and progressive pain and swelling of the right elbow and knee joints. Clinical examination revealed swollen, warm and tender right elbow and right knee with effusion and severe restriction of movements. A whole body bone scintigraphy, using technetium-99m-methylene diphosphonate (Tc99m-MDP), was done as well as a magnetic resonance imaging (MRI) scan of the right elbow and knee joints [Figures 1 and 2, respectively].

Multifocal septic arthritis of the right elbow and knee joints with osteomyelitis was diagnosed. The right elbow and knee joints were aspirated and drained thick greenish pus. A Ziehl-Neelsen stain of the aspirate from both sites showed sheets of acid fast bacilli. The patient underwent drainage and debridement of the affected bones; tissues were also sent for mycobacterium and fungal culture. Standard quadruple antituberculous therapy (ATT) and clarithromycin were started while awaiting the culture results. HAART was continued and non-steroidal anti-inflammatory drugs (NSAIDs) were prescribed.

A repeat CD4 count at this presentation showed it had risen to 185 cells/mm$^3$ and HIV-1 viral load had dropped to 3350 RNA copies/ml. The culture of the aspirate of both joints and the humeral and femoral bone biopsy revealed growth of non-Mycobacterium avium complex (non-MAC), non-tuberculous Mycobacterium which could not be further identified in the public health laboratory. The isolate was sensitive only to

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**Figure 1:** Three-phase bone scintigraphy delayed images show increased radiotracer uptake in right elbow and knee.

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**Figure 2a:** MRI Right elbow (STIR sequence in coronal plane) demonstrates bone marrow oedema in the lateral epicondyle associated with joint effusion and soft tissue swelling.

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**Figure 2b:** Magnetic resonance image of right knee T1W FS CE in the sagittal plane showing intra osseous abscesses in condyles (not in picture) and patella with joint effusion, synovitis and bone marrow oedema.
ciprofloxacin and clarithromycin while resistant to all standard antitubercular (ATT) drugs including rifampicin, ethambutol and aminoglycosides. The ATT treatment was stopped while ciprofloxacin and clarithromycin were continued in addition to HAART. The patient made a full recovery and has remained asymptomatic to date.

Case Two

A 38 year-old woman was diagnosed in December 2006 with HIV-1 infection after presenting with *Pneumocystis jiroveci* pneumonia (PCP) [Figure 3]. She had a CD4 count of 8 cells/mm³ and the HIV-1 viral load of 314,000 RNA copies/ml at diagnosis. Three weeks later she was started on HAART (zidovudine, lamivudine and efavirenz). About four weeks after commencing HAART, she developed fever, headache and vomiting. The clinical examination showed neck stiffness and positive Kernig’s sign. The computed tomography (CT) scan of the brain was normal. A lumbar puncture was performed and the subsequent India ink stain and *Cryptococcus* antigen were both positive in the cerebrospinal fluid (CSF). *Cryptococcus neoformans* was later isolated from the CSF. The patient received liposomal amphotericin 5mg/kg IV daily for 2 weeks followed by fluconazole 400 mg PO daily for 8 weeks and later maintained on oral fluconazole 200 mg daily. HAART was discontinued temporarily during the initial phase of treatment (the first two weeks), but was later restarted. At this presentation, the CD4 count was 35cells/mm³ and the HIV-1 viral load was 35,000 RNA copies/ml. The patient had a full recovery with follow-up CSF cultures documenting sterility.

Case Three

A 57 year-old man was diagnosed in August 2006 with HIV-1 infection when he presented with biopsy proven *Toxoplasma gondii* brain abscesses. The CD4 count at presentation was 85cells/mm³ and the HIV-1 viral load was >750,000 RNA copies/ml. He was treated with a combination of pyrimethamine 75 mg PO daily, sulfadiazine 1g PO 6 hourly and folinic acid 15 mg PO daily for 6 weeks. He was afterwards put on maintenance therapy. The patient’s symptoms improved as a result of this treatment. A follow up MRI brain confirmed resolution of the lesions. HAART (zidovudine, lamivudine, abacavir) was then started. Eight weeks later, he presented with fever, abdominal pain and diarrhoea. The clinical examination revealed jaundice and tenderness at the right upper quadrant of the abdomen. Acute cholangitis was suspected. An MRI scan of the abdomen was done [Figure 4] and revealed dilated intrahepatic bile ducts with the contrast uptake suggesting inflammation.

The patient underwent upper gastrointestinal (GI) endoscopy, which showed an ulcerative necrotic lesion and areas of hyperaemia involving...
the entire duodenum. A duodenal aspirate and biopsy showed the presence of *Cryptosporidium*. The stool was also positive for *Cryptosporidium*. The test for cytomegalovirus by polymerase chain reaction (CMV-PCR) was negative. The CD4 count at this presentation was 287 cells/mm³ and the HIV-1 viral load was 4670 RNA copies/ml. The patient was started on paromomycin 500 mg PO three times daily and azithromycin 500 mg PO once daily. The HAART was continued and a complete resolution of the symptoms was achieved. A follow-up upper GI endoscopy and MRI six weeks later were both normal.

**Case Four**

A 30 year-old woman was diagnosed in 2003 with HIV-1 infection during pregnancy. The CD4 count was 20 cells/mm³ and the HIV-1 viral load was >750,000 RNA copies/ml. She was then lost to follow-up. In 2005, she presented with severe CMV and *Candida esophagitis* (diagnosis made by upper GI endoscopy and oesophageal biopsy). She was treated with flucanozole 400 mg intravenously (IV) daily and ganciclovir 5mg/kg IV twice daily. Pentamidine 300 mg via aerosol once monthly was also started for prophylaxis against PCP as the patient had a severe allergy to sulpha. HAART (zidovudine, lamivudine, indinavir) was started. Three weeks later, the patient presented with generalised tonic-clonic seizures. The clinical examination was normal. An urgent MRI brain scan [Figure 5a & b ] was consistent with cerebral toxoplasmosis. The serology for toxoplasmosis was positive in both immunoglobulin G and M (IgG & IgM). A working diagnosis of cerebral toxoplasmosis was made and the patient was started on combination of pyrimethamine 75 mg PO daily, clindamycin 450 mg PO three times daily and folinic acid 15 mg PO daily for 6 weeks and later was put on maintenance therapy.

The HAART was discontinued temporarily. The CD4 count at this presentation had risen to 84 cells/mm³ and HIV-1 viral load had fallen to 7130 RNA copies/ml. The patient remained asymptomatic during this period with no recurrence of convulsions. A follow-up MRI brain scan at the end of 6 weeks of treatment confirmed complete resolution. The same HAART regimen was then resumed with no unwanted consequences.

**Case Five**

A 23 year-old man was diagnosed in October 2005 with HIV-1 infection when he presented with uncomplicated *Salmonella* bacteraemia. The CD4 count was 10 cells/mm³ and the HIV-1 viral load was >750,000 RNA copies/ml. The US Centers for Disease Control and Prevention (CDC) stage at diagnosis was B3. He received ciprofloxacin 400 mg IV twice daily for two weeks. HAART
We described five patients with advanced HIV-1 infection in whom initiation of HAART have resulted in unmasking of an underlying occult opportunistic infection. The five cases have all the classical features suggestive of immune reconstitution inflammatory syndrome (IRIS). All five had advanced HIV disease with a very high viral load and low CD4 count at the initiation of the therapy (HAART). The paradoxical worsening and the presentation, with an opportunistic infection 2–8 weeks after the initiation of HAART in all five cases, correlated with the drop in viral load and the rise in CD4 count. The variety of pathology in these five cases associated with IRIS is also remarkable and is consistent with the common rule of IRIS that ‘anything is possible’.

The HAART for HIV infection has been one of the most dramatic progressions in the history of medicine. Since its introduction, HAART has led to significant declines in AIDS-associated morbidity and mortality. These benefits are, in part, a result of partial recovery of the immune system, manifested by increases in CD4+ T-lymphocyte counts and decreases in plasma HIV-1 viral loads.

Soon after the introduction of HAART,
Clinicians noticed that some patients initiating HAART experienced unique symptoms during immune system recovery. In these patients, clinical deterioration occurs despite increased CD4+ T lymphocyte counts and decreased plasma HIV-1 viral loads. This clinical deterioration is a result of an inflammatory response of the immune system to both intact subclinical pathogens and residual antigens. Because clinical deterioration occurs during immune recovery, this phenomenon has been described as immune restoration disease (IRD), immune reconstitution syndrome (IRS), and paradoxical reactions. Given the role of the host inflammatory response in this syndrome, the term immune reconstitution inflammatory syndrome (IRIS) has been proposed.

IRIS can be defined as a pathological inflammatory response and paradoxical clinical deterioration as a result of HAART related immune recovery or reconstitution in HIV infected persons. In other words, IRIS is a syndrome that occurs because a patient develops an exuberant response to appropriate therapy. IRIS is now a widely recognised phenomenon that can complicate HAART.

How IRIS develops is not yet well understood and its pathogenesis remains largely speculative. Current theories concerning the pathogenesis of the syndrome involve a combination of underlying antigenic burden, the degree of immune restoration following HAART, and host genetic susceptibility. These pathogenic mechanisms may interact and likely depend on the underlying burden of infectious or non-infectious agents.

As illustrated in all the five cases above, the majority of patients who develop IRIS do so within the first 4 to 8 weeks after starting HAART. However, the interval between the start of HAART and the onset of IRIS is highly variable, ranging from less than 1 week to several months after HAART initiation. Furthermore, most patients who develop IRIS have had high viral loads and very low CD4+ T-lymphocyte (CD4+) counts. In this report, all five patients had CD4 counts of <100 cells/mm3 at the start of HAART. In addition, three patients had an HIV-1 viral load of >750,000 RNA copies/ml.

The frequency of IRIS has not been reported conclusively and the overall incidence of the syndrome itself remains largely unknown, being dependent on the population studied and its underlying opportunistic infections burden. In the largest study of IRIS to-date, 31.6% of HIV infected patients developed IRIS while on HAART. In a large retrospective analysis examining all forms of IRIS, 25% of patients exhibited one or more disease episodes after initiation of HAART. Other cohort analyses examining all manifestations of IRIS estimate that 17–23% of patients initiating HAART will develop the syndrome. Another study demonstrated that up to one third of patients with HIV/tuberculosis co-infection who begin HAART in resource-limited countries could be at risk of developing tuberculosis-associated IRIS (also known as TB-IRIS).

Collation of the clinical, immunological and immune-genetic data presented over the last 15 years has identified risk factors for IRIS. A higher viral load and low CD4 T cell count at the start of HAART along with a rapid drop in viral load with treatment, as shown in all five cases in this report, has been found to be a favourable setting for IRIS to occur. In the affected patients, CD4 T cell counts are often <50 cells/mm3 at the start of HAART and subsequently increase more than 2–4 fold during the 12 months after initiation of HAART, and a significant decrease in HIV load of more than 2 log10 copies/ml is often noted.

IRIS manifestations are diverse and depend on the infectious or non-infectious agent involved. These manifestations have not been precisely defined. In general, and as illustrated in the case reports above, they are characterised by fever and worsening of the clinical manifestations of the underlying opportunistic infection. These clinical manifestations may be at the site of a previously recognised opportunistic disease (none of our patients) or may “unmask” disease at new sites not previously known to be infected by the pathogen (none of our patients). They may also represent a response to a previously unrecognised additional pathogen (all five patients reported here). A paradoxical clinical worsening of a known condition or the appearance of a new condition after initiating therapy characterises the syndrome.

*Mycobacterium tuberculosis* (TB) is among the most frequently reported pathogens associated with IRIS. The commonest clinical manifestations of TB-IRIS are fever, lymphadenopathy and worsening respiratory symptoms. In most studies, TB-IRIS occurs within two months of HAART initiation. These studies suggest that the onset of
Mycobacterium-associated IRIS is relatively soon after initiation of HAART, and clinicians should maintain a high level of vigilance during this period.

The last patient in our report (Case Five) was a classic case of TB-IRIS. He presented with fever and cervical lymph-nodes enlargement shortly (four weeks) after initiation of HAART. Hepatosplenomegaly probably signifies widespread TB disease and its subsequent regression on ATT further supports this hypothesis.

In addition to Mycobacterium tuberculosis, nontuberculous mycobacteria are also frequently reported as causative pathogens in IRIS. Mycobacterium avium complex (MAC) remains the most frequently reported atypical mycobacterium linked with IRIS. Other atypical mycobacteria are rarely associated with IRIS.

The first patient in our series (Case One) had a nontuberculous Mycobacterium that was not MAC (and could not be further identified) which resulted in a severe form of multiple-site osteomyelitis. This disease manifested shortly after introduction of HAART (seven weeks later). Furthermore, it occurred at the time of marked immune recovery and marked improvement in CD4 count (CD4 count had risen 4 to 5 fold by then).

Soon after the introduction of HAART, it was observed that some patients presented with an initial or recurrent episode of cryptococcal meningitis during the first few weeks of therapy; however, IRIS with cryptococcal meningitis is rare. The most common IRIS syndrome associated with cryptococcal infection is lymphadenitis. IRIS was reported in 30–33% of HIV-infected patients with Cryptococcus neoformans after the initiation of HAART. Lower CD4-cell count and higher HIV-RNA concentrations at the onset of infection correlated with a higher risk of IRIS. Patients with IRIS also had a greater reduction in HIV-RNA concentration within 90 days of HAART initiation, and had a greater fungal burden at the onset of infection. Furthermore, initiation of HAART within 30–60 days of the treatment of fungal infection has been associated with a higher risk of IRIS.

The second patient (Case Two) in our report presented with an initial, first episode of cryptococcal meningitis while on HAART (3 weeks later). She had an extremely low CD4 count at the time of the initiation of HAART (CD4 8 cells/mm3) and had a dramatic immune recovery with a 4-fold CD4 count rise and >1 log reduction in the HIV-1 viral load at time of presentation.

The number of reports of IRIS associated with parasitic diseases is small but increasing. Only two cases of suspected IRIS associated with toxoplasma encephalitis have been reported in published literature. In one case, no clinical details were given. In the other, an HIV-infected patient with a nadir CD4 cell count of 83 cells/mm3 presented with a focal seizure after 3 weeks of HAART.

The fourth patient in this report (Case Four) presents, to our knowledge, only the third reported case in the literature of IRIS associated with toxoplasma encephalitis. This patient presented with generalised seizures three weeks after initiation of HAART. He had an exceptionally low CD4 count (CD4 count 2 years earlier was 20cells/mm3) and a very high HIV-1 viral load (>750,000 RNA copies/ml) at the time of initiation of HAART. He had a dramatic immune recovery with a 4-fold CD4 count rise and major reduction in the HIV-1 viral load of >2 log reduction at the time of presentation with cerebral toxoplasmosis. The diagnosis of cerebral toxoplasmosis was made on the basis of positive serology, multiple ring-enhancing intra-cerebral lesions on an MRI scan and a positive response to treatment for toxoplasmosis with complete resolution on follow-up brain imaging.

Of interest is that there are no frequent reports in the published literature of IRIS associated with Cryptosporidium infection. We here report a rare case of Cryptosporidium infection associated with IRIS.

Our patient (Case Three) presented eight weeks following initiation of HAART with fever, abdominal pain and diarrhoea. This occurred at a time where immune recovery was dramatic (CD4 count rise from 85 to 287 cells/mm3) and HIV-1 viral load reduction was substantial (HIV-1 viral load reduction >2 log). Endoscopy proven extensive duodenal inflammation and ulcers were found in addition to clinical, biochemical, and an MRI assisted diagnosis of cholangitis. Furthermore, the duodenal aspirate and multiple duodenal biopsies confirmed the presence of Cryptosporidium. The stool sample was also positive for Cryptosporidium. No other aetiologies were identified. The resolution of symptoms and normal subsequent endoscopy further supports the diagnosis of IRIS associated
Unmasking Immune Reconstitution Inflammatory Syndrome (IRIS)
A report of five cases and review of the literature

Cryptosporidium. To our knowledge this is the first reported case in the literature.

Diagnosis of IRIS is clinically challenging and involves differentiation between the progression of the initial opportunistic infection (OI) (including the possibility of antimicrobial resistance and treatment failure); development of a new OI; unrelated organ dysfunction, or drug toxicity. IRIS should be suspected in patients who show clinical or radiologic deterioration following initiation of HAART accompanied with improvement in CD4 count and viral load.

Since there is no diagnostic test for IRIS, confirmation of the disease relies heavily upon case definitions incorporating clinical and laboratory data. French et al.7 and Shelburne et al.22 published two of the most widely used IRIS case definitions in the literature. In a recent article, published in the Clinical Infectious Diseases journal, Haddow et al. proposed a revised case definition of IRIS incorporating both definitions along with separate definitions for unmasking and paradoxical IRIS.23 The current management of IRIS remains controversial and therapy for IRIS is largely empiric. There are no well-controlled trials concerning the management of IRIS. All evidence in the literature regarding the management of IRIS is found in case reports and small case series reporting on management practice. Furthermore, no clear guidelines exist regarding the continuation of HAART during IRIS; therefore, the decision to continue HAART in spite of IRIS should be based on the clinical scenario. If the pathogens involved in IRIS are not amenable to specific treatment, or if life-threatening events occur and steroids are ineffective, one should consider suspending antiretroviral therapy.24

Symptomatic therapy for IRIS can be tried with NSAIDs and steroids. Immune modulation in some instances is warranted, but specific drugs and protocols are lacking. Other modalities tried include thalidomide and intravenous immunoglobulin (IVIG)

In our report, HAART was discontinued temporarily in three patients (cases Two, Four and Five). These patients had cryptococcal meningitis, cerebral toxoplasmosis and TB lymphadenitis respectively. HAART was discontinued in the patient with cryptococcal meningitis during the induction phase of treatment (first two weeks) and was restarted thereafter with no complications. In the patient with cerebral toxoplasmosis, HAART was suspended until complete resolution of the brain abscesses and associated oedema (six weeks). HAART was then reintroduced with no problems. In both scenarios, HAART was suspended temporarily to minimise the inflammatory process and its potential unwanted consequences in a critical site (brain). In the patient with TB-IRIS, HAART was suspended during the initiation phase of ATT (first two months) only to enhance patient compliance with TB treatment. Both HAART and ATT were later combined resulting in cure.

HAART was continued without interruption in the remaining two patients (cases One and Three). In the patient with atypical Mycobacterium associated-osteomyelitis (Case One), in addition to continuing HAART, NSAIDs were used. Specific therapy with clarithromycin and moxifloxacin resulted in cure. In the patient with Cryptosporidium induced duodenitis and cholangitis (Case Three), HAART was continued and specific prolonged treatment with paromomycin and azithromycin was given resulting in clearance of the parasitic infection and subsequent cure.

Although some IRIS cases are short-lived, or cause minor clinical problems, others may result in significant morbidity and sometimes death. However, IRIS does not appear to have favourable or unfavourable implications about patient survival, with the possible exception of IRIS associated with cryptococcal meningitis.10

To date, with exception of the patient with TB-IRIS (Case Five), who died from disseminated Kaposi sarcoma, the other four patients have remained well and had optimal clinical, immunologic and virological responses on HAART.

Conclusion

Our cases establish the fact that IRIS is a significant problem in the post HAART era and is associated with several challenges for the treating physicians. As the use of HAART increases around the world (including developing countries where a large number of severely immune-deficient patients are being given HAART), physicians managing patients with HIV infection will encounter increasing numbers of patients with IRIS. The inclusion of IRIS in the differential diagnosis of a patient who
presents with an inflammatory process after initiating HAART allows for a focused approach to diagnosis and therapy. Further studies are needed to help us to understand how to deal with this clinically significant problem. Guidelines for the management of IRIS and the use of HAART during IRIS also need to be developed.

References


ASOACTIVE INTESTINAL POLYPEPTIDE (VIP) secreting tumours are a rare cause of chronic or occasionally acute secretory diarrhoea and more than 90 percent are found in the pancreas. The tumour incidence is estimated to be one in 10 million persons per year. They are now referred to as VIPomas; 10% of them are neural in origin and found mainly in children. The first description of an association between a pancreatic islet cell tumour and watery diarrhoea was by Priest and Alexander in 1957. One year later, Verner and Morrison described 2 similar patients. The association between watery diarrhoea and elevated VIP levels was reported by Bloom in 1973, and the clinical syndrome later confirmed when five healthy subjects developed profuse diarrhoea within 4 hours of receiving an infusion of porcine VIP. Synonyms include the watery-diarrhoea-hypokalaemia-achlorhydria (WDHA) syndrome, the Verner-Morrison syndrome and pancreatic cholera. Functioning neuroendocrine tumours (NETs) of the pancreas may secrete a variety of different polypeptides causing a variety of different syndromes; those that secrete an excess of VIP are termed VIPomas, and usually present with chronic refractory diarrhoea and are often malignant at presentation. Differential diagnoses include enterotoxin production by vibrio cholera and E.coli,
rectal vilous adenomas and bile salt entropathy.

Case Report

A previously fit Sri Lankan doctor presented to his local hospital with a history of well controlled diabetes mellitus for 5 years and hypertension for 15 years; his medications were valsartan, atenolol and mixtard insulin. He had noted loose watery motions for 2–3 years which he attributed to irritable bowel syndrome (IBS). Three months before being referred here, his diarrhoea had worsened and he was admitted with diabetic ‘ketoacidosis’ and treated with intravenous insulin (IV) and fluids. Two days later, he developed acute renal failure requiring haemodialysis for 3 days. He was treated with ciprofloxacin and discharged after two weeks. Three weeks later, he was again readmitted with profuse watery diarrhoea, confusion, vomiting and acute renal failure. Investigations revealed creatinine >300 µmol/L (normal range [NR] 59–106), potassium 2.5 mmol/L (NR 3.5–5.0) and calcium of 3.5 mmol/L (NR 2.2–2.5). During catheter insertion for haemodialysis he vomited and aspirated his stomach contents resulting in a cardiorespiratory arrest. He was intubated and ventilated for 12 days during which time he had haemodialysis for three days, together with upper and lower gastrointestinal (GI) endoscopies which revealed a fluid filled stomach and colon with normal appearing mucosa. On his final admission to the referring hospital, he was started on ciprofloxacin, metronidazole, octreotide 50 mcg twice daily as a neuroendocrine tumour was considered as one of the differential diagnoses. After several days, however, the diarrhoea still persisted and metronidazole was added to treat possible antibiotic-associated diarrhoea. Extubation failed on at least two occasions; he was then transferred to Sultan Qaboos University Hospital by aeroplane on a ventilator, intravenous fluid and ionotropes. During the flight his blood pressure fell to 30 systolic. On arrival here, he was gravely ill with a blood pressure (BP) of 45/30 on dopamine and IV fluids. The serum creatinine was now 180 µmol/L, potassium 2.8 mmol/L, HCO₃ 10 mmol/L (NR 22–29), Hb 19 gm/dl (n 11.5–15.0). The estimated stool volume before receiving octreotide was as much at 20 liters/day as within 12 hours he had passed 12 liters by rectal tube.

An endocrine consultation was asked for 12 hours after his admission and based on his history, the presence of secretory odourless diarrhoea, hypokalaemia and metabolic acidosis, a clinical diagnosis of VIPoma was made. The patient was then immediately started on a therapeutic trial of octreotide, 100 mcg 8 hourly, after taking blood samples to test for for VIP, chromogranin A and other hormone levels. His response was dramatic and similar to a patient treated with long acting octreotide in 1985 who also had an immediate cessation of diarrhoea. One day later, the diarrhoea had improved substantially; he was extubated, the dopamine stopped and his BP returned to normal. By day four, his electrolyte profile was normal without any additional therapy [Table 1].

An abdominal ultrasound revealed an 8 cm mass in the left upper quadrant. Computed tomography (CT) and magnetic resonance imaging (MRI) scans

### Table 1: Stool volume: response to octreotide 100 mcg 8 hourly.

<table>
<thead>
<tr>
<th>Days</th>
<th>Stool Vol in Litres</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
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<tr>
<td>4</td>
<td>10</td>
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<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

Figure 1: MRI scan showing pancreatic tail mass 8.2 x 7.5 cm with necrosis.
VIPoma Crisis
Immediate and life saving reduction of massive stool volumes on starting treatment with octreotide

[Figure 1] of the abdomen revealed an 8.2 x 7.5 cm well encapsulated and necrotic pancreatic tail mass with no evidence of metastases. After three weeks on octreotide, the patient had gained 7 kg and felt quite normal.

On day 21, he underwent a distal pancreatectomy and splenectomy [Figure 2]. No liver, peritoneal or lymph node metastases were seen. The patient had an uneventful postoperative course and the diarrhoea has not since recurred. The octreotide was stopped four days after surgery.

Histological examination revealed a NET with partial capsular invasion [Figure 3] strongly positive for chromogranin A [Figure 4] and VIP. A minority of cells were positive for glucagon and pancreatic polypeptide (PP). The margins of the pancreas were free of tumour and the spleen was normal. Two weeks post surgery, the repeated VIP test revealed a level of 46 and chromogranin A 37, both normal (Table 2). An octreoscan performed 14 weeks after surgery showed no evidence of recurrence.

Discussion
This patient's symptoms improved immediately with the introduction of octreotide in a dose of 100 mcg thrice daily having previously failed to respond to a much smaller dose. VIP is a 28 aminoacid polypeptide which is widely distributed throughout the body and normally functions as a neurotransmitter. In the gut, VIP regulates the blood flow, smooth muscle activity, pancreatic and intestinal secretions and inhibits gastric acid production. It also stimulates intestinal water, sodium and chloride secretion, inhibits resorption and increases colonic potassium secretion.

VIPomas are rare NETs with only 241 cases reported worldwide until 1998. Our patient's presentation was typical with copious watery odourless diarrhoea, hypokalaemia and metabolic acidosis. The secretory nature of the diarrhoea was confirmed by its failure to respond to fasting. Hypokalaemia results from losses in the stool and the acidosis from a combination of dehydration, hypotension and renal failure which was profound in our patient due to his massive stool volumes estimated to be up to 20 litres/day. This is much more than the usually reported volumes of 6–8 litres daily. Hyperglycaemia occurs in one third of patients and results from increased hepatic glucogenolysis caused by the elevated VIP levels. However, this was not the mechanism in our
patient as his insulin requirement did not change following treatment with octreotide, resection of the tumour, and normalisation of the VIP levels. The patient’s hypercalcaemia resolved during treatment with octreotide and intravenous fluids excluding associated hyperparathyroidism; it was related either to dehydration induced hyperalbuminaemia or to increased bone resorption caused by excessive VIP or other peptides produced by the tumour.13

Octreotide controls symptoms and normalises VIP levels in nearly 90% of patients often with extensive metastatic disease.4 Because our patient was so ill, octreotide was given as an immediate therapeutic trial before tumour localisation studies were carried out. The results were remarkable and gratifying; the diarrhea improving substantially after his first 100 mcg injection [Tables 1 and 2]. Octreotide was continued until 4 days after surgery at which time he had gained 7 kg. More than 70% of VIPoma patients present with metastatic disease.13 Fortunately, this was not the case here, but his tumour was large and histologically reported as a tumour of uncertain behaviour. He will therefore be followed up with serial VIP measurements and, if indicated, further octreotide scanning.

Conclusion

To conclude, this was a case of unexplained massive watery diarrhoea which responded immediately to octreotide treatment. Clinicians facing similar cases should prescribe a therapeutic trial of octreotide without waiting for the results of investigations as it might be life saving.

ACKNOWLEDGEMENTS

We thank Dr Dhia Al-Layla of Sultan Qaboos Hospital, Salalah, Oman, for suggesting the possible diagnosis of a neuroendocrine tumor (VIPoma) and referring the patient to us. We also thank Professor Gordon Stamp (Royal Postgraduate Medical School, London) for reviewing the slides and the VIP immunostaining.

References


Table 2: Serum peptide and electrolyte levels before (day 0) and upon discharge. CG-A: chromogranin A

<table>
<thead>
<tr>
<th>Time</th>
<th>VIP ng/L</th>
<th>CG-A µg/L</th>
<th>K mmol/L</th>
<th>Crea µmol/L</th>
<th>HCO³ mmol/L</th>
<th>Ca mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Admission Day 0</td>
<td>900</td>
<td>240</td>
<td>2.5</td>
<td>180</td>
<td>10</td>
<td>3.5</td>
</tr>
<tr>
<td>Post surgery Day 31</td>
<td>46</td>
<td>37</td>
<td>4.1</td>
<td>70</td>
<td>26</td>
<td>2.2</td>
</tr>
<tr>
<td>Normal range</td>
<td>&lt;65</td>
<td>&lt;100</td>
<td>&gt;3.5</td>
<td>&lt;104</td>
<td>&gt;18</td>
<td>&lt;2.6</td>
</tr>
</tbody>
</table>

**Table 2:** Serum peptide and electrolyte levels before (day 0) and upon discharge. CG-A: chromogranin A
Spongistically occurring epidural haematomas (SEDH) are an uncommon entity. The possible causes are neoplastic lesions, craniofacial infections, bleeding disorders, uraemia, sinus thrombosis, heart surgery, arteriovenous malformation and intra-diploic epidermoid cysts. The case reported by Ahmad et al. is the only case of spontaneous posterior fossa epidural haematoma which occurred in a child with a cardiopulmonary bypass. We report the first case of spontaneous posterior fossa epidural haematoma in a patient of sigmoid sinus thrombosis secondary to mastoiditis.

**Case Report**

A 40 year-old man was referred to the outpatients department of C.S.M. Medical University Hospital, India, with complaints of mild headache and incoordination. He had a 10-year history of recurrent right ear discharge which had been treated by oral antibiotics, but had no ear discharge at the time of presentation. He had no history of bleeding diathesis or trauma and was not a known case of any medical ailment. On examination, he was conscious, oriented and higher mental functions were normal. All his cranial nerves were intact with no signs of meningeitis. He had an impaired tandem gait with no positive cerebellar signs. His haematological parameters, including renal function test, liver function test, coagulation profile were normal. A magnetic resonance imaging (MRI) confirmed epidural sub-acute bleeding in the right side of posterior fossa with mastoiditis and right sigmoid sinus thrombosis. A right suboccipital craniotomy and evacuation of the haematoma was done and the patient was relieved of his complains.

**Keywords:** Haematoma, epidural; Cranial fossa posterior; Sinus thrombosis; Mastoiditis; Case report; India
function test and coagulation profile, were normal.

The magnetic resonance imaging (MRI) scan of his brain with a venogram was already available and showed a well defined extra axial lesion on right side of the posterior cranial fossa postero-medially to the right mastoid with hypointense signal on T1W, T2W and GRE images [Figure 1] and peripherally hypointense on T1W and T2W image suggestive of early subacute epidural bleeding. The right mastoid showed T1 hypointense and T2 hyperintense lesion suggestive of mastoiditis. A magnetic resonance (MR) venogram imaging scan demonstrated a right sigmoid sinus thrombosis [Figure 2]. As both the MRI and the venogram demonstrated a haematoma with thrombosed right sigmoid sinus, no other investigation was done and surgery was planned.

A retromastoid suboccipital craniotomy was done and an epidural organised clot of 30 ml volume evacuated. A craniotomy was preferred over craniectomy as peroperatively no evidence of infection was found and the craniotomy prevents a bone defect. The free bone flap was washed with chloramphenicol saline and kept in chloramphenicol saline during the surgery.

Mild oozing was noted from the transverse sinus region which was managed by packing with absorbable haemostat (Surgicel®, Ethicon, Inc., Summerville, NJ, USA). The patient was complaint free in the postoperative period and was subsequently transferred to the Ear Nose and Throat Department for the management of right mastoiditis and then modified radical mastoidectomy after 4 weeks. The haematoma was confirmed on histopathology and the culture was found to be sterile.

Discussion

Spontaneously occurring cranial epidural haematomas have been reported in the literature worldwide. Liedbeck et al. in 1848 reported the first case of SEDH in a patient with haemophilia. Schnieder et al., in 1951, were the first to report a case of SEDH in a case of craniofacial infection in autopsy of two patients. To date there have been 55 reported cases of SEDH [Table 1].

There are seven possible causes for SEDHs mentioned in the world literature. The commonest possible cause for spontaneous epidural haematoma reported in the international literature is neoplastic condition. The most common type of SEDH is hepatocellular carcinoma reported in seven cases. The other neoplastic conditions mentioned are eosinophilic granuloma, Langerhans' cell histiocytosis, lung carcinoma, ovarian carcinoma, malignant fibrous histiocytoma, and Ewing's sarcoma.

The second commonest possible cause for
Mastoiditis causing Sinus Thrombosis and Posterior Fossa Epidural Haematoma
Case report

SEDH reported is associated craniofacial infection which was present in 17 of the 55 reported cases.12-19 Two mechanisms have been proposed for such bleeding. The first is arteritis, which results in weakening of the meningeal vessel wall and causes subsequent bleeding. The second mechanism proposed is progressive detachment of the dura mater from the inner table of the skull by the accumulation of exudates, pus, or air.

The next most common cause reported is bleeding disorders. There are 11 case reports of bleeding disorders causing SEDH. The possible causes mentioned are haemophilia,20 thrombocytopenia, hypofibrinogenemia21 and vitamin K deficiency. Epidural haematomata (EDH) are reported to be caused by chronic renal disease and uraemia.22 The coagulation abnormalities associated with uraemia or heparin use during haemodialysis may be the factors responsible for the EDH in such patients. Platelet dysfunction, as has been reported in uraemia, may further explain EDH formation in these patients.

There are two case reports of SEDH in patients of cardiopulmonary bypass.1,23 Ahmad et al. reported a case of spontaneous posterior fossa epidural haematoma in a child who had undergone a cardiopulmonary bypass. This was the first reported case of spontaneous posterior fossa extradural hematoma.3 Other causes of spontaneous extradural haematoma reported are dural vascular anomalies and intradiploic epidermoid cysts.11

Knopman et al. reported a case of SEDH secondary to venous sinus thrombosis.24 The mechanism for the development of the haematoma is that the venous haemodynamic pressure increases secondary to poor outflow, causing tension on the proximal venous system. This tension causes blood to dissect between leaflets of the proximal sinus and into the epidural space.

Mastoiditis is known to cause sigmoid sinus thrombosis.25 Our patient developed spontaneous posterior fossa epidural haematoma secondary to sigmoid sinus thrombosis which was due to mastoiditis. This is the first reported case of SEDH of the posterior fossa in sigmoid sinus thrombosis due to mastoiditis.

### Table 1: Reported cases of spontaneous epidural haematomas with aetiology

<table>
<thead>
<tr>
<th>Aetiology</th>
<th>Example</th>
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<tbody>
<tr>
<td>Neoplastic condition</td>
<td>Hepatocellular carcinoma³</td>
</tr>
<tr>
<td></td>
<td>Eosinophilic granuloma²</td>
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<tr>
<td></td>
<td>Langerhan’s cell histiocytosis⁶</td>
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<tr>
<td></td>
<td>Lung carcinoma³</td>
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<td></td>
<td>Ovarian carcinoma³</td>
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<td></td>
<td>Malignant fibrous histiocytoma⁷</td>
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<td></td>
<td>Ewing’s sarcoma⁸</td>
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<tr>
<td></td>
<td>Intradiploic epidermoid¹</td>
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<tr>
<td>Craniofacial infection</td>
<td>Fontal sinusitis¹</td>
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<td></td>
<td>Middle ear suppuration¹</td>
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<td></td>
<td>Orbital cellulites¹</td>
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<td>Pansinusitis¹</td>
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<td></td>
<td>Maxillary sinusitis¹</td>
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<td></td>
<td>Cleft palate with frontal sinusitis¹</td>
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<td></td>
<td>Sphenoiditis¹</td>
</tr>
<tr>
<td></td>
<td>Nasal polyps¹</td>
</tr>
<tr>
<td>Coagulation abnormality</td>
<td>Haemophilia³</td>
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<tr>
<td></td>
<td>Hypofibrinogenemia²</td>
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<tr>
<td></td>
<td>Uraemia²</td>
</tr>
<tr>
<td>Heart surgery</td>
<td>Open heart surgery¹</td>
</tr>
<tr>
<td></td>
<td>Cardiopulmonary bypass surgery⁹</td>
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</tbody>
</table>

Conclusion

Spontaneously occurring EDH is an uncommon entity. Certain conditions have been reported to be associated with SEDH such as craniofacial infections, hepatocellular carcinoma and bleeding disorders. Craniofacial infections are the most commonly associated condition. This is the first case report of sigmoid sinus thrombosis secondary to mastoiditis thus causing EDH of the posterior cranial fossa.

### References


Stump Appendicitis following Laparoscopic Appendectomy

Suresh Parameshwarappa, Gabriel Rodrigues, Raghunath Prabhu, Charudutt Sambhaji

Abstract:
Stump appendicitis (SA) is a rare clinicopathologic entity characterised by inflammation of the appendiceal remnant after incomplete appendectomy. The diagnosis is not routinely suspected in patients who have previously undergone appendectomy. We report a case of SA in an adolescent boy who had previously undergone laparoscopic appendectomy. The case necessitated surgical completion of the appendectomy.

Keywords: Appendicitis; Stump; Surgery; Case report; India

Stump Appendicitis (SA) is a rare post-appendectomy complication whose exact incidence and prevalence are not clearly defined. The incidence of SA may be increasing, possibly because of widespread utilisation of laparoscopic appendectomy. This may result in a long appendiceal stump which is the commonest aetiology for stump appendicitis. Confirmation is either by imaging studies or diagnostic laparoscopy. Only a few cases of SA have been reported in English medical literature.

Case Report
An eighteen-year-old boy who had undergone laparoscopic appendectomy one year previously presented at Kasturba Medical College Hospital, Manipal, India. He had lower abdominal pain of three days duration which was most acute in the right iliac fossa. It was not associated with fever and vomiting. He had had a similar episode six months previously, which had subsided with conservative treatment. The general physical examination was unremarkable. Per abdominal examination revealed healed scars of laparoscopic surgery, along with tenderness in the right iliac fossa with no palpable mass. Laboratory investigations were within normal limits. A contrast enhanced computed tomography (CECT) scan of the abdomen and pelvis showed a short length tubular structure at the caput caecum with enhancing wall and stranding of adjacent perappendiceal fat consistent with an inflamed appendiceal stump [Figure 1]. A preoperative diagnosis of stump appendicitis was made and an open ‘completion’ appendectomy was performed with an uneventful postoperative period.

Discussion
Stump appendicitis is rare following previous appendectomy, the known causative factor being a long residual stump. The true incidence and prevalence of SA are not known largely due to under-reporting and its poor definition. The first case of SA was reported by Rose in 1945 and since then thirty-seven cases of ‘residual appendicitis’

Departments of Surgery and Radiology, Kasturba Medical College, Manipal University, Manipal, Karnataka, India.
*Corresponding Author email: rodricksgay@yahoo.co.in
have been reported in English medical literature. The age at presentation of SA ranged from 11 to 72 years (mean 39 years) with an equal incidence in both sexes. The time of onset ranged from two weeks to decades after the appendectomy.

Although no relationship between laparoscopic appendectomy and SA has yet been conclusively demonstrated, the occurrence of SA is more prevalent following laparoscopic appendectomy than conventional appendectomy. The causes of SA are insufficient inversion of the stump, a long proximal remnant of the appendix, incomplete removal of the distal remnant and partial laparoscopic or laparotomic appendectomy. The growing utilisation of laparoscopic appendectomy may increase the frequency of stump appendicitis due to the potential limitations of this technique. These include a smaller field of vision, lack of three-dimensional perspective and the absence of tactile feedback, thus potentially leaving a longer stump. Other causes reported in the literature include inadequate identification of the appendiceal base because of severe local inflammation; a retrocecal or sub-serous appendix; simple ligation of the appendix without invagination of the stump; leaving a long stump due to fear of caecal injury, and difficult dissection and local ulceration due to faecolith. A stump longer than 5 mm serves as a reservoir for the faecolith, becomes ischaemic and eventually perforates. Inversion of the long stump into the caecal wall does not necessarily prevent SA and other complications. Clinically, SA patients present with signs and symptoms similar to those of appendicitis or acute abdomen and with a previous history of appendectomy. The presence of an appendectomy scar does not absolutely rule out the possibility of SA.

Ultrasonography (USG) and a CT scan of the abdomen constitute the modalities of choice for preoperative diagnosis of SA. USG can reveal a thickened appendix stump, fluid in the right iliac fossa and caecal oedema. A CT scan of the abdomen and pelvis is more useful than USG for the accurate preoperative diagnosis of SA, as it also excludes other aetiologies. The CT features of SA are similar to those of acute appendicitis, which include pericecal inflammatory changes, abscess formation, fluid in the right paracolic gutter, cecal wall thickening, and an ileocecal mass. A specific diagnosis of SA can be made preoperatively when inflammatory changes surround a distended appendiceal stump. Barium studies and colonoscopy have also been reported as being helpful in diagnosing SA. Diagnostic laparoscopy is gaining popularity and can be helpful for the diagnosis of SA.

SA is most commonly treated by an open operation, but cases successfully treated using laparoscopic intervention, have been reported. Very rarely, an aggressive surgery such as ileocolic resection may be obligatory. Laparoscopy seems to be more successful than conventional laparotomy as it permits a global inspection of the abdominal cavity and an easier adhesiolysis. Accurate visualisation of the base and leaving a stump less than 3 mm in depth can minimise the incidence of SA. Use of angled scopes may provide good visualisation of the appendix.

**Conclusion**

The diagnosis of SA is often missed or delayed. It should be considered in patients presenting with right lower abdominal pain and a history of previous appendectomy. CT imaging studies help in preoperative diagnosis of SA. Completion of the appendectomy by conventional or laparoscopic means is the treatment of choice for SA.
References


Odontogenic Cutaneous Fistula
Report of two cases

*Nafisa Samir,1 Abdulaziz Al-Mahrezi,1 Salim Al-Sudairy2

A fistula is an abnormal pathological pathway between two anatomic spaces or a pathway that leads from an internal cavity or organ to the surface of the body. A sinus tract is an abnormal channel that originates or ends in one opening. An odontogenic cutaneous fistula is a pathologic communication between the cutaneous surface of the face and the oral cavity. In the literature, the terms fistulas and sinuses are often used interchangeably. A cutaneous fistula of dental origin is a rare entity, but well documented in medical, dental and dermatological literature.1,3 As the lesion develops, it is usually not thought to be of dental origin and the patient seeks treatment from a dermatologist, a family physician or a general surgeon, often undergoing multiple antibiotic regimens, surgical excisions and biopsies. It has been estimated that half of the patients with odontogenic cutaneous fistulas are submitted to multiple dermatological surgical operations and long-term antibiotic therapy before the correct diagnosis is established.4 Delay in diagnosis adds to the chronicity of the lesion and sometime leads to cosmetic deformities secondary to cutaneous scarring.

An odontogenic cutaneous fistula usually arises as a sequel to bacterial invasion of the dental pulp through a breach in the enamel and the dentine by a carious lesion, trauma, or other causes.5,6 If treatment is not initiated at this stage, the pulp becomes necrotic and infection spreads beyond the confines of the tooth into the peri-radicular area resulting in apical periodontitis that subsequently dissects along the path of least resistance and erupts through the skin.6 Once diagnosed, the treatment is simple and effective, consisting of removal of the infected pulp tissue and filling of the root canal with biocompatible material resulting in minimal scarring of skin.

CASE ONE - DESCRIPTION
A 25 year-old man with no known co-morbidity presented to the Family Medicine Clinic of Sultan Qaboos University Hospital (SQUH), Oman, for the evaluation of a chronically draining, dimpled, crusted small nodule on his right cheek.

Abstract:
Odontogenic cutaneous fistula or sinus is an uncommon, but well documented condition, which is often initially misdiagnosed as a sole cutaneous lesion and inappropriately treated. The misdiagnosis as a skin infection often results in inappropriate management. We here present two cases of odontogenic cutaneous fistula that were seen after being treated unnecessarily with antibiotics.

Keywords: Odontogenic; Cutaneous fistula; Case report; Oman

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was initially diagnosed as a superficial skin infection and was treated for several months with a number of systemic and topical antibiotics which provided only temporary relief. Physical examination revealed a small retracted skin lesion on lower part of right cheek of about 5 mm in diameter. The skin opening of the lesion was crusted, but with minimal swelling. Gentle pressure on the surrounding tissue elicited a scanty non-purulent bloody discharge on the surface. There was also a cord-like palpable tract extending from the cutaneous lesion to the oral cavity inside. Intra-oral examinations showed poor oral hygiene and dental caries in the right lower premolar and first permanent molar (teeth nos. 45 and 46). The patient was referred to an oral and maxillofacial surgeon. His orthopantomogram (OPG) demonstrated well defined radiolucency around the apex of teeth 45 and 46 [Figure 1].

CASE TWO – DESCRIPTION

An 18 year-old male student was referred from the SQUH Surgery Department to an oral and maxillofacial surgeon in the Oral Health Department regarding a chronic, but painless, draining lesion on the left side of the cheek. Like the first case, this patient had also been treated for several months with antibiotics. On examination, there was a crusted skin lesion of about 0.8 cm in diameter on left side of the face [Figure 2]. Again, non-purulent discharge was expressed on pressing the surrounding skin. Intra-oral examination showed poor oral hygiene and caries in the lower left second premolar (tooth no. 35) and lower left first permanent molar (tooth no. 36). His OPG also showed radiolucency at the apex of teeth numbers 35 and 36 [Figure 3].

CASES ONE AND TWO – TREATMENT

The intra-operative findings for both patients showed a fluid-filled cyst around diseased teeth with a draining tract extending from the cyst to the outside skin. Both patients were booked for surgery under general anaesthesia (GA), as not only was the extraction of diseased teeth required, but also the excision and repair of the skin lesions. To excise the fistula, first an elliptical incision was made on the skin layer only. In this way, the facial nerve was protected as it lies in the deeper fascia. Then the dissection was made below the skin until the fistula tract was reached. The dissection was continued through the muscle until the intraoral origin of the tract was reached. The affected teeth were removed and the cystic lesion and granulation tissue were completely enucleated. The incision site was irrigated and closed in layers (muscles and oral mucosa with 3.0 vicryl suture and skin with 4.0 nylon suture). The elliptical shape of the incision has the effect of minimising post-surgical scar formation as compared to the disfiguring scar which may result if the lesion is left to heal on its own by granulation. Both patients were followed up postoperatively and both showed significant improvement [Figure 4].
Discussion

The clinical cases of odontogenic cutaneous lesions described in this study had been previously misdiagnosed and managed inappropriately. The cutaneous fistula of dental origin is an uncommon, but well documented, condition in the literature. Diagnosis is challenging for many reasons. This can be due to the fact that these lesions do not always arise in close proximity to the underlying dental infection and only about half of patients ever recall having had toothache. A clinician’s high index of suspicion can lead to early and correct diagnosis. A thorough history taking and intraoral examination are critical for making the appropriate diagnosis and may spare the patient much unnecessary treatment. In suspected cases, early consultation with a dentist is of great importance in providing appropriate differential diagnosis and clinical care.

The classic lesion is an erythematous, smooth, symmetrical nodule, 1–20 mm in diameter with or without drainage. The chronic lesion often leads to retraction of skin secondary to scarring. A cord like tract can be felt attached to the underlying bony structure. Many of above mentioned typical findings were present in the two cases described in this report. Patients may experience intermittent remission of the symptoms. Intraoral examination may reveal dental caries or restorations and periodontal disease, but the examiner should keep in mind that even the tooth involved can appear normal. The initial working diagnosis is confirmed by radiographs and pulp vitality tests, diseased teeth responding negatively to the latter. In the clinical cases described here, the radiographs clearly revealed obvious periapical radiolucencies which are associated with diseased teeth. As far as definitive treatment is concerned, root canal therapy or surgical extraction is the treatment of choice.

Usually, no systemic antibiotics need to be prescribed as the lesion is a localised entity, but they should be considered in patients with diabetes, immunosuppression, or signs of systemic infection. Most authors believe that once the primary cause is removed the cutaneous lesion heals without treatment, usually resolving in 1 to 2 weeks. The residual scarring can be surgically revised if it is cosmetically unappealing for the patient. Delay in the treatment of dental infections can sometimes lead to the infection spreading to fascial spaces in the orofacial area or deep in the head and neck (peripharyngeal space infections). In addition, odontogenic infections may cause osteomyelitis of the jaw or systemic illness.

Many patients seek evaluation from several physicians before an accurate diagnosis is made. It has been estimated that half of all patients undergo multiple surgeries and trials of antibiotics before definitive diagnosis. This emphasises the importance of communication between medical sub-specialists and dentists in the evaluation of patients with head and neck lesions.
Conclusion

These cases highlight the fact that dental aetiology should be considered as a part of a differential diagnosis for any orofacial skin lesions. It also serves to underline the importance of communication between medical subspecialties. Although many non-odontogenic disorders may also produce an extra-oral fistula, the opinion of a dentist in cases of a cutaneous sinus tract is of great importance in providing appropriate clinical care. Endodontic treatment or dental extraction may eliminate the infection and lead to resolution of the lesion and prevent unnecessary ineffective antibiotic therapy and/or surgical intervention. These cases would also suggest that dentists may need to educate their medical colleagues and medical students on the importance of oral examination and role of the dentist in the management of oro-facial infections.

References

A 7-year-old boy from South India presented with recurrent left motor seizure with secondary generalisation. The computed tomography scan of his brain [Figure 1] demonstrated a solitary enhancing cystic lesion with eccentric mural nodule (scolex) in the right frontal region. Based on the epidemiological profile (he came from an endemic area), as well
as the clinical and imaging findings, a diagnosis of solitary cerebral cysticercus granuloma was made. He was treated with a short course of anticycticercal treatment (albendazole), prednisolone (after excluding spinal and ocular cysticercosis) and phenytoin. Follow-up imaging at 6 months revealed significant improvement.

Solitary cysticercal cysts are an important cause of symptomatic seizure in endemic areas. Other clinical manifestations include headache and focal neurological deficits. In the natural history of neurocysticercosis, the following stages of evolution of parenchymal larval cysts can be observed on neuroimaging: viable cyst, granulomatous cysticercosis and disappearance of cyst with or without residual calcification.

In the viable cyst stage, the cyst wall is not visible on imaging and the cyst demonstrates little or no perilesional oedema. Ring-like or nodular areas of enhancement with prominent perilesional oedema mark the phase of granulomatous cysticercosis.

The image shown here demonstrates the granulomatous cysticercosis as a ring-enhancing lesion with scolex and peri-lesional oedema. Ultimately, the remnant of the cyst is either not visible on the imaging or observed as calcified lesion(s).

Medical treatment for viable or granulomatous cysticercosis includes antihelminthic medication, standard anticonvulsants for seizures and symptomatic medication such as anti-inflammatory drugs such as steroids. Antiparasitic therapy for solitary cysticercus granuloma is shrouded in controversy for the following reasons: 1) solitary cysticercus granuloma may resolve spontaneously without antihelminthic treatment; 2) the parasite cannot grow and develop further in the cerebral parenchymal location, and 3) antihelminthic treatment kills the parasite that can potentially cause neurological complications such as a transient increase in seizure frequency, headaches, and raised intracranial pressure. The latter complication is observed especially in patients with multiple cysticercus granulomata. The arguments for cysticidal therapy include rapid disappearance of cyst(s) and the possibility of less residual calcification. Albendazole in a dose of 15 mg/kg/day divided into two doses is the antihelminthic drug widely employed. The duration of treatment was 1 month in older studies, but this has been reduced to 15 days and even 1 week in later studies. It is usually administered along with corticosteroids to prevent the neurological complications associated with the degeneration and death of the parasite as mentioned above. A recent Cochrane Database systematic review found evidence for a reduction both in the number of viable lesions and in seizure frequency in those patients with non-viable cysts on albendazole therapy. The other available antihelminthic drug is praziquantel, usually given in dose of 50 mg/kg/day for 2 weeks. A single day course of praziquantel has also been employed. Surgery is usually reserved for extraparenchymal neurocysticercosis such as an intraventricular cyst, spinal cysticercosis causing spinal cord compression, for hydrocephalus, or for ophthalmic cysticercosis.

In general, the seizure outcome in solitary cerebral cysticercosis is good in view of the symptomatic nature of the seizure. The optimal duration of anticonvulsant prophylaxis has not been finally decided. Some authors favour anticonvulsant prophylaxis for 6 months and repeat the neuroimaging scan (by CT or magnetic resonance imaging) to look for resolution of the lesion with a view to tapering anticonvulsants in patients who are fit free, while others have continued anticonvulsant therapy for 2 years. Patients with residual calcification may be at risk of recurrent seizures.

The image is mainly presented here to highlight the characteristic appearance of cysticercus lesion termed ‘hole with a dot’, the hole represents the cysticercus ring lesion itself and the dot the scolex. For physicians in non-endemic regions such as Oman, awareness of this imaging characteristic aids in the recognition of cerebral cysticercosis in patients who originate from or have travelled to endemic areas.

References


A 52 year-old right-handed man, from South India, presented with left hemiparesis of one day duration. He had vascular risk factors such as hypertension, ethanol consumption and cigarette smoking. There was no family history of left handedness. Neurologically, he demonstrated left faciobrachial (muscle power: grade 0–1/5), crural weakness (grade 3–4/5), global aphasia, left hemisensory impairment and left hemianopsia, with a National Institute of Health (NIH) Stroke Scale score of 19. The cardiac evaluation did not disclose valvular lesion/vegetation, mural thrombus, or significant ischaemic lesion. The computed tomography (CT) scan of his brain [Figure 1A] showed recent infarction in the distribution of the right middle cerebral artery, but there were no additional left hemispheric infarcts (also confirmed subsequently...
by a diffusion weighted magnetic resonance imaging scan). As he presented beyond the time window for thrombolytic therapy, he was treated with an antiplatelet drug, statins, antihypertensive medication, speech therapy and physiotherapy. A repeat computed tomography CT brain scan [Figure 1B], performed 7 months later, demonstrated a chronic infarction in the same location with no additional left hemispheric lesion. Although his left hemiparesis had significantly improved (arm power: grade 3–4/5), his global aphasia continued to pose significant problem for verbal communication over the subsequent years.

Crossed aphasia (CA) refers to a language (symbolic communication with words) disorder resulting from a unilateral right hemispheric lesion in dextrals (right-handers). CA is rare with an estimated prevalence of 0.4–3.5% of all aphasic syndromes. In right handers, language function is often lateralised to the left hemisphere. Atypical cerebral dominance for language in our patient accounted for the right hemispheric stroke-related aphasia that was persistent and functionally disabling. The pattern of his clinical course suggested complete lateralisation of language to his right hemisphere. Apart from ischaemic strokes, other aetiologies include aneurysmal subarachnoid haemorrhage, multiple sclerosis, direct cortical stimulation, migraine with aura and focal dementia.4–8

References

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This article is based on an interview with the parents of the child conducted in August 2010 by Theo Poulton, a Year 12 student at The British School Muscat, on placement at Sultan Qaboos University Hospital. It was edited by Dr Prakash Mandhan, his supervisor and the neonatal surgeon in charge of the patient.

“Having children is not only a blessing, but also a joy for parents and grandparents” said Mrs. A. Her son AH, now an eight month-old boy, has spent almost 90% of his life in Sultan Qaboos University Hospital (SQUH). AH and his twin brother were to be the couple’s first children so, naturally, they were very excited as they awaited the birth of their sons. However, this excitement was dampened when they learnt that their son, AH, would need an operation.

AH was born at SQUH in January 2010 and since then has undergone three major and several minor operations. He was born prematurely as the second twin; this meant that he not only weighed less than a normal newborn, but was also more susceptible to infections. Any procedures he underwent would be higher risk to him compared to a full-term baby.

When he was 12 days-old and in the incubator in the Neonatal Intensive Care Unit (NICU), the nurse noted that his abdomen was enlarged and soon green coloured bile started pouring from his mouth. A quick assessment by doctors in the NICU together with X-ray and blood test findings suggested that he had an intestinal infection. The neonatal surgeon on duty was called and AH immediately underwent an emergency operation to save his intestine. “When the neonatal surgeon told us about AH’s condition, the necessity of urgent operation and the expected outcome, we were very worried and prayed to Almighty God to save his life” said AH’s parents. “I was so worried because as it was such a big operation for such a small baby (1.4 kg). I wondered, how can surgeon operate on such a small baby?”, added AH’s mother. While the senior neonatal surgeon, two senior anaesthetists and a couple of senior nurses were trying hard to save AH’s life, his parents and family members were praying and waiting for news about the baby.

Nearly 95% of his small intestine was seriously infected and the germs had already affected his whole body. The neonatal surgeon had to remove his dead small intestine and then tried to save some of other affected parts of intestine in order to give AH a chance to live and grow unaided. After the operation, AH was given very strong antibiotics and full intensive care support to give him a chance to fight against the deadly infection. Unfortunately, within 48 hours, he deteriorated further and needed a second operation. During this procedure, all except the first ten centimetres of the small intestine was found to be dead. It was a very hard decision for the surgeons to make, as well as for the parents, but since this was the only option to save his life, his entire dead small intestine was removed in the hope that once he was cured of the infection, he could be offered other alternatives. This left AH with only a very small percentage of the 250 cms of intestine of an average full-term newborn. “I remember, my wife was shocked after she had this news about
AH and so was I, but since we had no other option and we wanted him to survive, we made this hard decision. It helped to hear that there would be some options afterwards for AH to get an intestine” said AH’s father, an employee in a private company.

Despite this second operation, AH’s health was not good in the following few days. His father said, “We feared that we were very close to losing him, but we had great confidence in the doctors in SQUH and also hope from Almighty God that he would be alright”. He also said, “It was through our hope and the efforts of the surgeons, doctors and nurses that he survived and within a week he was a different boy.”

During this difficult period, AH’s father said that they had great moral support from family and friends. AH’s mother agreed and added, “The most important thing was that my husband was there for me and that everyone in the Unit was trying his/her best for our son”. She continued, “When we looked at our twin sons in the neonatal unit, we always felt more protective of AH due to his condition. “I used to look at him, touch and cuddle him for hours to give him loads of support and love as he was my very tiny and cute baby,” said AH’s mother.

Due to the shortness of his small intestine, AH was now dependent on intravenous (IV) total parenteral nutrition for his supply of basic nutrients with the paediatric gastroenterologist taking over this special care. After a few weeks, it was time for the surgeon to restore AH’s intestine so that he could be started on oral feeds. “Based on our previous experience, this time we were more confident and less worried about the operation since we had great trust in AH’s surgeon, who had already operated on him twice”, AH’s father commented. “We had more hope now that AH would be able to have some feed and grow like his brother”. AH’s third operation successfully rejoined his 10 cm of small intestine to his large intestine and also made a temporary hole in his stomach for supplementary feeds. “AH could be started on small feeds ten days after his third operation and we were very excited on that day” said his parents. “When I saw the nurse giving him his first feed, I was thrilled”, said AH’s mother. “I was imagining joyfully that soon I would be able to feed him myself”. After a few days, his feed was increased slowly and he was also removed from the incubator as he was improving and looking well.

Time flew by. At eight months-old, AH now weighs 5.0 kg and receives nutrition from IV drips as well as from the tube into his stomach. “Although, he does have very minor problems with the tube in his stomach and with the central line for his IV feeding (which is very close to his heart), overall he is doing well”, said AH’s mother. By August 2010, AH had had four operations and his parents had more hope for his future health.

AH has spent most of his life in SQU Hospital, his mother almost constantly with him. She is a teacher, but unable to fulfil her professional commitments due to AH’s condition. Her husband has been a great support and they share the care of AH and his twin brother at hospital and home. AH’s mother said that although the care in the ward was good, she sometimes felt frustrated at not being given all the details about her son’s condition, “I like to know everything about my baby and his condition. Maybe more junior staff are not used to being asked about every little thing. It’s not only the doctor’s decision. As his mother, I would like to be involved in his care plan”. She felt that the consultants did understand her feelings and did provide the information she wanted. Her husband agreed, saying, “For us, we would like to make the decisions. If they decide to operate on him and we are not convinced, then we will say no. I remember, when the surgeon said AH needed one more operation (to connect AH’s small and large intestines). He explained the risks and benefits. It took us some time to decide, but finally we agreed with him. I think it is better to make the decisions yourself rather than leaving it to others”.

As for the overall care and support given to AH at SQUH, AH’s mother said, “I feel it is really very good especially from the consultants, such as the paediatric surgeon, the paediatric gastroenterologist, the neonatologist and the paediatrician”. It has been a great experience for us to see what a great team of doctors and nurses we have here. Everyone at different levels has been very helpful and supportive to us. The hospital administration has also been very cooperative for the supply of consumables, IV feeding and medicine for our son”.

AH is now at home on IV nutrition, but regularly comes to SQUH for evaluation. The hope is that AH can have an intestine transplant, using part of the small intestine from his twin brother or a parent, so that he can have a chance to grow and survive without IV nutrition. One of the major disadvantages of IV nutrition is that it affects the
liver functions and can eventually cause liver failure, and the need for a liver transplant. Up to now, there have been quite a few intestinal and liver transplants in children all over the world and the results are very encouraging. However, this treatment modality still needs more development before it becomes safe and easily available.

“Since there is no facility for intestine and liver transplant in Oman, we have been in touch with transplant teams in Europe and America for our son”, said AH’s parents. “The doctors from Europe have asked us to send the medical reports and soon, inshallah, we will go and see a transplant team in order to get an opinion for AH. “I have done a lot of research and reading on the Internet about intestine and liver transplant”, said AH’s mother. “Although, I am not very comfortable with the final results, I have gradually become quite convinced about the need for it. Earlier, I was totally against the proposition when the consultants first suggested it. It is going to be a huge burden on both of us financially as well as socially as we are the only earning members in the family. Finally, we are a bit concerned about the care AH will need after the transplant. It is going to be very tough and hard on both of us to travel to Europe for post-transplant care hence we hope that transplant doctors in Europe will make a team with our surgeons and gastroenterologists in SQUH, so that we can get very similar care right here in SQUH. I have seen the hard work of our doctors and nurses in SQUH and we have a lot of confidence in all of them. We only wish this transplant could happen as soon as possible so that our son can have a more normal life”, concluded AH’s mother.
Sickle cell haemoglobinopathy is the third most common genetic blood disorder in Oman.\(^1\) The genetic background of the Omani population is heterogeneous and hence there are several haplotypes including African (e.g. Benin, Bantu) and the Arab-Indian resulting in different disease severity ranging from very mild to very severe.\(^2\) In our population, there are some other factors that can modify the course of the disease like co-inheritance of alpha thalassemia that is highly prevalent in the Omani population (amounting to about 50%) and is believed to be associated with development of avascular necrosis (AVN) of the hip.\(^3\)

AVN of the femoral head is a common complication in patients with sickle cell disease (SCD), and collapse of the femoral head occurs in 90% of the patients within five years of the diagnosis of osteonecrosis.\(^4\) In our hospital, we follow more than 500 children with SCD and 32 of them have developed AVN over the last 10 years. We have tried several treatment modalities, starting from early childhood, to improve or prevent progression of the disease in these patients. For milder grades of the disease, Steinberg stages I, II & IIIa (15 cases), we practice conservative measures such as non-weight bearing exercises using crutches, and physiotherapy including anti-gravity muscle strengthening exercises and a non-joint loading range of motion exercises.

For more severe forms of the disease (17 cases), many of our patients (11) have undergone surgical intervention either locally or abroad and came back to us for follow-up. Following published reports of some success, we used concentrated autologous bone marrow injection in 4 cases, with 3 failures and improvement of the hip in one child [Table 1]. In many of our patients (13/32), the disease has progressed to warrant hip replacement which is a major surgery. Since these patients are young, they may need to undergo revision 3 to 4 times in their life. We tried to address the reasons for the poor outcome in our children and we found that they do not use the crutches supplied and are not very compliant with the pre and postoperative physiotherapy rehabilitation programme.

Despite the seriousness of the problem and its life long effect on the patient’s life with increasing pain, decreasing mobility, increasing liability to overweight, psychological stress and catastrophic economic impact on the patient, AVN in paediatric SCD patients has been inadequately addressed in the literature with only 19 papers cited in PubMed. In addition, there is no consensus in the literature on the best treatment options for the paediatric SCD patient with femoral head AVN. Though strong evidence is lacking, hydroxurea, despite

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**LETTER TO EDITOR**

**Avascular Necrosis of the Hip in Sickle Cell Disease in Oman**

*Is it serious enough to warrant bone marrow transplantation?*

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its very positive effects on many aspects of SCD, has been implicated as a possible precipitator of AVN as it increases haemoglobin level in these patients. Nowadays, more indications are evolving for bone marrow transplantation (BMT) in SCD. However, the major problem for experts is to identify which patients require BMT earlier in life before the development of serious and life long complications.

The management of SCD–AVN, in our experience and in this part of the world, has been frustrating and associated in most instances with progression or recurrence of the disease. However, in Oman, we have many extended families with high rates of consanguinity thus increasing the possibility of human leukocyte antigen (HLA) matched sibling donors. The cost of an HLA matched transplant is much less in our setup (US $45,000–50,000) than in Western countries.

Since SCD vasculopathy is the main factor for developing cerebrovascular accidents (CVA) and AVN, it is plausible to speculate that BMT will be useful in AVN as in the case of CVA. We believe that AVN of the hip is a severe complication of SCD that warrants haematology experts considering it among the indications for BMT.

Table 1: Outcome of different surgical interventions to treat sickle cell disease children with avascular necrosis of the hip in Oman

<table>
<thead>
<tr>
<th>Intervention</th>
<th>No. of Patients</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autologous bone marrow injection</td>
<td>4</td>
<td>1 success, 3 failures (progression)</td>
</tr>
<tr>
<td>Core decompression</td>
<td>4</td>
<td>1 success, 3 failures (progression to collapse)</td>
</tr>
<tr>
<td>Vascularised bone graft</td>
<td>2</td>
<td>1 success, 1 failure (non union)</td>
</tr>
<tr>
<td>Non-vascularised bone graft</td>
<td>1</td>
<td>Failure (progression to collapse)</td>
</tr>
<tr>
<td>Femoral osteotomy</td>
<td>1</td>
<td>Success</td>
</tr>
<tr>
<td>Pelvic osteotomy</td>
<td>1</td>
<td>Failure (progression to collapse)</td>
</tr>
<tr>
<td>Hip joint fusion</td>
<td>1</td>
<td>Failure (non-union)</td>
</tr>
<tr>
<td>Distraction arthrodiatasis</td>
<td>1</td>
<td>Failure (regional osteoporosis/collapse)</td>
</tr>
</tbody>
</table>

References

To the Editor,

I read with great interest the article “I found it on the internet” Preparing for the e-patient in Oman,1 published in SQUMJ in August 2010. Briefly, this article reviews the e-patient, medical student and the relationship between them. I would like to share with you and the readers my views regarding the above article. In my opinion, an e-patient is easy to deal with as he/she listens, understands and does what his/her doctor asks him/her to do properly. He/she is not difficult. This is because I find e-patients similar to university students. University students have high levels of knowledge and good communication skills and therefore cooperate well. Their unanswerable questions may be of great benefit as they may lead the teacher to undertake more research. In my opinion, the issue of preparing medical students for the e-patient in Oman is not a demanding one. In general, medical students, from SQU or the Oman Medical College, are well educated and have wide training experience. In addition, there is no specific data on the number of Omani patients seeking for health information via the Internet as Dr Masters only gave figures for general Internet use in Oman. On the other hand, non-university students, like non-e-patients, have less understanding and are less inclined to cooperate.

I also think that e-patients should be faced with e-doctors. In addition to their existing knowledge, e-doctors can enhance and increase their medical knowledge using various and wide Internet resources. Thus they will strengthen their ability to answer many questions posed by either e-patients or regular patients. Thanks to the various number of medical websites, the e-doctor can now play an even more beneficial role in the consultation than the e-patient, in particular as regards the treatment plan.

A far more pressing concern in Oman is the number of patients going abroad for treatment, even for appendicitis or tonsillitis. The number of patients that are treated abroad at Ministry of Health expenses in 2009 was 4522 and the number is on rise. Governmental and non-governmental sponsorships for medical travel abroad are limited. It is important to note that the majority of Omani patients who go abroad for treatment therefore do so at their own expense. E-doctors can play a major role in reducing the number of these patients. If I can trust my doctor, then he can start treating me. Trust in treatment starts with a patient or e-patient, and then it goes to friends and family, then community and finally the whole country. Trust is an easy word to say, but it is very hard to achieve and takes a longer time to take root. Prospective and retrospective studies should be undertaken to highlight currently hidden information about e-patients and e-doctors in Oman.

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Authors’ Response

Dr. Alwahaibi’s close reading of our article, and the resultant comments, are appreciated. He raises several points, and we would like to respond to each of them.

First, Dr. Alwahaibi’s experience with e-patients is encouraging, and reflects a similar pattern that has been found elsewhere. In my own research,1 I found some doctors relishing these consultations, finding them stimulating, and one even likened it to an enjoyable objective structured clinical examination (OSCE). To get patients to this stage, however, frequently requires patience and guidance from the doctor. It is this patience and guidance that our article wishes to encourage.

In addition, unfortunately, my own and other research has found that e-patients are not always like this. For example, some patients who would normally be confrontational have used the information found on the Internet to raise the confrontation to a new level, in an attempt to intimidate the doctor. Patients can also become confused or misled by the information they find on the Internet if their searches are not guided. And then, there are the so-called “cyberchondriacs.” Our article is aimed at ensuring that medical students are alert to these issues, and can manage the resultant situations competently.

Second, we agree wholeheartedly that e-patients should be faced with e-doctors. Indeed, the Medical Informatics courses taught to Sultan Qaboos University students in both Phase I and II are aimed at equipping the students with these skills. I currently teach on those courses, and I will gladly share the curriculum outline with Dr. Alwahaibi (or any other reader) on request.

Third, as Dr. Alwahaibi points out, our article does not provide figures on the number of Omani patients seeking health information on the Internet. Unfortunately, no research has yet been published on that topic. Indeed, part of the motivation for the paper was to provide the impetus for a research project to do just that. Until we have such data, however, there is no reason to believe that the proportion of e-patients (compared to Internet users) is significantly different in Oman from other countries, so proportions of total Internet users can be used as a guide.

Moreover, in our article, we made the prediction that the number of Omani Internet users would follow Roger’s diffusion curve;2 and was set to increase at an accelerated pace in the very near future. The latest figures3 indicate that this acceleration has occurred. Our article used 2009 figures, which estimated the number of Omani Internet users at a little less than 500,000. By 2010, this figure had increased to more than 1.2 million.3 In the light of this dramatic increase, the need to accommodate the e-patient is possibly of greater urgency than was the case when the article was written.

Finally, I am not qualified to comment on the reasons for Omani patients seeking medical treatment elsewhere, but I certainly agree with Dr. Alwahaibi that a student who has been well trained to deal with the information will undoubtedly inspire confidence in any e-patient. Further, I agree that studies of e-patients and e-doctors should be undertaken in Oman; if Dr. Alwahaibi would like to collaborate on such a project, I would welcome further correspondence on the subject.

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References


B ook vendors, whether it is Amazon.com or outlets in the shopping mall, are stocked with books entertaining us with promises to lose weight or increase our virility and, more relevant to some of us, a quick fix for androgenic alopecia. The preoccupation with self-help books pervades all spheres of our lives, and now the fad appears to be encroaching even upon the ivory tower of the academic world.

Judging from the title of the book, Getting Research Published - an A-Z of publication strategy by Elizabeth Wager, a freelance medical writer, editor and trainer, this indeed appears to be just another self-help book hitting the shelves of bookstores, or does it have more to offer?

Skimming through the pages, one would think that this book should belong to the same series as the popular “For Dummies” reference books. However, whoever bothers to read this book, will realize that it is a gold mine of information on the mystery of the publication process. This book, the second edition, is meant to alleviate the common sources of anxiety we may have regarding the publication process and provide a guide to manoeuvring round the numerous pitfalls that occur on the road to publication.

Yes, there are many books written for that purpose, but what distinguishes this book from other the books, at least among those that I have so far come across, is that it manages to cover all the essential topics while keeping the language both simple and witty. If you are well versed in the process of medical publication, you will find that this book expresses in print your inner thoughts and experiences in a way that you would yourself find hard to explain. Inevitably, the book contains much jargon relevant to the publication process,
but each term is clearly defined and the large majority of them are worthwhile for any aspiring authors to know, for example, ‘lead time’, ‘nitpickers’ and ‘ombudsman’. The book is also up-to-date with all the new terms and concepts emerging due to the increased complexity of the world of publications with its electronic publications and open access journals, leading to terms such as “Digital Object Identifier systems”, “young reviewers” and “zealot reviewers”.

Another feature of this book, which makes it clear, concise and easy to understand, is its structure. Wager has divided her book into two parts. Part one is entitled “Publication Strategy – An Overview” and Part Two the “A-Z of Publication Strategy”. The first part is divided into five chapters and contains a guide to publication strategy, tips on how to grasp the complexity of multi-centre studies and, finally, a cautionary tale exemplified by a mythical figure known as Dr. Seymour. Being a well-known expert in medical publishing with a track record extending for many decades, Wager also gives the reader ample opportunities to learn about the pros and cons of working with a medical writer. The second part of her book is encyclopedia-like with relevant topics arranged alphabetically from A to Z, as the title of the book would suggest. Its coverage ranges from a guide on how to write an abstract, to a word of caution on the processes of publication.

This book also touches upon some interesting topics that might raise eyebrows, such as the topic of ‘xenophobia’. This is not a reference to the rise of the right-wing, ideological thugs who are sweeping across the world as recession gets hold of many economies. In medical publication terms, ‘xenophobia’ or ‘geographical bias’ is a furtive activity that insidiously exists in some journals. For example, you send a manuscript from Oman and you are rejected simply because you come from a far away land. Indeed, there are anecdotal reports of journals that are known to make negative editorial decisions on articles from this region.

Today, research is becoming more prominent in Oman, and Sultan Qaboos University is getting its own ‘perestroika’ attitude to research culture. The clique of older academics who function as administrative professors are loosening their hold on the system. In the world which we now inhabit, I strongly believe that this book should be considered essential reading for the new members of the ever-growing and ever-changing research body, where the slogan that you either “publish or perish” is being trumpeted loudly. This book would also be an asset to those who are teaching research methodology or running newly established publications. And for those of us who are reaching the end of our career, this book will only rekindle the lament, “I wish I’d known all of this before”. But on a more positive note, I cannot wait to see what more Elizabeth Wager will have to offer in the third edition!

**REVIEWER**
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OVER THE PAST THREE DECADES, THE field of cardiovascular intervention has strikingly expanded in terms of technique, technology and information. It has gained sound acceptance owing to both its short and long-term proven benefits. Consequently, the interventionalist should be aware about these procedures in a tactile, cognitive and clinical sense.

The authors’ philosophy in writing this book is to discuss the potential clinical benefits of interventional versus surgical modalities for adult cardiovascular disease patients who require either percutaneous or surgical interventional procedures.

This textbook has 64 chapters covering a wide range of topics related to cardiovascular and extracardiac interventional techniques. They are organised into five sections: 1) septal defects and valvular heart disease; 2) coronary interventions; 3) aortic coarctation, aneurysms and dissections; 4) carotid and cerebral artery interventions, and 5) renal and peripheral arterial interventions.

In general, the chosen topics are well written, with easily understood subtitles. The selected coloured as well as black and white photos and images are lucid with self-explanatory legends. Each chapter has an extensive bibliography to support the review of the materials presented thus making the book attractive to readers.

The data presented provides the latest scientific information in techniques and technology as well as new surgical alternatives. The chapters on chronic total occlusions and bifurcation lesions show that interventional cardiology can be a very sophisticated art when fully supported by new and refined technology.

The main strength of this book, as compared to others in the same field, lies in the sections on the application of new technologies like optical coherence tomography (OCT), the increasing role of magnetic resonance imaging (MRI) in cardiology, as well as new procedures such as transcatheter aortic valve implantation (TAVI). In my opinion, most interventional cardiologists will find all
chapters worth reading, either to refresh their basic knowledge or to refine techniques.

Training and hands-on experience are the fundamental foundation for the interventionalist and no single text can displace this. There are some topics that I found missing in this otherwise valuable book. First, vascular access approaches, the first and most crucial step in any percutaneous technique. Second, the difficulties associated with each procedure and tips and tricks to overcome these. Third, given that complications related to interventional procedures were Gruentzig’s (the father of interventional cardiology) greatest concern. I think the editors did not give the prevention and management of complications the central role that they should merit.

This book describes the recent advances in the field of cardiovascular intervention. The intended audiences, according to the authors, include students, residents, general practitioners, cardiologists, neurologists and surgeons. I believe that the book is not suitable for students, general practitioners or even for cardiology residents in their early clinical practice.

Despite these few negative points, a textbook like Cardiovascular Interventions in Clinical Practice can advance the knowledge of an experienced interventionalist performing percutaneous coronary and peripheral interventions as well as being of interest to cardiac surgeons. The editors, authors, and publisher must be congratulated on delivering a first edition of such high quality. Personally, I consider this book to be a tremendous addition to the library of cardiologists, cardiothoracic surgeons and radiologists interested in intervention.

REVIEWER
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Medical disorders in pregnancy may cause complications and have adverse effects on the mother, foetus and the newborn. The physiological changes that occur during pregnancy may aggravate a maternal disease process thus increasing maternal morbidity and mortality rates. It is essential that all who care for pregnant women have insight into medical disorders and the way that these are influenced by pregnancy. Adequate knowledge of these medical disorders helps and guides the obstetricians in managing complicated pregnancies. Several text books on medical disorders and pregnancy have been published.

One of the foremost books in this field, Medical Disorders in Obstetric Practice, was first published by Professor Michael de Swiet more than 35 years ago. It was one of the first international text books to focus exclusively on providing expert guidance to obstetricians, medical specialists and anesthesiologists for the care of medical illness during pregnancy. The goal of this book is to provide obstetricians with information in instances in which an optimal medical consultant may be lacking. This fifth edition has been entirely revised, co-written and co-edited by an expert team of practising clinicians from all over the world, including a high risk obstetrician, a medical subspecialist and an obstetric anesthesiologist when required. The team approach provides a broad interdisciplinary practical perspective to the care of medical illness in pregnancy that addresses the entire period from preconception to postpartum follow-up.

The current edition’s 50 chapters cover a wide variety of diseases in pregnancy, divided by different body systems. For example, the first five chapters deal with pulmonary, haematological, coronary and thromboembolic diseases in pregnancy as well as thrombophilia and pregnancy. An entirely new section has been added to the fifth edition. This provides brief, practical, and evidence-based advice from highly experienced clinicians about the proper investigation and safe management of
the most common medical problems in pregnancy. Topics covered include syncope, palpitations, headaches and abnormal liver function tests. Additional chapters have also been added on a wide range of topics including cancer, critical care, obesity, advanced maternal age and prescribing in pregnancy and lactation. This edition makes much greater use of tables, algorithms, text boxes and figures to summarise and illustrate key points for busy clinicians. A special section of each major chapter now addresses issues related to the provision of anaesthesia care to obstetric patients.

Almost all chapters include details that cover the needs of an obstetrician. However, the chapter on prescribing in pregnancy has limited details on medications and risks to pregnancy compared to their risks with lactation, a subject that is addressed well in the appendix section.

Overall, this book is a useful resource for general and high risk obstetricians. It covers many aspects of medical diseases and problems in pregnancy. I would definitely use this book as a reference and I do recommend it to others.

REVIEWER

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Hirschsprung’s Disease Scientific Update
Sultan Qaboos University Hospital

25th October 2010

Introduction to the Update and to Hirschsprung’s Disease
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Hirschsprung Disease (HSCR) is a developmental disorder of the enteric nervous system, characterised by an absence of ganglion cells in the distal colon resulting in a functional obstruction. Although this condition was described by Ruysch in 1691 and popularised by Hirschsprung in 1886, Swenson in 1949 described the first consistent definitive procedure for HSCR.

Early diagnosis of HSCR is important to prevent failure to thrive, enterocolitis, colonic perforation and dilatation of distal gut, which not only affects the type and number of surgical procedures, but also helps to prevent post-surgical morbidity. Patients need to be monitored closely after surgery for years after surgical treatment of HSCR. With early diagnosis and timely treatment, most children with HSCR will not have long-term adverse effects and can live normally.

Our observation has been that there is paucity of the knowledge about HSCR in our region. This observation has been based on the late referral of children with chronic constipation from primary health care teams to tertiary hospitals in Oman. The diagnosis of HSCR has been a dilemma due to many constraints. The management has been hampered both by local beliefs and by inadequate community and parental education about the impact of delaying surgical treatment in these children. The result is poor outcome with or without surgical intervention and hence the cycle goes on. To address this complex socio-medical issue, we organised this educational session for general doctors, nurses and specialists, who are involved in care of children, to highlight the necessity of early referral, diagnosis and the management.

Currently, approximately 90% of patients with HSCR are diagnosed in the newborn period. HSCR should be considered in any newborn, who fails to pass meconium within 24–48 hours after birth, or in any child with a history of chronic constipation since birth. Other symptoms include bowel obstruction with bilious vomiting, abdominal distention, poor feeding, failure to thrive and poor weight gain as shown in Figure 1 below. The exact worldwide frequency of HSCR is unknown, but reported occurrence is approximately 1:1500–1:7000 newborns. HSCR occur more often in males; however, long-segment disease is common.

Figure 1: Hirschsprung’s disease patient with abdominal distension and visible bowel loops.
in females. HSCR is uncommon in premature infants. Approximately 20% of infants will have one or more associated abnormalities involving the neurological, cardiovascular, urological, or gastrointestinal systems.\(^5\)

Children may present with diarrhoea caused by enterocolitis, which is related to stasis and bacterial overgrowth. This may progress to colonic perforation, causing life-threatening sepsis.\(^6\) Plain abdominal radiographs may show distended bowel loops with a paucity of air in the rectum. A barium enema will demonstrate a narrowed distal colon with proximal dilation, a classic finding of HSCR, [Figure 2]. Another positive radiographic finding of HSCR is the retention of barium for longer than 24 hours after the barium enema has been performed. Anorectal manometry, which detects the relaxation reflex of the internal sphincter after distension of the rectal lumen and this normal inhibitory reflex is thought to be absent in patients with HSCR, is not commonly used for HSCR due false positive results and other limitations.\(^7\)

The gold standard to diagnose HSCR is rectal biopsy. The current practice is to perform a bedside simple suction rectal biopsy in the newborn to obtain tissue for histologic examination by a special device [Figure 3]. On histology, both the myenteric (Auerbach) plexus and the submucosal (Meissner) plexus are absent from the muscular layer of the bowel wall and hypertrophied nerve trunks enhanced with acetylcholinesterase stain are also observed throughout the lamina propria and muscularis propria.

Once the diagnosis is confirmed, the treatment is to remove the poorly functioning aganglionic bowel [Figure 4] and to create an anastomosis to the distal rectum with the healthy innervated bowel (with or without an initial diversion). A number of definitive procedures have been used, all of which have demonstrated excellent results in experienced hands. The three most commonly performed surgeries are the Swenson, Duhamel, and Soave endorectal pull-through procedures. Recently, the transanal pull-through in which no intra-abdominal dissection is performed has also been popular.\(^8,9\) Another addition to the surgical armamentarium is the laparoscopic approach to the surgical treatment of HSCR,\(^10\) where the transition

![Figure 2: Barium enema of Hirschsprung’s disease patient showing narrow rectosigmoid with proximally dilated bowel. The drawing is a demonstration of the radiological findings.](image1)

![Figure 3: Suction rectal biopsy procedure for patients with suspected Hirschsprung’s disease.](image2)

![Figure 4: Operative findings in a Hirschsprung’s disease patient showing hugely dilated distal gut proximal to the aganglionic segment of bowel.](image3)
zone is first identified laparoscopically, and then the mobilised rectum is brought down through the anus. Short-term outcomes for both, transanal and laparoscopic approaches have been similar to open single stage approaches with the benefits of minimal analgesia and shortened hospital stays.\textsuperscript{11,12} Postoperatively, although patients may encounter one or more problems such as anastomotic leak, anastomatic stricture, intestinal obstruction, pelvic abscess, wound infection, chronic constipation, incontinence and enterocolitis, the long-term follow-up studies have shown that greater than 90% of children experience significant improvement and will do relatively well.\textsuperscript{8,11,12} Patients with an associated syndrome have been found to have poorer outcomes.\textsuperscript{13,14}

The future of children with HSCR is looking promising. The possibility of stem cell transplantation into the aganglionic gut and the reactivation of dormant stem cells in the gut to regenerate the enteric nervous system are being actively investigated.\textsuperscript{15} Experiments have demonstrated that neural crest stem cells (NCSC) are present, even in the adult gut, and are capable of proliferation and differentiation. In addition, researchers have been able to inject neural crest stem cells and later identify them in the native rectum. Whether or not injected stem cells or reactivated native progenitor cells will have the capability to recreate a functional enteric nervous system remains to be elucidated.

SPECIAL CONCERNS

Total colonic aganglionosis is a more severe form of HSCR in which the entire colon and even some of the small intestine is aganglionic. These children have increased morbidity and mortality.\textsuperscript{16,17} Ultrashort-segment HSCR is characterised by a few centimeters of aganglionic bowel in the rectum, adjacent to the anus. Recognizing this condition can be very difficult. These patients are not typically diagnosed until they are older and most patients can be satisfactorily treated with a surgical myomectomy, which involves resecting a longitudinal strip of the posterior muscular wall of the rectum.

References

What is Hirschsprung Disease – A surgeon’s perspective
Dr. Madhvan Nayer

Hirschsprung’s disease (HSCR) is a common cause of intestinal obstruction that affects 1:1500 to 1:5000 live births. It is more common in male children. It usually presents in the newborn period as intestinal obstruction or in older children as constipation and abdominal distention. HSCR occurs due to the absence of ganglion cells in the muscle wall at muscle and submucosa levels, hence causing a failure of relaxation of the circular muscle and internal sphincter, leading to functional constipation. It occurs in the rectosigmoid in 85% of cases; however, HSCR may be more extensive and may extend into the entire colon (total colonic HSCR). The diagnosis of HSCR is made by demonstration of absence of ganglion cells in the submucosa or in muscle layer of the intestine. A plain X-ray of the abdomen will show a distended bowel with no gas shadows in the pelvis. When a barium enema is done, it will delineate a dilated proximal colon and a very narrow “corkscrew” rectum. Rectal biopsy is performed after bowel decompression and the specimen is then subjected to a histopathological examination by the usual staining technology (haematoxylin and eosin) or enzymehistochemistry methods that demonstrate an abundance of acetyl cholinesterase (ACh) in the nerve bundles. Once diagnosis is confirmed, either a one-stage procedure involving resection of the aganglionic bowel and restoring continuity or a multi-stage procedure (colostomy, pull-through and reversal of stoma) is carried out. In patients who have failed to achieve good decompression of the proximal bowel or in whom there is refractory enterocolitis, a preliminary diverting colostomy is mandatory. There are quite a few techniques to perform definitive (pull-through) procedure in HSCR and the recent development is minimal invasive surgery. Irrespective of the type of surgery technique, the outcome is promising and fairly good long term results are achieved in over 85% cases. Constipation and/or occasional soiling have been noted in about 15% of children in the post-operative period. One of the most serious early complications is anastomotic leak and intestinal obstruction and the most serious late complication is enterocolitis. Enterocolitis in HSCR can be serious and life threatening, hence it is addressed very aggressively and has been seen more often in patients who have had enterocolitis prior to definitive treatment or those in whom there has been persistent obstruction. It is possible to do re-surgery in patients who have had inadequate results or in those whose colon becomes much dilated due to inadequate post-operative bowel management. The role of parents, patient and community nurses in ensuring good regular evacuation cannot be overemphasised.

Medical Aspects of Hirschsprung’s Disease
Dr. Siham Al-Sinani

This paper includes a brief introduction about the history of Hirschsprung disease (HSCR), as well as an introduction to its pathogenesis and epidemiology as described in the literature. The typical clinical manifestation of HSCR might not be evident in all patients, which poses major difficulties in diagnosing children with atypical features of HSCR and therefore delays its treatment. Criteria for the diagnosis of HSCR in different age groups is discussed with proposal of an algorithm for patients with delayed passage of meconium according to their accessibility to medical care and opportunities for close follow-up in the Omani health system. The different presentations of HSCR are explained and studies of differences between children with idiopathic constipation and children with constipation secondary to HSCR. These studies did not show major differences in the clinical features based only on constipation, once again making it even more difficult to identifying HSCR based solely on clinical features without further investigations. The only exception is a child with constipation with no other organic features, normal natal history, normal growth with normal physical examination and normal rectal examination. To most pediatricians, this presentation is not commonly seen in Oman. Very few children with a history of constipation, who are referred to us at the pediatric gastroenterology unit of Child Health Department at Sultan Qaboos University Hospital, have this negative history and physical examination. Studies comparing diagnostic investigations commonly used for HSCR have revealed significant differences in the sensitivity and specificity of such investigations. Suction rectal biopsy (SRB) and anorectal manometry (ARM) (when the expertise is available) have the highest sensitivity and specificity. SRB and ARM are therefore preferred over contrast studies if HSCR is to be ruled out with greater certainty. HSCR is not a disorder seen in children only. Different age groups (including adults) can have HSCR, with interesting studies comparing the initial presentation, different pathogenesis and outcome in older patients compared to children. Case reports describe adults with long standing constipation to have catastrophic complications with enterocolitis and gut perforation found on postmortem examination. Adults with long standing constipation should be investigated for HSCR. Hirschsprung’s associated enterocolitis (HAEC) is a known major complication of HSCR. The discussion covers it definition, pathogenesis, when to suspect it and how to diagnose and treat it as a pediatrician (and not a surgeon). Appropriate and timely medical intervention avoids morbidity and mortality from HAEC.

Hirschsprung’s Disease and Syndromes
Dr. Adila Al-Kindy

Hirschsprung’s disease (HSCR) is an important genetic cause of functional intestinal obstruction. About 20% of HSCR cases have another congenital anomaly either isolated or part of a syndrome (mainly monogenic Mendelian inherited). HSCR is a congenital malformation of the hindgut, a neurocristopathy, resulting from failure of migration of the neural crest cells that form the enteric nervous system between 5–12 wks gestation. Neurocristopathies are a group of diverse disorders resulting from defective growth, differentiation, and migration of the neural crest cells. Neural crest is a multipotent embryonic structure that gives rise to neuronal, endocrine and
Hirschsprung's Disease (HSCR) is a common intestinal anomaly that presents mainly in the neonatal age. The diagnosis may prove difficult in patients with no classical symptoms. The gold standard of diagnosis remains rectal biopsy. Anorectal manometry (ARM)

Embryological and Molecular Mechanisms of Hindgut and Enteric Nervous System Development

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Hirschsprung disease (HSCR) and anorectal malformations result from alterations in hindgut development. Progress in the understanding of the genetic basis of HSCR and other anorectal malformations has been made by the application of the findings from genetic and chemical animal models of altered hindgut and neuromuscular development. Several genes have been shown to be important for the hindgut and enteric nervous system development and work is going on to identify genetic alterations and interactions that may explain the variable phenotypes of HSCR and ARMs. We used ethynlethenioirea (ETU) rat model in our lab to study the embryological and molecular mechanisms of hindgut and enteric nervous system development. Our experiments have shown that the downregulation of shh, BMP4 and hox genes in developing hindgut of ETU-exposed fetal rats. When the immunohistochemical studies of the neurons and glia that comprise the enteric nervous system (ENS) (the intrinsic innervations of the gut) were performed, we found that there was marked reduction in the immunoreactivity of NSE, VIP and SP in the hindgut of experimental foetuses as compared with the controls. Our observations are that the expression of shh and its target genes in ETU-exposed fetal rats is downregulated and intramural nerves, stained by VIP and SP-100 antisera, were decreased in various phenotypes of hindgut developmental derivatives. The embryological and cellular mechanisms of hindgut development in ETU-exposed fetal rats will be presented as well as similar work done in other laboratories.

Current Molecular Biological Understanding of Hirschsprung’s Disease

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People have appreciated the hereditary contribution to Hirschsprung’s (HSCR) disease at least since the 1960s. At about the same time, the association with Down syndrome was noted. Since then, particularly in the last 20 years, a number of karyotype abnormalities have been observed on a number of chromosomes. The observation of an interstitial deletion on chromosome 10 about 20 years ago was an important clue to the identification of the first and most important gene responsible for Hirschsprung’s disease—RET. At the same time gene screening for mutations in this gene never identified more than a minority of patients with significant sequence changes in the coding region. It became apparent that HSCR risk was determined by the sum of a number of risk alleles, as well as, more rarely, by mutations of large effect in RET and also other genes, such as the endothelin B receptor gene. There are about a dozen relatively important genes, and perhaps many dozens more less important genes, contributing to HSCR. Some of these genes are associated with particular and recognisable phenotypes, such as the so-called Mowat-Wilson syndrome described by us. Other gene mutations carry implications that are not necessarily immediately obvious: some mutations in Phox2b for instance carry a risk of neuroblastoma. Particular mutations in RET are responsible for multiple endocrine neoplasia type 2 (MEN2) cancer syndromes. It is of interest that a long-term follow-up in Scandinavia has picked up a higher rate of medullary thyroid cancer in adult survivors of Hirschsprung’s disease. Although I do not believe that gene therapy will ever be possible in the majority of cases, gene screening for known multi-case families is certainly possible, and may be useful, particularly as the cost of sequencing comes down. In particular, mutation screening would be of some use if it detects that minority of patients who may be at risk for malignancy, or other as yet undiscovered late effects. We already screen RET for several of the known MEN associated mutations in Hirschsprung’s patients on occasion. At present, we are studying the lethal spotting rat, a Hirschsprung’s disease model with a mutation in the endothelin B receptor gene, and a phenotype like the Shah-Waardenburg syndrome in humans. This animal suggests clues as to where our attention should be directed in further human studies and follow-up.

Anorectal Manometry in Hirschsprung’s Disease

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Hirschsprung’s Disease (HSCR) is a common intestinal anomaly that presents mainly in the neonatal age. The diagnosis may prove difficult in patients with no classical symptoms. The gold standard of diagnosis remains rectal biopsy. Anorectal manometry (ARM)
Hirschsprung’s Disease (HSCR) is a group of disorders characterised by a lack of propulsive peristalsis in the distal colon resulting from an absence of ganglion cells in the wall (submucosal and myenteric). An increase in adrenergic and cholinergic nerves is associated in the aganglionic segment. Colonic smooth muscle relaxation is also deficient due to the disturbed function of vasoactive intestinal polypeptide and nitrous oxide mediated inhibitory nerves. The HSCR can be of short or long segment type, total bowel aganglionosis, ultra-short segment HSCR and zonal aganglionosis. Diagnostic work up requires a full-thickness rectal biopsy specimen for haematoxylin and eosin staining and various special stainings. Inadequate tissue samples and low rectal biopsy are major pitfalls for histopathology. Pathologic diagnosis includes pre-operative and intra-operative biopsies. The presentation of ganglion cells in rectal or colonic biopsy rules out the possibility of HSCR. Multiple serial sections need to be examined before a definitive diagnosis of HSCR can be rendered. The special stainings such as acetylcholinesterase staining, calretinin immunostaining and rapid intra-operative immunoperoxidase staining for synaptophysin have been found to diagnose HSCR much faster and more accurately. In Hirschsprung associated enterocolitis (HAEC), microscopic examination reveals cryptitis, crypt abscesses, mucosal necrosis and transmural necrotising inflammation and perforation. The co-existence of neuronal intestinal dysplasia (NID) above the aganglionic segment of HSCR can complicate the interpretation of intra-operative biopsies specimen for HSCR. The diagnosis of intestinal motility disorders remains a challenge for both clinicians and pathologists.
Looking at the last 40 years, we can see that survival from Hirschsprung’s disease has improved from about 70% in the 1960s, to close to 100% now. Not only has survival improved, but the mean age at diagnosis has also improved, and plateaued about 20 years ago. I will discuss results first presented during my Ph.D. more than 10 years ago, then some results presented more recently after we started regularly to use laparoscopic assisted transanal pull-throughs, and give a roundup of my impression of the literature of the last few years. Generally speaking, functional results after pull-through are not quite as good as the older generation of surgeons liked to believe. There is a substantial incidence of at least some degree of soiling and constipation. Survival, however, has improved and modern laparoscopic surgery means that patients are subjected to much less surgical trauma and fewer operations with less time in hospital. I want to stress that long-term follow-up does not only include the functional results for defecation and continence, but also should include an assessment of general quality of life, psychological well-being, and the possibility of an increased risk of associated illnesses as foreshadowed in my talk on the genetics of Hirschsprung’s disease. We do not know how our patients will do as they enter middle and old age. This knowledge requires decades of follow-up. Well-constructed studies of this sort are difficult to achieve, and there are few
of them in the literature. Hirschsprung's enterocolitis is still a poorly understood problem, and a source of much of our remaining post-operative morbidity. It may very well be multifactorial, and it has been suggested that Hirschsprung's disease patients may have immune deficits, altered motility and functional obstruction, or simply technical anastomotic problems giving them low-grade obstruction. All of these factors may then singly or in combination result in enterocolitis. Although in most cases the attacks decrease with age, while in some patients there are persistent problems. I will discuss several ideas concerning this entity. Several authors and research groups are investigating the possibility of stem cell rescue in the animal model. The development of the enteric nervous system is complex and guided by signals that are ordered in both space and time. It remains to be seen whether injected neuroblasts will be able to order themselves in a functionally useful way. If this sort of therapy is possible, it would make bowel resection and anastomosis a thing of the past. Despite the promise of the new genetic technologies, in practice progress at present is more evolutionary than revolutionary and good results continue to depend on attention to all the details of care.
What is ‘A High Quality Pathology Service’ when the Budget is Limited?

Prof. Peter N. Furness

Vice-Chair & Revalidation Lead, Academy of Medical Royal Colleges and President, The Royal College of Pathologists, UK

As a result of the impact of the international banking crisis, state-funded pathology services in the UK are currently facing a Government-imposed cut in funding equivalent to 20% of total cost. Even before this, we had seen calls for ‘consolidation’ of pathology services into fewer, larger laboratories, to improve efficiency. This process is now being forced forward, with the UK Government putting increasing emphasis on the involvement of private companies in what was previously a mainly state-run service. The simultaneous introduction of reorganisation and the introduction of the profit motive are generating great concern and controversy. In this situation, the role of the Royal College of Pathologists, as a charitable organisation, is to advocate the highest possible standards of pathology service for the benefit of patients. All concerned with these changes claim that the plans that they advocate will maintain or increase ‘quality’, even if resources are cut. They cannot all be right, but how should the quality of a pathology service be measured? Pathologists will usually emphasise getting the diagnosis right or producing accurate measurements. Clinicians may emphasise speed of delivery of results. Managers will emphasize cost and efficiency. Commercial organisations emphasise customer satisfaction; but who is the customer, and is the customer sufficiently well informed to decide what should generate such satisfaction? If we consider patient safety, data collected by the UK National Patient Safety Agency show that adverse patient safety incidents in relation to laboratory services almost all occur in getting the specimen to the laboratory and in getting the result to the clinician. How often are these ‘interface’ problems considered when laboratory quality is discussed? Ultimately, the only true measure of the quality of a laboratory service is the improvement of patient outcomes that it achieves. But patient outcome is influenced by so many factors that the contribution of the laboratory is impossible to assess; so surrogate measures have to be used. In which case, we need an open discussion of what such markers really assess and which of them are of value. It is clear that any simple method of measuring laboratory quality is likely to be incomplete or simply wrong. Identifying the best way to measure quality in pathology is a considerable challenge. Persuading all concerned that this really is the best way to measure quality is an even greater challenge.

Quality Assurance and Quality Management Systems in Laboratory Medicine

Dr. Robby Bacchus

World Association of Societies of Pathology and Laboratory Medicine, Education Secretariat (WASPaLM), UK

The pursuit and concern for quality in health care is an issue that transcends national boundaries. Of all the health professions, the laboratory based pathology disciplines have done most and continue to do most to monitor the quality of what they do, and to anticipate and correct sources of analytical error. In addition to recording their performance both within their own departments and as members of peer groups, they educate themselves, their peers and clinical colleagues in an effort to improve the effectiveness of the service they provide, set analytical goals and subject themselves to external proficiency testing. Quality assurance is the whole programme...
of activities mounted by laboratories, regions, countries, professional groups, commercial and industrial companies in an attempt to improve clinical laboratory performance generally. They include: 1) encouragement of the constant use of internal quality control in every laboratory; 2) support for external quality assessment (EQA) schemes; 3) all measures taken to increase within laboratory reproducibility and between-laboratory comparability by means of training courses, conferences and other collaborative activities. These are based on hypotheses derived from internal quality control and from EQA results accumulated over time. There are now growing public demands for accountability, finite fiscal resources, provider resistance or apathy towards changes and an imbalance in the supply and demand equations for health care organisations in today's world. Active quality assurance programmes are critical to meet this challenge.

Research in Sickle Cell Disease, from Bench to Bedside
Dr. Salam Al Kindi

In Oman, 5.7% of Omani people carry the gene for sickle cell disease (SCD) and about 0.2% manifest the disease. Although SCD is traditionally looked at primarily as a disorder of red cells, it is a disease demonstrating a model for red cell interaction with white cells and endothelial cells. Recent work from our laboratory on acute chest syndrome, which is one of the major causes of death in SCD and vaso-occlusive crises (VOC), the most frequent presentation of this disease, has demonstrated this. An alteration in the level of nitric oxide as well as lymphocytes and monocytes activation play a significant role in both conditions. Similarly the sickled red cells, causing perturbed platelet and haemostatic functions, play an important role in stroke development, complicating further the hereditary component of thrombophilia in this syndrome. These changes are promising focii for studies on the various therapeutic interventions that are available for this disease such as hydroxyurea, low molecular weight heparin, nicosan and other agents that are under study in clinical trials. Good progress made in reduced intensity conditioning (RIC) bone marrow transplant for patients with SCD is seen in the recent experience in our centre, enabling the sickled and normal cells to co-exist together and ameliorate symptoms of SCD. Currently the use of stem cells is in progress to help patients with avascular necrosis of the hips (AVN), a crippling complication seen in some of our patients.

Benefits of Accreditation of Medical Laboratories – A global perspective
Paul Stennett

Chf Executive, Clinical Pathology Accreditation (UK) Ltd and Chief Executive of the United Kingdom Accreditation Service, UK

This paper covers the accreditation of medical laboratories starting with a detailed review of what the Clinical Pathology Accreditation (CPA) standard assesses during a laboratory visit and some typical outcomes of assessments. It also looks at how to prepare for accreditation, what is involved and an indication of the costs of accreditation. The role of Peer Assessors in the assessment is also examined. Next, there is a review of the benefits that medical laboratories in the UK have reported in terms of reduced risk and improved quality. To conclude, there is a short preview of how the accreditation standards might be further improved to continue to meet the quality requirements of medical laboratories in the future.

The National External Quality Assurance Programme in Oman
Dr. Suleiman Al Busaidi

Director of Laboratories, Central Public Health Laboratory, Oman

The presentation outlines the historical development of the National External Quality Assessment Scheme (NEQAS) in Oman. We will describe the goals and objectives of the program as well as currently available disciplines and future plans for the programme. In this presentation, we will focus on the microbiology scheme since NEQAS focuses on agents of diseases of public health and clinical importance. We will therefore, also give an overview of how panels are simulated, quality checked, packed and shipped to various participating laboratories as well as the strategic use of results in planning purposes. The evaluation guidelines and the marking scheme will be presented together with some examples of performance of laboratories in some panels. The microbiology scheme covers 26 laboratories including all Ministry of Health laboratories, other government sister institutions, some private laboratories and four international reference laboratories outside the country. The Quality Assurance Unit prepares the panels which consist of simulated samples as well as instructions for processing and response forms. The simulated specimens are spiked with relevant pathogens and inoculated in appropriate transport media. The specimens are quality checked for contamination and viability before and after dispatch. After passing the quality checking the specimens are packed and dispatched by courier to various participating laboratories. The participating laboratories process the specimens and report the results to the External Quality Assessment Unit at the Central Public Health Laboratory within two weeks. A confidential report on the expected results, together with overall performance (including marks obtained), is then issued to each participating laboratory.
Laboratory Services in Oman – Past, present and future
Dr. Nayyar Ali
Head of Diagnostic Laboratory Services, Ministry of Health, Oman

The Diagnostic Laboratory Services of the Ministry of Health have seen rapid development and a dramatic increase in laboratories and staffing. By end of 2009, there were 198 laboratories with a staff of 1,147 and the number of pathologists has increased to 80. There has also been a marked increase in sophistication and modernisation of the service with the introduction of automation to most extended health centres and hospitals. As a result of the increase in the numbers of laboratories, the increase in automation and the general population trends, there has been a significant increase in the numbers of tests done. In 1992 there were just under 6 million tests done. This had increased to 13.8 million by end of 2009. In conclusion, the laboratory service has come a long way in a relatively short time. Our aim is to provide the highest quality laboratory services at the lowest possible cost offering a comprehensive diagnostic service relevant to the needs of the people and available close to all communities in the country. Therefore, we do recommend the introduction of external quality control, an increase in staff to meet the increased work load and the introduction of new tests at regional hospitals.

Are we Ready to Use Portfolios as an Assessment Tool of Professional Development?
Dr. Arundathi Kurukulasuriya
Senior Specialist, Department of Haematology, Royal Hospital, Oman

Assessments currently in practice use comparative achievements to place knowledge, skills and attitudes of individual students in a rank, by using grades, test scores and grade percentiles, which are often obtained at summative end of course assessments. They are designed by trainers often with no constructive feedback to the learner. Usually, the lower cognitive learning styles, such as memorising and reproducing, are used by the student when preparing for these assessments. Portfolios are self portraits of students demonstrating personal development over a period of time, with guidance by the trainers and peers, where the learning is student centered. They foster skills in self evaluation, problem solving, lifelong learning, communication, writing and reflective practice. They promote higher cognitive learning strategies such as application, interpretation and reflection.

The portfolio can be also be integrated easily with instruction; however, the guidelines for developing them must be aligned well with the learning outcomes of the course. Trainers must also provide guidance and monitor the development of the portfolio in order to avoid the student spending too much time developing it, and neither learning from it nor producing a worthwhile collection of his/her work for assessment. Hence it is vital to train the trainers to guide students in portfolio development. Oman is in a phase of nation building and need professionals who have been educated by modern educational tools, such as assessment by portfolio, rather than by the archaic summative assessment.

ABSTRACTS BIOCHEMISTRY SESSIONS

Second and Third Generation PTH Assays in Secondary Hyperparathyroidism worth the Upgrade
Dr. Daniel Holmes
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It is well-known that second generation “intact” PTH (iPTH) assays have cross-reactivity with C-terminal PTH metabolites, often collectively referred to as “7-84PTH”. This becomes particularly relevant in patients with chronic kidney disease (CKD) because these fragments accumulate in the plasma. This has led to several companies developing third generation assays variably referred to as “whole PTH”, “biointact PTH” or “1-84PTH”. Is the move to a third generation PTH assay worth the effort? We have recently performed intact PTH, 1-84PTH (Diasorin Liaison), 25(OH)VitD, Bone ALP, Ca, and PO4 in a large cohort (~2500) of patients from the CanPREDDICT study. Estimated GFR in this cohort was between 14 and 45 ml/min (stage 3 and 4 CKD); none were receiving dialysis at the time of recruitment. Relationships between these bone markers and both iPTH and 1-84PTH will be reviewed. Based on this data, we will look at the performance and value of the 1-84PTH assay in the setting of non-dialysis-dependent CKD.

Vitamin D: Growing Role in Medicine
Dr. Meenu Kaur
Specialist Biochemist, Central Public Health Laboratories, Oman

Vitamin D deficiency has serious health consequences beyond rickets and osteomalacia. Evidence from clinical, epidemiological studies and randomised controlled trials shows that vitamin D has a role in prevention of many chronic diseases and its deficiency is associated with an increased risk of several diseases like osteoporosis, autoimmune disorders, diabetes, hypertension, autism, infections and various cancers. More than 200 genes are known to be regulated by 1,25-dihydroxyvitamin D[1,25(OH)2D], the active metabolite of
vitrein D. The serum concentration of 25-hydroxyvitamin D [25(OH)D] is the indicator of vitamin D nutrition status. Although the cut-off value to define vitamin D deficiency remains controversial, the current consensus points to a goal of ensuring the 25(OH)D level of 30-100ng/ml. Studies addressing vitamin D deficiency, supplementation and toxicity issues indicate the need to amend the existing advice on vitamin D requirements in all age groups. Vitamin D supplementation is also advised for the maternal and child health care. Lack of sun exposure is widely accepted as the primary cause of worldwide epidemic of vitamin D deficiency. Other causes include dark pigmentation, use of sunscreens, skin covering, obesity, old age, insufficient dietary intake, malabsorption and certain medications. Sunlight exposure, food fortification and supplementation with vitamin D are effective in preventing and treating vitamin D deficiency. Monitoring 25(OH)D levels in patients receiving high doses helps to guard against toxicity.

**Standardisation of Endocrine Assays**

Dr. Catherine M. Sturgeon

Clinical Biochemist, Royal Infirmary of Edinburgh, UK

High quality health care provision encourages the development of evidence-based guidelines, many of which include recommendations about laboratory testing. For some tests, analyte concentrations that define the need for clinical intervention are specified (e.g. parathyroid hormone in chronic kidney disease). This commendable drive to improve comparability in clinical practice will only succeed if the analytical results on which these recommendations depend are properly standardised. Improving between-method comparability is particularly challenging in immunoassays. Difficulties encountered generally reflect the characteristics of particular analytes, contributed factors include errors of calibration, antibody selection, assay design and properties of the assay matrix. The lack of established international standards for some analytes is also problematic. It is interesting to consider how these factors influence between-method comparability for several representative analytes, together with some of the international initiatives addressing these issues. Achieving comparability of results for analytes with well-defined structures should, in theory, be possible. However, despite the availability of highly purified analyte preparations, isotope-dilution gas chromatography-mass spectrometry (ID-GCMS), reference methods and reference laboratories, poor comparability of results for these analytes is still regularly demonstrated in external quality assessment (EQA) schemes. While short incubation times and absence of automated extraction steps probably also contribute, isotope dilution gas chromatography-mass spectrometry (ID-GC-MS) targeting exercises confirm that poor assay calibration is responsible for much of the poor agreement observed. ID-GCMS methods may ultimately replace immunoassay of steroids in the routine hospital laboratory. In the meantime, encouraging manufacturers to use the available reference measurement systems to assess and improve their assays is a major priority that is being actively addressed. Establishing internationally recognized reference panels of patient sera and assigning these ID-GC-MS target values, as has been done for cortisol under the auspices of the International Federation of Clinical Chemistry (IFCC), should also help to encourage between-method comparability. While the same factors that contribute to poor between-method agreement for the steroid hormones are relevant to molecularly heterogeneous analytes such as the peptide hormones, the relative importance of these factors differs. EQA data demonstrate coefficients of variation of >10% for luteinizing hormone (LH), follicle stimulating hormone (FSH), human chorionic gonadotrophin (hCG) and prolactin, but incorrect assay calibration does not appear to be the major cause of poor between-method agreement for these analytes. This suggests that while correct calibration is essential for improved method comparability, antibody specificity and assay design are also of considerable importance. Addressing these requires knowledge both of what present assays are measuring and of what is clinically desirable to measure. In a prototype IFCC project, highly purified international reference reagents for six important hCG-related molecules have been prepared and calibrated in molar units. Their primary purpose initially is to assist manufacturers and users alike in characterising what current hCG assays are measuring. By combining the results of such studies with those of antibody-mapping studies carried out by the International Society of Oncology and Biomarkers (ISOBM), broad recommendations have been made regarding hCG antibody combinations likely to be most appropriate for particular clinical applications. Assessing results obtained for panels of patient sera should now permit validation of the predictions made. This two pronged approach with hCG, aimed both at standardising antibody specificity and improving the accuracy of calibration, may serve as a model for a broad strategic approach to improve between-method comparability of other molecularly heterogeneous analytes, and hence their clinical utility.

**Tumour Marker Guidelines**

Dr. Catherine M. Sturgeon

Clinical Biochemist, Royal Infirmary of Edinburgh, UK

Increasing pressure to provide health care based on “best practice” has stimulated the development of guidelines in cancer medicine. The recommendations of the American Society of Clinical Oncology (ASCO) and the National Comprehensive Cancer Network (NCCN) have recently been complemented by more detailed guidelines from the National Academy of Clinical Biochemistry (NACB) in the United States and the European Group for Tumor Markers (EGTM) which provides a more laboratory-oriented perspective. In the pre-analytical phase, as well as ensuring the integrity of specimen collection and identity, and maintaining awareness of the effect of treatment or other conditions that may influence interpretation, the laboratory can encourage appropriate test requesting by promoting the recommendations made by the NACB for fifteen major cancer types. The NACB and EGTM guidelines also include detailed recommendations for best practice in the analytical phase, where well validated methods, rigorous internal quality control (IQC) procedures and participation in well-designed external quality assessment (EQA) schemes are all essential. Excellent precision and reproducibility (intra-assay variability <5%; inter-assay variability <10%) are important especially at concentrations close to critical clinical decision points, as is long-term assay stability. Awareness of clinically relevant interferences is highly desirable, with active dialogue between laboratory and clinical staff facilitating early identification of erroneous results. In the post-analytical phase, laboratory
Measurement of proteinuria is a traditional and established test for kidney injury. It facilitates risk stratification and appropriate management of patients with chronic kidney disease identified based on estimated glomerular filtration rate (eGFR) that will prompt early referral and initiation of renal protective therapy. Testing for proteinuria or albuminuria in at-risk groups has been recommended by many international guidelines. Measurement based on reagent strips is accompanied with many pitfalls and should be abandoned in favour of laboratory measurements reflected as urine protein or albumin concentration as a ratio to urinary creatinine. Methods for total protein are more sensitive to albumin, having poor precision at low concentrations, being insensitive, non-specific and subject to a range of false-positive and false-negative problems. Urinary albumin measurement provides a quantitative, relatively standardised measurement for the loss of an important single protein in most nephropathies. It is widely accepted as the test of choice for the detection of diabetic nephropathy that is commonly requested by physicians, although total protein measurement continues to be used by specialists investigating kidney disease particularly in non-diabetics. The use of urine albumin measurement as a front-line test for detection of diabetic nephropathy that is commonly requested by physicians, although total protein measurement continues to be used.
External Quality Assessment

Dr. Catherine M. Sturgeon

The primary function of an external quality assessment (EQA) service must always be to provide each participating laboratory with an objective assessment of its own performance, enabling comparison with that of other laboratories. Data from EQA schemes influence laboratory decisions when considering changes of method, particularly for complex analytes such as tumour markers and the glycoprotein hormones. Increasingly, data generated by EQA schemes inform the strategic decisions of professional organisations, including those involved in setting standards for laboratory accreditation, those working to improve analytical standardisation and comparability of clinical results for specific analytes (e.g. the International Federation of Clinical Chemistry) and those specifying quality requirements for health programmes (e.g. the National Health Service Prostate Cancer Risk Management Programme in the United Kingdom and the Kidney Disease: Improving Global Outcomes (KDIGO) initiative in the United States). Such reliance on EQA data places a considerable responsibility on EQA providers to ensure that specimens distributed are appropriate, i.e. as similar as possible to patient specimens and of clinically relevant concentrations. The validity of target values (usually trimmed consensus means) should also be regularly confirmed, by assessing recovery of international standards, by assessing linearity on dilution, and by determining their reproducibility on repeat distribution of the same pool. Assessment of long-term assay stability is particularly important for tumour markers. Experience suggests that major factors contributing to between-method differences in results include errors in calibration, differences in antibody specificity, and method design. Between-method agreement has improved for some analytes, including prostate specific antigen (PSA), for which between-method coefficients of variation in the UK National External Quality Assessment Scheme for PSA have fallen from 21.9% to 9.5% following the widespread adoption of International Standard 96/670 for PSA and highly commendable efforts by diagnostic companies to calibrate their PSA methods accurately in terms of this standard. However there are still significant method-related differences in results for many other analytes. Attention to method design is also required, e.g. to achieve equimolarity of recognition of different isoforms where relevant (as is desirable for complexed and free PSA and for HCG and its free beta-subunit) and to minimise the risk of clinically relevant interferences (e.g. heterophilic antibodies and high dose hooking). Through occasional issue of ‘special’ EQA samples designed to probe these potential pitfalls, aspects that need to be addressed, e.g. better blocking in vulnerable methods or protocols to identify hooking in some laboratories can be highlighted to manufacturers and users. This essentially educational remit of EQA can be further extended beyond the monitoring of analytical performance to incorporate assessment of other aspects of laboratory provision. In the pre-analytical phase, EQA surveys of practice provide a means of assessing the quality of advice given by participants to clinical users about test requesting. Interpretative exercises in which participants are asked to interpret their EQA results in the context of a given clinical history enable assessment of post-analytical advice including reference intervals, recommendations for further testing and interpretative comments.

Disorders of Aldosterone

Dr. Daniel Holmes

The laboratory serves as the main diagnostic tool for screening, diagnosis and tumour localisation in patients with primary aldosteronism. Since aldosterone and plasma renin activity (PRA) methods demonstrate such wide inter-method bias, appropriate aldosterone, PRA and aldosterone:PRA ratio thresholds need to be established at each laboratory. These cannot be taken from literature without careful comparison of methods. Appropriate use of screening tests will be reviewed along with the use and interpretation of provocative diagnostic tests. Strategies for successful analysis and useful reporting of samples taken from selective venous sampling of the adrenal veins will be reviewed.

Paediatric Growth Hormone Disorders

Dr. Aisha Al-Sinani

Growth assessment is essential in child care and short stature can be recognised only with prompt history and accurate measurements of growth data. The hallmark of endocrine disease is linear growth failure that occurs to a greater degree than weight loss. There are three key diagnostic features of growth hormone disorders (GHD): abnormal growth demonstrated by auxological evidence of height < -2 SD, subnormal growth velocity and low GH in response to two dynamic tests. Primary insulin-like growth factor (IGF) deficiency can be missed as constitutional short stature if the dynamic stimulation is not interpreted cautiously. Measurement of IGF-1 and IGFBP-3 can be performed in our laboratory at the Royal Hospital, which is helping us to establish a diagnosis in IGF deficiency. Unfortunately, genetic analysis for growth hormone receptor genes is not available; it is recommended for those patients with access to laboratories expert in these techniques.
Vitamin B12 – Insight
Dr. Manal Al-Kindi
Chemical Pathologist, Royal Hospital, Oman

Serum B12 remains the most common vitamin investigation in clinical practice, it included in investigations of patients who present with symptoms of anaemia, glossitis or neurological dysfunction. Vitamin B12 acts as a cofactor for enzyme that catalyses methyl group transfer. This includes DNA methylation and the conversion of homocysteine to methionine. B12 is transported in blood predominantly bound to haptocorrin and smaller amount transported by transcobalamin. Only B12 carried by transcobalamin is available for cellular uptake and hence it is consider physiologically relevant. Understanding the pathophysiology of B12 and the different causes of B12 deficiency helps in solving the challenges in medical diagnosis and management. Biochemical tests for diagnosis of B12 deficiency, including total serum B12, holotranscobalamin, intrinsic factor antibodies and homocysteine, will be discussed in the presentation. Lack of standardisation in B12 assay results in a wide variation in B12 results between different laboratories. In addition different laboratories use different methods, report in different units and use different reference intervals making the use of the serum total B12 test alone to diagnose B12 deficiency of limited value.

ABSTRACTS HAEMATOLOGY SESSIONS

Bone Marrow Transplantation in Thalassaemia – How can we improve outcome?
Prof. Alok Srivastava
Professor of Medicine, Head, Department of Haematology & Centre for Stem Cell Research, Christian Medical College, Vellore, India

Allogeneic bone marrow transplantation (BMT) is the only currently established way to cure beta thalassaemia major and other severe haemoglobinopathies. The rate of success of BMT in these patients has been traditionally correlated with their risk stratification based on the extent of liver damage prior to BMT as assessed by the adequacy of chelation (whether started within 2 years of first transfusion), degree of hepatomegaly (> 3 cm) and presence or absence of fibrosis on liver biopsy. The long term event free survival for class I patients (least risk) is over 90%; for class II patients (intermediate risk) ~85% and for class III patients (high risk) ~60%. The causes of failure of BMT, particularly in class III patients, are mostly related to two major categories of complications – regimen related toxicities and immunological problems such as graft versus host disease (GVHD) or graft rejection. Our work over last few years has been directed at understanding their biological basis and finding ways to mitigate them. We have extensively evaluated the pharmacokinetics of busulfan and cyclophosphamide, (Balasubramaniam et al., 1999) assessed their genetic basis and correlated them with clinical outcomes (Srivastava et al., 2004; Chandy et al., 2005). We have recognized that our class III patients can be further classified into those who are at extremely high risk of failure of treatment. (Mathews et al., 2007). We are now able to dose adjust busulfan doses based on the first dose kinetics of the individual. We are also attempting to use newer conditioning regimens in these patients. We have also evaluated the immunological aspects of GVHD and graft rejection and shown the role of dendritic cells and natural killer cells in these events. (Rajasekar et al., 2009, 2010).

Laboratory Aspects of Bone Marrow Transplantation
Dr. David Dennison
Senior Consultant and Director BMT, Sultan Qaboos University Hospital, Oman

Haematopoietic stem cell transplantation has advanced considerably over the last four decades to provide curative therapy for patients with a wide range of disorders. The success of any transplant program is dependent upon high quality laboratory support with both basic and specialised services. A transplant program linked to a busy multispecialty hospital has the advantage that the four basic laboratory disciplines of haematology, biochemistry, microbiology and histopathology should be already well-established and can provide the routine services required for the transplant patient. The technology and expertise within these laboratories need to be enhanced over time to cope with the development of the transplant programme. Rapid viral diagnostics and expertise in the histopathology of graft versus host disease are some examples. The haematology laboratory is unique for the spectrum of support it provides for a transplant programme. In centres worldwide, the haematology laboratory services could range from routine blood counts and peripheral smear examinations to immunomagnetic t-cell depletion or CD34 selection in haploidentical transplants, DNA fingerprinting for chimerism and sophisticated techniques for the detection of minimal residual disease following haematopoietic stem cell transplantation for malignant disorders. The laboratory aspects of haematopoietic stem cell transplantation are diverse. A successful transplant program needs a well-coordinated basic and specialized laboratory support. Finally, the interaction of the transplant clinician and the biomedical scientist is important for growth and development of any transplant service.
The Incidence of Alloimmunisation in the Omani Sickle Cell Disease Patients Undergoing Red Cell Exchange Transfusion

Dr. Sulayma Al Lamki

Senior Consultant, Haematology, Royal Hospital, Oman

Patients with sickle cell disease (SCD) require several transfusions in their lifetime and hence are prone to develop multiple antibodies. The incidence of alloimmunization among such patients varies in different countries. We analysed patients with SCD in our centre who had exchange transfusions between December 2001 and May 2007. The incidence of alloimmunisation was 14.5%, and 75% of these patients had developed only one antibody. The most frequently observed alloantibody was anti K followed by anti C and anti E. These findings are similar to the data observed internationally.

Clinical Transplantation of Stem Cell Research – The way forward

Prof. Alok Srivastava

Professor of Medicine, Head, Department of Haematology & Centre for Stem Cell Research, Christian Medical College, Vellore, India

The last decade has seen major advances in our understanding of the biology of embryonic (ESC) and adult stem cells (ASC). What has evolved more recently is our ability to differentiate them ex-vivo into cells, tissues and potentially organs of our interest. However, considerable controversy exists with regard to the source of ESC. The science of differentiating them into therapeutically relevant cells also needs to develop further. Finally, ways will need to be found to overcome the immunological barriers after transplantation. Therefore, much effort has also been directed towards identifying, characterising and expanding adult stem cells from different organs. Many organ specific stem cells are also being evaluated for the treatment of several degenerative disorders. This has led to the development of a whole new science that has been termed regenerative medicine. The recently recognised immunomodulatory properties of mesenchymal stem cells have also opened new possibilities of their use for tolerance-induction in organ transplantation and treatment of other disorders associated with immune dysfunction. In the last few years, there has been tremendous excitement around the potential of reprogramming adult stem cells by introducing certain critical transcription factors that confer to them the property of pluripotent differentiation similar to embryonal stem cells. These have been called induced pluripotent stem cells. The science of these cells is rapidly evolving and holds tremendous promise for regenerative medicine. However, as we tread this path to developing stem cell therapies, it is critical that we understand that none of these potential applications, apart from HSC transplants for haematological diseases, has reached the status of being widely offered as standard therapy at present. Their safety and efficacy need to be assessed in preclinical models and clinical trials. Offering such therapies at this time, therefore, through advertised services that give the impression of efficacy beyond what is established is wrong. The scientific and medical community and indeed the regulatory authorities in every country need to guard against such exploitation of vulnerable patients. Apart from potential harm to the individual, this approach will bring disrepute to science in the long run. The International Society for Stem Cell Research (ISSCR) has developed guidelines (http://www.isscr.org/clinical_trans/pdfs/ISSCRGLClinicalTrans.pdf) for clinical translation of stem cell research as well as a website to provide appropriate information to patients (http://www.isscr.org/about/Stem_Cell_Treatments.htm). While the path may seem long and difficult, we need to remain positive and persevere to develop these therapies in an ethical and scientific manner.

Polychromatic Flow Cytometry in the Clinical Laboratory

Prof. Brent Wood

PACTor, Haematopathology Laboratory, Department of Laboratory Medicine, University of Washington, Seattle, USA

Multicolour flow cytometry has become a routine method for the multiparametric analysis of cellular populations in both the research and clinical laboratories. The advent of clinical instruments that allow for the routine analysis of up to 10 simultaneous fluorochromes is rapidly transforming clinical flow cytometry and offers a number of advantages including: improved assignment of cell lineage and maturational stage, more definitive assessment of immunophenotypic abnormality, increased laboratory efficiency and the ability to acquire large numbers of events, and ultimately standardisation of diagnostic approach. The increased degree of technical complexity of instrumentation and reagents, as well as decreased sensitivity for the simultaneous detection of multiple antigens on the same cell population for certain fluorochrome pairs, are the principle disadvantages. While it is now possible to generate reliably high-level multiparametric data, software tools to allow for the efficient and optimal use of this increased level of information have lagged behind and are now a primary focus of technology development. This talk will discuss technical details required for the successful performance of high-level multicolor flow cytometry, approaches under development for the analysis of multiparametric data in a clinical context, and recent technological advances likely to impact the field in the near future.

The Molecular Diagnosis of Haematological Disorders

Mr. Shoaib Al Zadjali

Senior Scientist, Department of Haematology, Sultan Qaboos University Hospital

Over the last decades nucleic acid-based molecular diagnostic procedures have evolved astoundingly from the time-consuming labor-intensive Southern/Northern blot technology to cost-effective high throughput methodologies. This was made possible with the advent
of polymerase chain reaction (PCR) technology which allowed unlimited supply of target nucleic acid molecules. PCR-based diagnostic analysis (PCR-RFLP, Allele-specific PCR, Gene dosage, GeneScan, DNA Sequencing, Quantitative PCR and MLPA) are endowed with very high sensitivity, specificity and versatility. After an initial period of research and development, followed by quality assurance, the Department of Haematology of Sultan Qaboos University Hospital spearheaded the application of these technologies to a variety of inherited or acquired haematological disorders which include notably: chronic myeloid leukaemia (CML), acute lymphoblastic leukaemia (ALL), acute promyelocytic leukaemia (APL), myeloproliferative disorders, chimerism studies following bone marrow transplantation and haemoglobinopathies. Given the high rate of consanguinity of our society, further complicated by the genetic complexity of inherited disorders, a constant research component in designing/updating our diagnostic strategy was mandatory in order to translate them from bench to bed side. Such achievements were possible thanks to the fruitful and close interactions among the staff of the department at all levels.

Leukaemia Stem Cells in Acute Myeloid Leukemia: Implications for therapy and potential targeting strategies
Dr. Adhra Al Mawali

Chief of Medical Laboratory Science, Department of Haematology and Blood Transfusion, Royal Hospital, Muscat, Oman

A better understanding of leukaemic stem cells and molecular biology will lead to more effective therapies for leukaemic diseases. Malignant stem cells have been identified in acute myelogenous leukaemia, chronic myeloid leukaemia and some types of acute lymphoblastic leukaemia. Like normal stem cells, these leukaemic stem cells (LSCs) are able to self-renew, differentiate, and proliferate extensively. Evidence suggests that LSCs are critical for the initiation and perpetuation of leukaemic disease. Leukemia contains a subpopulation of cells that display characteristics of stem cells. These cells maintain tumour growth. The properties of LSC indicate that current conventional chemotherapy, directed against the bulk of the tumour, will not be effective. LSC are quiescent and do not respond to cell cycle-specific cytotoxic agents used to treat leukaemia and thus contribute to treatment failure. New strategies are required that specifically target this malignant stem cell population. LSCs are likely responsible for disease relapse and therefore represent an ideal target for effective therapy. Further characterisation of LSCs, using molecular and immunological techniques, is required to develop such therapies and monitor disease before relapse. LSCs are reported to over express the alpha subunit of the IL-3 receptor (CD123) compared to normal CD34+/CD38- haematopoietic stem cells, however, it has not been demonstrated whether the CD123 positive (CD34+/CD38-) subpopulation is enriched for any clonal markers of AML or any LSC properties. In our study, using five-colour flow cytometry, we confirm significant expression of CD123 in 32/34 cases in the total blast population (median expression = 86%). CD123 was also strongly expressed in the CD34+/CD38- cells (96 ± 2% positive) from 28/32 primary positive specimens for CD123 taken from consecutive cases of adult AML at our institution. CD123 was not expressed/low in normal bone marrow CD34+/CD38- cells (median expression = 0%, range (0-.004%). Samples were tested for the presence of FMS-like tyrosine kinase 3 (FLT3) internal tandem duplication (ITD) by PCR as a tracking marker for leukaemic clones (10 positive /25). FLT3/ITD Positive AML samples were sorted into two putative LSC populations according to the expression of CD123 and then analysed for the presence of FLT3/ITD. Interestingly, FLT3/ITD was only detected in the CD34+/CD38-/CD123+ (7/7) and not in the CD34+/CD38-/CD123- subpopulation (6/7). These results suggest CD123 immunoprofiling provides further delineation of the FLT3 positive leukaemic stem cell clone and maybe useful to combine with CD34 and CD38 markers in tracking residual and relapsed disease at the level of the LSC. Combinational therapy targeting both CD123 and FLT3 in this population may result in more effective anti-LSC eradication.

Detection of Minimal Residual Disease in Paediatric Acute Lymphoblastic Leukaemia
Prof. Brent Wood

Director, Haematopathology Laboratory, Department of Laboratory Medicine, University of Washington, Seattle, USA

Minimal residual disease (MRD) detection is increasingly recognised as an important prognostic factor for patients with pediatric precursor B cell lymphoblastic leukaemia/lymphoma (acute lymphoblastic leukaemia, ALL). This presentation will review the experience of ALL MRD detection by flow cytometry with a focus on the experience of the Children's Oncology Group (COG). Data from the most recently completed generation of COG trials confirms the prognostic significance of ALL MRD in a large cohort of patients with pediatric ALL. In particular, these trials demonstrate a clear association between increasing levels of MRD detection and reduced event free survival when assessed at day 8 after initiation of therapy in peripheral blood, end of induction (day 29) in bone marrow aspirates or end of consolidation in bone marrow aspirates. The combined use of MRD assessment at day 8 in peripheral blood and day 29 in bone marrow allows for the identification of a subset of patients with very low risk of relapse for whom therapeutic reduction might be a consideration. MRD is also shown to be prognostically significant within subsets of good cytogenetic risk patients or trisomy 4 and 10, and is associated with both short and long term relapse after therapy. In a multivariate analysis, the detection of minimal residual disease by flow cytometry is found to be the single most important prognostic indicator. The current generation of clinical trials in COG is now focused on determining whether risk adapted therapy based in part on MRD detection can favorably impact outcome for this disease.
Diagnosis of Red Cell Enzymopathies
Dr. Kanjaksha Ghosh

Director, National Institute of Immunohaematology, Mumbai, India

Red cell enzymopathies, when severe, can present with diverse clinical presentations, hence a single diagnostic strategy is unlikely to suit all clinical situations. G6PD deficiency which reaches polymorphic proportions in many populations as a result of balanced polymorphism against falciparum malaria infection needs to be detected by simple population screening methods. For many haemolytic anaemias, due to red cell enzymopathies, the red cell enzyme level needs to be measured spectrophotometrically or fluorimetrically. However, certain red cell enzymes are present in high concentration in reticulocytes, neutrophils and lymphocytes hence the enzyme level is measured when the acute state of haemolysis has passed and the blood is depleted of white cells by using suitable techniques. Certain red cell enzymes like Pyrimidine 5’ nucleotide deficiency can be suspected when coarse basophilic stippling is noted in blood films. This enzyme may become deficient in lead poisoning. Spectrophotometric measurement of red cell enzymes are performed in haemolysates made from leukocyte depleted blood samples and is used as the source of the red cell enzyme. A substrate solution for the given enzyme is incubated with the haemolysate along with coenzymes like NAD or NADP After a constant period of incubation either the (i) remaining substrate (ii) amount of product produced by enzyme action or (iii) quantitative changes in the coenzymes, i.e. NAD NADPH2 and vice versa, are measured by using suitable colorimetric or fluorimetric techniques. Glycolytic pathway enzyme defects causing haemolytic anaemia used to be screened in the past by auto haemolysis with correction of haemolysis by different compounds; however, this was cumbersome. Complete deficiency of NADP dependent methaemoglobin reductase and certain glycolytic enzyme deficiencies associated with cytochrome B 5 deficiency in the nervous system may lead to severe mental retardation. Red cell catalase deficiency can be associated with recurrent mouth ulcers without haemolytic anaemia. Porphyrin synthesis in the red cells is driven by innumerable enzymes and finally leads to synthesis of haem. Acute erythropoietic porphyria and erythropoietic proto porphyria are two important conditions. Both of them can be associated with moderately severe haemolytic anaemia. The diagnosis of these cases is done by screening the red cells for porphyrin metabolites fluorimetrically. Hence a combination of clinical features, red cell morphology and screening tests discussed above should guide the investigators in selecting the diagnostic techniques for a specific red cell enzyme deficiency.

The Role of the Pathologist in the Diagnosis of Lymphoma
Dr. Ibrahim Al Hadabi

Consultant, Department of Histopathology, Sultan Qaboos University Hospital, Oman

Immunohistochemistry plays an important role in the classification and diagnosis of malignancies. According to the World Health Organization, the classification of Hodgkin’s and non-Hodgkin’s lymphomas depends on many factors including clinical features, morphology, cell lineage, stage of maturation and immunophenotype. The panel of markers is selected according to the differential diagnosis initially made by morphology. The approach to interpretation and diagnosis using immunohistochemistry and the pitfalls in diagnosis by this tool is discussed.

Community Screening for Haemoglobinopathies: Technical and logistic considerations
Dr. Kanjaksha Ghosh

Director, National Institute of Immunohaematology, Mumbai, India

Community screening to identify carriers and couples at risk is an important component of a haemoglobinopathies control programme. An effective implementation of screening programmes first requires adequate outreach for information, education and communication (IEC) both in urban and rural areas. The target groups should include: 1) Medical professionals and nurses; 2) Medical Social Workers and Community Leaders; 3) Students and Teachers in schools and colleges, and 4) General population. A combination of different approaches would have to be used keeping in mind the social, cultural and religious sentiments of multiethnic populations. Mass media should reach even remote areas. Other approaches would include talks during conferences and CME programmes for medical personnel. Inclusion of topics on haemoglobinopathies in school and college curricula, booklets, pamphlets and posters for distribution in different regional languages are other means of the dissemination of knowledge. Once this is in place, community screening should be undertaken after due consideration of logistical issues. The following need to be considered. If resources are inadequate, preliminary screening can be done using a combination of NESTROFT, solubility test and DCIP test where carriers with beta thalassemia, Hbs and HbE respectively will be picked up and HPLC analysis can be done in the individuals who are positive in any of the 3 tests. For screening of beta thalassemia carriers, the most suitable approach would be to do a complete blood count, HPLC on all individuals with MCV<80fl and/or MCH <27 pg. At least 5 to 6 regional centres should be identified where training can be given for manpower development in that region as well as for undertaking regular quality control programmes to avoid any misdiagnosis. It is impossible randomly to screen the large population in India and it would be most appropriate to initially screen newlywed couples and women in antenatal care who would be at immediate risk of having an affected child. The other groups which could be screened are school and college going students as they are easily accessible. Multicentre studies have been undertaken to screen all these 3 groups by the Indian Council for Medical Research and a few centres in six different states, mainly in medical colleges under the Jai Vigyan Programme. These need to be expanded to other states and ultimately facilities should be established at the district level in every state. Eventually more centres for prenatal diagnosis need to be established to successfully initiate a community control programme for haemoglobinopathies at the
national level.

**Clinical Aspects of Bleeding Disorders**

Prof. Alison Street

Vice President, Medical World Federation Haemophilia, Alfred Health, Melbourne, Australia

When reviewing a patient for symptoms of a bleeding disorder it is important accurately and precisely to define any abnormality both for the selection of appropriate treatment and for family counselling. Population screening by laboratory testing is neither specific nor cost-effective for the detection of clinically significant bleeding disorders. The most important clues come from the person's history of bleeding with past surgery, but where there has not been experience of such a homostatic challenge it can be very difficult to predict whether a person will bleed or not with any particular procedure. Working parties from various societies, such as the International Society of Thrombosis and Haemostasis have developed clinical questionnaires in attempts to standardise "bleeding scores", but there is a large overlap between the normal population and people with bleeding disorders. The history of past experience of bleeding remains the most important factor in deciding whether to proceed to targeted testing for the presence of a bleeding disorder. A family history of bleeding and the mode of inheritance may also help, for example transmission of haemophilia through the maternal line or an autosomal dominant pattern in families with von Willebrand disease (VWD). The type of bleeding, for example mucosal bleeds from nose, mouth and other parts of the gastro-intestinal tract may suggest a problem with platelet adhesion/aggregation such as VWD, whereas joint and deep muscle bleeds are more characteristic of a plasma factor deficiency. Algorithms for testing can be developed and the more specialised tests of von Willebrand factor function, genetic mutation analysis and identification of plasma factor inhibitors are often centralised to reference laboratories. Treatment is most safely and efficiently co-ordinated through centres where clinicians from multiple disciplines are co-located and trained to deliver comprehensive care. Treatment products are the most costly budget item for which protocols need to be locally designed, implemented and audited to maximise effectiveness of the investment.

**Challenging Cases in Thrombosis and Haemostasis: SQUH experience**

Dr. Khalil Al Farsi

Consultant, Department of Haematology, Sultan Qaboos University Hospital, Oman

Disorders of thrombosis and haemostasis are leading causes of morbidity and mortality world-wide. Thanks to the explosion in both clinical and translational research, our understanding of thrombosis and haemostasis has expanded from thinking of them as simple vessel leakages or occlusions to knowing the details of the molecular mechanisms behind them. The new insights gained from these, and the availability of specialists in the field with years of experience dealing with such disorders, have helped us establish some guidelines. These have been of great help in the diagnosis and management of such disorders. However, despite all this, we are still faced with cases that pose challenges both in the laboratory and the clinic i.e. in diagnosis and management. I will discuss a few challenging cases and try to provide a step-by-step approach that might be of help in managing such cases.

**Developing Comprehensive Care Programs for the Diagnosis and Treatment of Haemophilia and other Bleeding Disorders**

Prof. Alison Street

Vice President, Medical World Federation Haemophilia, Alfred Health, Melbourne, Australia

Comprehensive care is defined as the continuing supervision of all medical and psychosocial factors affecting the person with a bleeding disorder. Patients with bleeding disorders and their families are a "minority population" with special health needs for diagnosis, (including carrier status) and treatment. They develop trust and confidence in clinicians who show interest and develop competence in diagnosis and management of these disorders. The principles and practice of comprehensive care are those of "chronic disease management" with particular emphasis on training and multidisciplinary teamwork. The services provided within a comprehensive haemophilia care center will depend on locally available human and financial resources. They usually involve leadership from a haematologist or rehabilitation specialist, with input from laboratory technologists, nurses, physiotherapists, dentists, surgeons, etc. Services may also be integrated with those required for managing other inherited disorders such as thalassemia and haemoglobinopathies and thrombosis. A patient registry with systematic data entry supports effective and efficient clinical management both of patients and treatment products. Managing patients with haemophilia through a comprehensive care centre has proven benefit for both patient and financial outcomes. Adoption and adaptation of the services to local needs and resources requires trust and commitment between funders, clinicians and patients.

**Screening Strategies for the Detection of Mutations in Haemophilia**

Dr. Shrimati Shetty

National Institute of Immunohaematology (ICMR), Mumbai, India

Haemophilias represent the most common and severe inherited haemorrhagic disorders caused by mutations in the F8 and F9 genes,
which lead to deficiency or dysfunctional factor VIII and IX protein respectively. The F8 gene is 186 kb long; it has 26 exons and encodes a 9-kb mRNA transcript while the F9 gene is 33 kb in size with 8 exons and 7 introns with a 2.3 kb mRNA. Complicating the molecular characterisation of this disease is the complexity of the genes, the mutational heterogeneity, and technical limitations of the current mutation detection techniques. The mutations causing both haemophilia A and B are spread throughout the genes and are mostly represented by point alterations. However, the inversion of intron 22 was found in 40–50% of patients with severe HA and the inversion of intron 1 was reported with a prevalence of about 2-5% in different populations studied. Except the intron 1/22 inversions in severe HA cases, mutation detection in the F8/F9 genes is challenging so that it is only partially met by conventional screening methods such as single stranded conformational polymorphism (SSCP); conformational sensitive gel electrophoresis (CSGE) and chemical mismatch cleavage (CMC); high resolution melting analysis (HRM), and denaturing high performance liquid chromatography (DHPLC). Each of these have a varying applicability and efficiency; however, they all suffer from incomplete detection rates in the range of 70–90%. Moreover, each method places variable demands on the technical skills and time investment of the investigator. Direct sequencing of the gene is now the most accepted method of mutation detection in haemophilias. A F8 / F9 DNA micro array platform is an alternative gene mutation analysis approach that has a high sensitivity, and reproducibility. The methodology is, however, expensive and time consuming and, with the reduction in sequencing costs, direct sequencing is now the most cost and time efficient strategy for haemophilia A/B mutation analysis. Even extensive scanning of the exonic areas, promoter and splicing areas fail to detect mutations in 2-3% of the cases suggesting the role of other genes in reducing factor levels. Reports of double mutations in both haemophilia A and B add further to the complexity of genetic diagnosis in these disorders. These factors undetected, the phenotypic diagnosis has not been totally abandoned in many of the laboratories who routinely perform genetic diagnosis of haemophilia families.
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الدلالات المختصرة لظواهر الكبوات وأثار النزوح الكروي عند مرضى التصلب الديمي الكبير
 دراسة نقطية CLI الحالات الساقطة

وعد الله شريف ملا عبد، هدى سعيد الهاشمي، مهنا ناصر الصليبي

ملاحظة الطفيف ضد الهام فيروس النزلات والاستجابة المناعية عند
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 ضد عامال التخزن مثال العملي: خالد الرصادي، رياض بومي، باحثينكا باليري

طارح حالات

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تغري خمس حالات ووراحة
عبد الله باهت، سدير أحمد، دليل سرحنا

أزمته فوقيا
تفاعل غرامي لجين لشخص يقاس خطوة من البرازيميد، العلاج الجزع أكيتارد
أبيه المذيع كريستوفر غران، عائشة الحمداني، راجيف جاين، كريستوفر غرانت

التهاب المثلا، يتسبب في خثار عمي
ورم دموي فوق جافية الحفرة الخلفية
غير حالة
محمد كلاف

التهاب جدوعة الراحة (التهاب الخلاصة من التخزين المذبوش)
بعد عملية نزع الراحة بالمذر
سورييل باراميشونارا، جابيريل رودريجز، رافين نانو، نانو تشالوم، سامس باي

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