If you can look into the seeds of time
And say which grain will grow and which will not,
Speak then to me…
William Shakespeare, Macbeth (Act I, Scene III)

Embarrassingly for the medical profession, the century that began so promisingly with the sequencing of the human genome may conclude with lower life expectancy than in the previous century.[1] Obviously, something is not right with our current approach to health. Many believe the answer lies in a population-based approach to disease, with prevention and early detection at the fore. As a result, great resources have been deployed in screening for the major killers, namely cardiovascular disease and cancer.

Medical screening is the work of the ambitious. The aim to decrease the burden of disease through early detection and subsequent early treatment has intuitive appeal. However, some within the medical profession are hesitant to proclaim universal approval, expressing pragmatic concerns over the effectiveness of large-scale screening programmes. Some screening methods, for example, the simple heel-prick used in the detection of newborn PKU, are an agreed success; however, the value of mass screening in breast and prostate cancer remains controversial.[2]

The recently instituted BreastCheck programme in Ireland offers mammograms every two years to women aged 50 to 64 years. This is similar to that offered in the UK by the National Health Service, which provides mammography every three years for women in the same age group. However, in the United States the American Cancer Society recommends yearly mammograms for all healthy women aged 40 years or older. This discrepancy illustrates the lack of agreement among national institutions on the most effective strategies for breast cancer screening. While other factors, including economic considerations, no doubt play a role in this discrepancy, some analysts believe the justification for population-wide mammography screening remains to be secured in high-quality evidence.[3] Further examination of this and other important issues is discussed later in this edition of the TSMJ.

Perhaps better established is the evidence for cardiovascular risk profiling. Heart disease is the leading cause of death in industrialised nations and promises to remain so for the next generation at least. Cardiovascular risk assessment has become an integral part of general medical practice and has proven to be an effective adjunct for both primary and secondary disease prevention. However, it is important to note that screening for traditionally accepted risk factors fails to predict more than 50 percent of acute cardiovascular events.[4] This illustrates two key points: firstly, our understanding of this major disease is incomplete; secondly, epidemiological discovery of new risk factors may lead to greater understanding of the disease – a "bed to bench" approach. This search for novel risk factors has resulted in an explosion of studies on the subject. Among these are examples illustrated by two articles featured in this edition of the TSMJ. Whether these or other novel markers find their way into routine practice remains to be seen, but the search will continue.

For systems of medical screening to achieve their desired goals, programmes must be evaluated with honesty and rigour. Without evidence of clear benefit, it would be unethical to encourage patients to participate in screening programmes, just as would it be unethical to prescribe drugs without proof of safety and efficacy. However, we must also remain optimistic that appropriate screening tools and evidence for their effectiveness will be established. It is the hope of all of us that we will look after our patients knowing we are truly helping them.

References

1. Olshansky SJ, Passaro DJ, Hershow RC, et al. A potential decline in life expectancy in the United States in the 21st century. *N Engl J Med* 2005;352:1138-45.
2. Law M. Screening without evidence of efficacy. *BMJ* 2004;328:301-302.
3. Olsen O, Gotzshe PC. Screening for breast cancer with mammography. *The Cochrane Database of Systemic Reviews* 2001;4:1877.
4. Ridker PM, Glynn RJ, Hennekens CH. C-reactive protein adds to the predictive value of total and HDL cholesterol in determining risk of first myocardial infarction. *Circulation* 1998;97(20):2007-11.

A Case of Fournier's Gangrene

Elaine Neary
4th Year Medicine

ABSTRACT
Fournier's gangrene is a rare, necrotising fasciitis of the genitals and perineum caused by a mixture of aerobic and anaerobic microorganisms. The complications of this synergistic infection are multiple organ failure and death. I report a case of a gentleman who presented to Accident and Emergency with gangrenous and necrotic penile, scrotal and perineal areas. Due to the aggressive nature of this condition, early diagnosis is crucial. Treatment involves extensive soft tissue debridement and broad-spectrum antibiotics. Despite appropriate therapy, mortality is high.

INTRODUCTION

Fournier's gangrene is an uncommon, rapidly progressive infection of the male genital, perineal and perianal regions with occasional cranial extension to the abdominal wall. It is characterised by a synergistic, necrotising fasciitis leading to the thrombotic occlusion of small subcutaneous vessels and the development of gangrene.[1] There are two types. Type I is due to a mixture of aerobic and anerobic organisms usually following an abdominal operation or associated with diabetes mellitus.[2] Type II is due to Group A *Streptococcus* synergistic with a second organism (*Staphylococcus aureus*, coliforms, *Bacteroides* spp.).[2,3] The majority of patients with Fournier's gangrene are immunocompromised and thus the primary wound might have been minor or might have arisen from an otherwise uneventful operation.[3, 4] Malignant disease, obesity, diabetes mellitus, peripheral vascular disease, local trauma, urethral stricture and perianal disease have been cited as the main predisposing factors.[5] Early presentation and diagnosis, supportive measures and the use of broad-spectrum antibiotics with prompt and aggressive surgical debridement remain the cornerstone of management.[6] In spite of these advancements in management, mortality is still high and averages 20-30 percent.[4]

CASE REPORT

A sixty-three year old gentleman was admitted by ambulance to the Accident and Emergency Department of a university teaching hospital in Dublin, Ireland, in January, 2005. He had been found lying in faeces in his room, which was fly-infested. No medical, surgical or family history was available, nor knowledge of any drug allergies. Collateral social history revealed that he lived on his own. Initial vital signs indicated that the patient was hypothermic (30.1^{o}C), hypotensive (54/34mm Hg), bradycardic (45 beats per minute) and dehydrated. He was moving all four limbs and scored 13/15 on Glasgow Coma Scale. Physical examination of his abdomen revealed a palpable bladder. Perineal examination revealed the entire penile skin to be gangrenous and necrotic. His scrotum was hard, swollen, erythematous and the left hemiscrotum was gangrenous. The perineal area was cellulitic and gangrenous. The inguinal area was swollen and cellulitic bilaterally, with a necrotic ulcer and lymphadenopathy. Digital rectal examination revealed a firm prostate, laena and small polyps. No other masses were palpable.

Investigations

Table 1.

Full Blood Count and Platelets

		Reference Ranges
White Cell Count	*30.5 x10 ^{9}/1	(4.0 – 11.0)
Neutrophils	*29.0 x10^{9}/ l	(2.0 – 7.5)
Lymphocytes	*1.2 x 10^{9}/l	(1.5 – 3.5)
Monocytes	0.3 x 10^{9}/l	(0.2 – 0.8)
Eosinophils	0.0 x 10^{9}/l	(0.0 – 0.4)
Basophils	0.0 x 10^{9}/l	(0.0 – 0.1)
Red Cell Count	*4.23 x 10^{12}/l	(4.60 – 5.70)
Haemoglobin	13.7 g/dl	(13.5 – 18.0)
Haematocrit	*0.384 (Ratio)	(0.430 – 0.510)
Mean Cell Volume	90.8 fl	(83.0 – 99.0)
Mean Cell Haemoglobin Content	*35.7 g/dl	(30.8-34.6)
Red Cell Distribution Width	13.6	(11.0-15.0)
Platelets	434 x 10^{9}/l	(140-450)

Renal Profile

Urea	*90.0 mmol/L	(3.0 – 7.0)
Sodium	*128 mmol/L	(135 – 145)
Potassium	*7.8 mmol/L	(3.5 – 5.0)
Creatinine	*894 umol/L	(50 – 115)
Bicarbonate	Not available	

Liver Profile

Total Protein	73 g/L	(60 – 80)
Albumin	*25 g/L	(35 – 50)
Total Bilirubin	10 umol/L	(0 – 17)
Alkaline Phosphatase	*441 IU/L	(40 – 120)
Gamma – GT	*156 IU/L	(10 – 55)
LDH	*595 IU/L	(230 – 450)
AST	*72 IU/L	(7 – 40)

Wound Swab of Scrotum

Culture	Ampicillin	**Ciprofloxacin**	**Gentamicin**
+++A.lwofii	Sensitive	Sensitive	Sensitive

Note: Further swab revealed the wound to be MRSA positive, which was likely to represent a nosocomial complication.

Blood Film

Neutrophil leukocytosis
Neutrophils contain toxic granular echinocytes (burr cells)
Lymphocyte morphology appears normal
Echinocytes could represent a storage artefact, or associated liver or renal disease

Hospital Course

The Urology team established the diagnosis of Fournier's gangrene of the penile skin and subcutaneous tissue and perineum, complicated by acute urinary retention, acute renal failure and septic shock. It was hypothesised that the infection started as a small perianal collection which progressed. He was treated with broad-spectrum antibiotics (penicillin, gentamicin and metronidazole) and debridement of the necrotic skin with near total resection of the penis. The team inserted a suprapubic urinary catheter, a faecal drainage tube and a fine bore nasogastric-feeding tube. A tracheostomy was created to facilitate airway and pulmonary management. Following surgery, the patient was transferred to the ICU for monitoring and support. He required dialysis, inotropic support and morphine infusions. Postoperatively, wound care was performed with twice daily dressing changes with Dakin's solution. One week later, the patient returned to theatre in order to establish if any necrotic tissue remained. The rectus sheath was found to be partially gangrenous. Hydrogen peroxide was used intraoperatively to destroy the gangrenous tissue through the formation of free radicals. Due to extensive blood loss his haemoglobin dropped to 7.3g/dl and blood pressure to 81/24 mm Hg. The patient received two units of blood and intravenous Hartmans solution. Several litres of normal saline were used intraoperatively to wash out the wound in order to minimise bacterial counts. The extensive wound was initially packed, with the intention of closing later by means of skin grafting. Meanwhile, the testes were placed in subcutaneous pockets in the upper border of the wound for coverage.

DISCUSSION: FOURNIER'S GANGRENE

Gangrene is defined as the local death of soft tissues due to disease or injury. The dead tissue is nearly always colonised by bacteria. Gangrene can be of two types:[7]

1) Non infected/dry
2) Infected or wet/gas gangrene.

Gas gangrene is a rapidly progressive infection with a high mortality rate that has been recognised for much of medical history. Hippocrates described necrotising infections that caused 'flesh, gnew and bones to fall away in great quanity.'[8] There are a number of subdivisions including: Clostridium cellulites, Clostridium myonecrosis and necrotising fasciitis. However, these entities represent part of a continuum, not all of which are due to clostridial species. Fortunately, deep soft tissue infections from gas-forming pathogens are relatively unusual and clinicians encounter this problem infrequently during their surgical careers.[8] These infections can infect multiple systems in the body including the skin, exocrine and cardiovascular systems. There is no gender, age or genetic predisposition, although they are more common in debilitated elderly patients with compromised immune systems.[5]

Necrotising fasciitis is a fulminant, rapidly progressive spreading infection. Widespread tissue destruction and necrosis of fat and fascia with overlying secondary necrosis of skin are characteristic of the infection (2-3 cm/hour at its most severe). Jean Alfred Fournier, a French venerologist, described a subgroup of necrotising fasciitis in 1883.[9] Originally, it was thought to be an idiopathic infection of healthy males. However, modern use of the term Fournier's gangrene has been expanded to include all perineal necrotising infections in both men and women.[8] The majority of infections documented occur in diabetic men aged 50 to 70 years.[9] It is a serious condition characterised by cellulitis of the scrotal skin leading to subcutaneous necrosis, all of which are preceded by subcutaneous crepitations. The testes are unaffected.[3] Fournier's gangrene can be classified as a synergistic necrotising sepsis if the tissues of the abdominal wall below the deep fascia, such as the anterior sheath of the rectus muscle, are involved.

Signs and Symptoms

The illness begins with a prodromal period of genital discomfort and pruritis followed by sudden onset of perineal pain out of proportion to the stimulus applied.[5,8] However, as the gangrene progresses this pain is replaced by anaesthesia.[8] Initially, the skin may appear to be normal and the extent of the subdermal gangrene may not be apparent.[3,8] This is important because it may delay diagnosis as the infection is centered at the fascial level. Progression through the fascial layers results in deep tissue necrosis and gangrenous skin changes, resulting in drainage of the affected area and demarcation between viable and dead tissue.[5,8] A by-product of anaerobic metabolism is the formation of crepitus, composed of hydrogen, hydrogen sulphide, nitrogen and nitrous oxide, which may be detected on x-ray, thus indicating the presence of dead tissue.[8,9] All of these signs are associated with a foul odour. There is rapid development of severe toxaemia with associated signs, for example, pyrexia with or without hypothermia, leukocytosis, thrombocytopenia, raised blood urea and nitrogen, tachycardia, hypotension and reduced urine output.[8,9] This may occur in just a few hours progressing to organ failure and death so the admitting physician must have a high index of suspicion when assessing the case.[8]

Causes

One of the most important causes of this infection is poor hygiene, which was evident in the case of the patient described above (Figure 1). Usually it is due to a less aggressive, more routine infectious process through some point of entry involving the colon, urinary tract, prostate or anorectal area, for example, a skin abscess, incarcerated inguinal hernia or fistulous tract.[5,8] It can also proceed from cellulitis or traumatic injury involving cutaneous structures in the perianal region.[8] A primary infectious focus can be determined in 95 percent of cases.[6] Obstetric events such as vaginal deliveries with episiotomies and Caesarean section, carcinoma of the large intestine, hematological malignancies, severe neutropenia and burns have also been implicated.[8] Predisposing factors for the development of necrotising soft tissue infections include: poor perfusion, hypertension, renal insufficiency, trauma, diabetes mellitus, malnutrition, immune suppression, cigarette smoke, intravenous drug abuse, obesity and spinal cord injury.[8,10] Old age itself is not a predisposing factor; however, elderly patients with poor self care and poor nutritional status are more susceptible to Fournier's gangrene and have a poorer prognosis.[5]

Diagnosis

Differential diagnosis includes cellulitis, balanitis, orchitis, epididymitis, torsion, strangulated hernia and benign scrotal oedema.[1] A range of investigations can combine to confirm the diagnosis, however it is usually a clinical diagnosis and the role of diagnostic imaging is limited. A full blood count is appropriate which in this case will reveal anaemia and leukocytosis. Renal profile is impaired with high urea and creatinine, which is typically secondary to septic shock. Coagulopathies, such as disseminated intra-vascular coagulopathy, secondary to sepsis/septic shock may also be seen. A mid-stream urine sample is taken to exclude urinary tract infection. The source of infection should be investigated by whatever means necessary. An intravenous pyelogram, barium enema, sigmoidoscopy and/or cystoscopy may be appropriate and the underlying cause should be treated.[3] Tissue biopsies and pus are sent for culture and sensitivity tests to detect gas-producing microorganisms. Synergy and interaction among organisms is seen in Fournier's gangrene, resulting in increasing virulence and local vascular thrombosis.[3] Streptococci release toxins such as streptokinase and hyaluronidase which cleave the fascial planes and allow the

Physiologic Variable/ Point Assignment	High Abnormal Values				Normal	Low Abnormal Values			
	+4	+3	+2	+1	0	+1	+2	+3	+4
Temperature (°C)	> 41	39-40.9	-	38.5-39	36-38.4	34-35.9	32-33.9	30-31.9	<29.9
Heart rate	> 180	140-179	110-139	-	70-109	-	55-69	40-54	<39
Respiratory rate	> 50	35-49	-	25-34	12-24	10-11	6-9	-	< 5
Serum Sodium (mmol/L)	> 180	160-179	266-159	350-354	130-149	-	120-129	111-119	<110
Serum Potassium (mmol/L)	> 7	6-6.9	-	5.5-5.9	3.5-5.4	3-3.4	2.5-2.9	-	<3.5
Serum Creatinine (mg/100/ml x 2 for acute renal failure)	> 3.5	2-3.4	1.5-1.9	-	0.6-1.4	-	<0.6	-	-
Hematocrit	> 60	-	50-59.9	46-49.9	30-45.9	-	20-29.9	-	<20
WBC (total/mm^3 x 1000)	> 40	-	20-39.9	15.19.9	3-14.9	-	1-2.9	-	< 1
Serum bicarbonate (venous, mmol/L)	> 52	41-51.9	-	32-40.9	22-31.9	-	18-21.9	15.17.9	<15

Table 2. *Fournier's gangrene severity index. Reproduced with permission of Lippincott Williams and Wilkins from Laor E, Palmer LS, Toila BM et al: Outcome prediction with Fournier's Gangrene. J Urol 154: 89-92, 1995.*

infection to spread rapidly.[7] The clinical picture is similar regardless of the bacterial species involved. The administration of antibiotics prior to culture can alter these results. A frozen section is useful for visualising soft tissue necrosis and dense infiltration of the involved area with polymorphonucleocytes. MRI will show oedema but is not necessary for diagnosis.

Treatment

These infections are a surgical emergency; a delay in treatment is associated with a significantly higher mortality rate. Radical debridement should be carried out, aggressively removing all necrotic tissue and any marginally perfused tissue, dissecting through all the fascial planes. Under normal circumstances, it takes significant force to separate soft tissues from fascia. However, if a blunt probe slides through this plane effortlessly, it is indicative of necrotising fasciitis. Debridement should be continued until all remaining tissues are adherent and viable.[8] However, this can pose a problem if the necrosis is extensive. In the case above, the rectus sheath was found to be partially gangrenous during the second operation, but its removal was not feasible as to do so would entail great risk to the patient. As a rule, the penis, testes, bladder and rectum are spared if possible. Spirnak *et al.* reported that patients who undergo multiple operations have greater mortality.[11] The authors believed that was due to a greater extent of disease in these patients. Appropriate intravenous broad-spectrum antibiotics which are effective against both anaerobic gram-negative and facultative aerobic organisms are important therapeutic adjuvants, but are not substitutes for proper surgical debridement. Empirical therapy is given initially which can then be modified on the basis of culture and sensitivity tests. Therapeutic drug monitoring is appropriate. The patient's results should be reviewed daily and creatinine phosphokinase (CPK) levels should be obtained to monitor for myonecrosis. As mentioned above, the patient was receiving enteral nutrition. Patients with large open wounds should be fed at a rate of 1.5 to two times greater than the basal requirement. Nutritional parameters such as albumin, prealbumin and transferrin should be monitored to assure proper caloric intake. The use of hyperbaric oxygen therapy is debatable as its therapeutic value is unclear.[8] The wound is closed later with skin grafting. Previously, unmeshed spilt-thickness skin grafts were used, but there is a vogue for the use of meshed spilt-thickness skin grafts due to 100 percent uptake rates, safety and efficacy.[12, 13] The aim is to restore function quickly and provide a good cosmetic outcome.[12] The resultant scar may predispose patients to squamous cell carcinoma after a long latent

Author	Year	Patients	Overall Mortality	Mortality with Early Débridement	Mortality with Delayed Débridement	Comment
Wang and Shih	1992	33	33%	20%	75%	Extremities only
Cartwright el al.	1995	6	33%			All strep*
Tsai et al.	1995	54	22%	0%	40%	
Shupak et al.	1995	37	32%	with HBO; (Hyperbaric Oxygen Therapy) 36%	(without HBO Therapy) 25%	
Moss, Musemecheaa and Kosloske.	1996	20	25%	0%	100%	Paediatric
Lille et al.	1996	29	14%	6%	25%	
Kaul et al.	1997	77	34%			Age, hypotension, bacteraemia correlated with mortality/all strep*
Bilton et al.	1998	68	15%	4%	38%	
Elliott, Kufera and Myers	2000	182	25%			Bacteraema, delayed surgery, organ dysfunction correlated with mortality
Brandt, Corpron and Wahl	2000	37	24%			Age, ventilator use correlated with mortality
Futes-Morales et al.	2002	39	18%			Paediatric
Sharma, Khatib and Fakih	2002	9	33%			Toxic shock predicted mortality/all strep*
Dahl et al.	2002	7	71%			Toxic shock predicted mortality/all strep*
Total		598	25%	6%	40%	

Table 3. *Mortality of Necrotising Fasciitis in Series Published After 1990.*

** All patients in this selected series had a streptococcal infection.*
^ All patients in this series were younger than 18 years.
From: Cameron JL. Current Surgical Therapy 8th edition, Philadelphia: Elsevier Mosby; 2004: 1079-1085

period. This time lag differentiates it from other scar carcinomas or Marjolin's ulcer.[14]

Expected Course/Prognosis

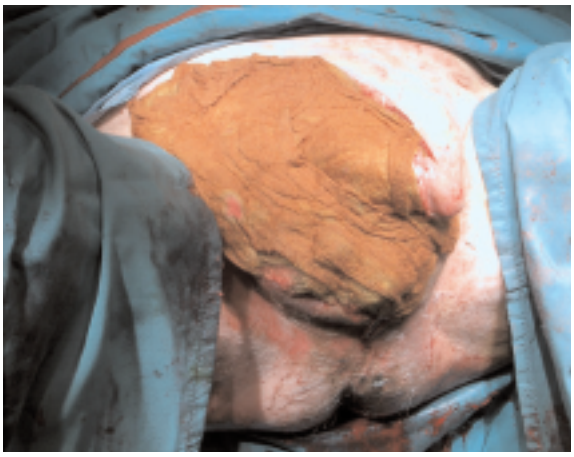
Fournier's gangrene is a life threatening illness requiring emergency surgery, despite which the patient may still not survive. Mortality rates as high as 20 to 30 percent have been described in some studies.[4] Certain factors influencing the survival of these patients, primarily relating to the patient's metabolic status and the extent of the disease, were evaluated by Laor *et al.* from which the Fournier's gangrene severity index (FGSI) was formulated.[15] In the FGSI, nine parameters are measured and the degree of deviation from normal is graded from 0 to 4. The individual values are summed to obtain the FGSI score (FGSIS). These parameters are: temperature, heart rate, respiratory rate, levels of serum sodium, potassium, creatinine and bicarbonate, haematocrit and leukocyte count (Table 2). In a study by Yeniyol *et al.* the accuracy of this index was tested. They found that the duration of symptoms before presentation was statistically important. Of the nine parameters, temperature, heart rate and respiratory rate were considered to be the most important by these authors. In patients who died, however, all the parameters were abnormal. The authors also found that lower serum albumin and total protein levels indicated the degree of debilitation and a poor prognosis. However, early diagnosis and treatment and the arrest of the gangrene at an early stage markedly improve outcome. The mortality from these infections has dropped significantly in the last century.[8] The most common error in the management of these lesions is delayed or inadequate surgical debridement. Other factors associated with increased mortality in necrotising infections are advanced age, co-existent systemic sepsis and development of organ failure (Table 3). Co-morbid conditions such as cancer, renal insufficiency and congestive heart failure also contribute to an increased mortality rate.[8]

CONCLUSION

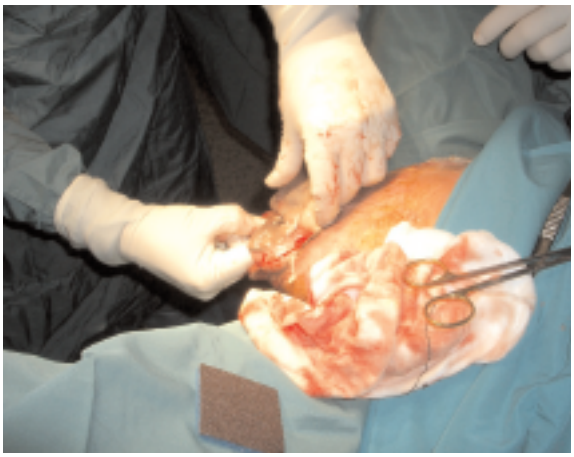
Despite the fact that Fournier's gangrene is a rare condition, it is essential that physicians know how to recognize it. Early recognition and diagnosis, followed by emergency surgery, are the keys to treating these cases and to the prevention of systemic sepsis, potential organ failure and death.

REFERENCES

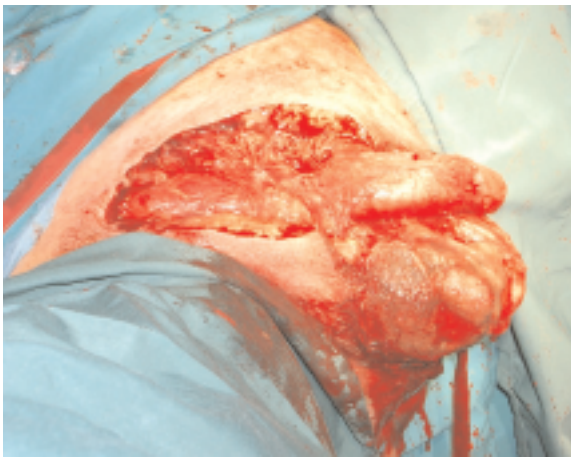
1. Patankar SP, Lalwani SK. Fournier's gangrene. *J Indian Paediatr* 2004;41:511.
2. Kumar P, Clarke M, eds. Clinical Medicine. 5th ed. Edinburgh: WB Saunders, 2002:66-7.
3. McLatchie GR, Leaper DJ, eds. Oxford Handbook of Clinical Surgery. 2nd ed. Oxford UK: Oxford University Press, 2003:53,890.
4. Pawlowski W, Wronski M, Krasnodebski IW. Fournier's gangrene. *Pol Merkuriusz Lek* 2004; 17(97):85-7.
5. Yeniyol CO, Suelozogen T, Arslan M, Ayder RA. Fournier's gangrene: Experience with 25 patients and the use of Fournier's gangrene severity index score. *Urology* 2004;64(2):218-22.
6. Muqim R. Necrotising fasciitis: management and outcome. *J Coll Physicians Surg Pak* 2003;13(12):711-4.
7. Henry M, Thompsen J, eds. Clinical Surgery. 1st ed. Edinburgh: WB Saunders, 2002:113,126.
8. Cameron JL, ed. Current Surgical Therapy. 8th ed. Philadelphia: Elsevier Mosby, 2004:1079-85.
9. Upport RN, Levy HM, Patel PH. Case 54: Fournier's Gangrene. *Radiology* 2003;226:115-7.
10. Vaidyanathan S, Soni BM, Hughes PL et al. Necrosis of the scrotum (Fournier's gangrene) in a spinal cord injury patient - a case study. *BMC Fam Pract* 2002;3; 20.doi10.1186/1471-2296-3-20.
11. Spirnak JP, Resnick MI, Hampel N. Fournier's gangrene: report of 20 patients. *J Urol* 1984;131:289-92.
12. Black PC, Friedrich JB, Engrav LH, Wessells, H. Meshed unexpanded split-thickness skin grafting for reconstruction of penile skin loss. *J Urol* 2004;172(3):976-9.
13. Maguina P, Palmieri TL, Greenhalgh DG. Split thickness skin grafting for recreation of the scrotum following Fournier's gangrene. Doi:10.1016/j.burns. 2003.07.001.
14. Chintamani, Shanker M, Singhal V, Singh JP, Bansal A, Saxena S. Squamous cell carcinoma developing in the scar of Fournier's gangrene-Case Report. *BMC Cancer 2004*;4:16 doi:10.1186/1471-2407-4-16.
15. Laor E, Palmer LS, Toila BM et al. Outcome prediction in patients with Fournier's gangrene. *J Urol* 1995;154:89-92.



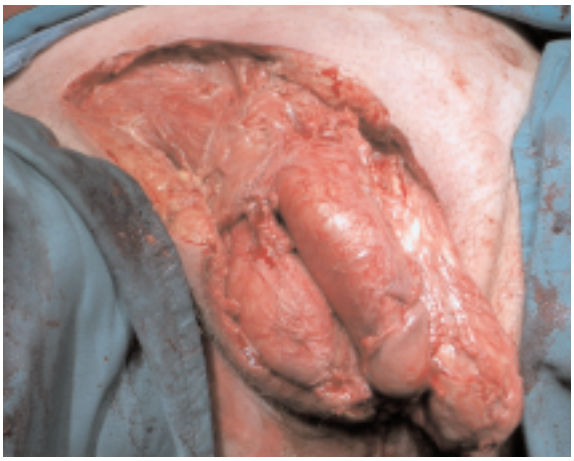
(A)



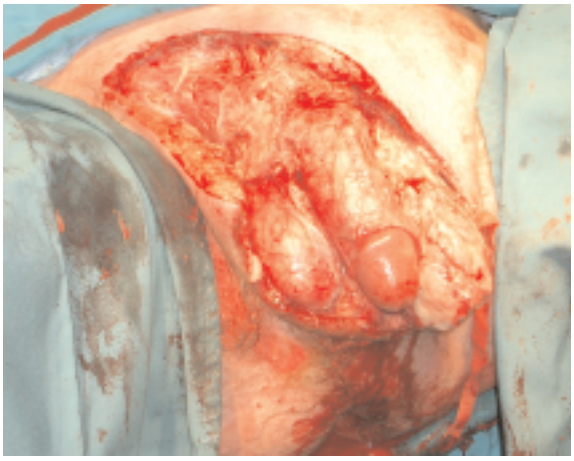
(B)



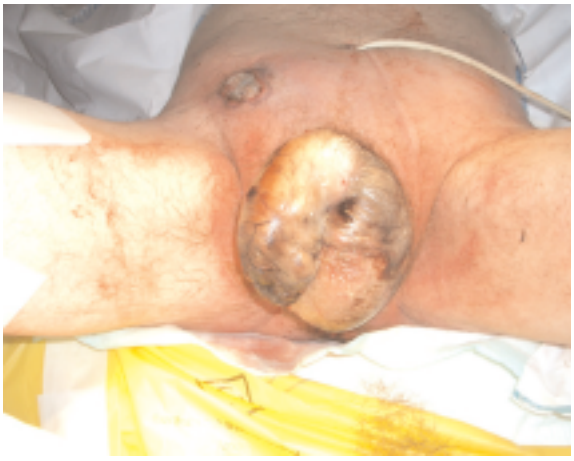
(C)



(D)



(E)



(F)

Figure (A)-(F). *Fournier's Gangrene as seen intraoperatively.**
**Photos provided by Rustom Maneksha.*

HIV - A Dental Perspective

Michael O'Halloran

5th Year Dental Science

ABSTRACT

The treatment of patients infected with HIV (human immunodeficiency virus) is a very interesting discipline within both medicine and dentistry. Recent advances in antiretroviral therapies have resulted in infected patients now having much longer symptom-free life spans and treatment planning now challenges the clinician to be cognisant of all the oral and dental aspects of the disease and its treatment. As well as influencing the epidemiology of the classical HIV-related lesions and infections, these drugs are associated with many drug interactions and adverse side effects. This review aims to describe the significant oral manifestations of the disease and highlight difficulties in managing this subset of patients.

INTRODUCTION

Since acquired immunodeficiency syndrome (AIDS) was first recognized in 1981, there has been a global pandemic with devastating consequences. Patients with AIDS were unlikely to survive more than a year or two and they had to live with the dreadful social stigma associated with the disease.[1] Since then, scientists have developed an effective arsenal of drugs against the causative agent, human immunodeficiency virus (HIV). Dr. David Ho introduced highly active antiretroviral therapy (HAART) in 1995[2] and it has transformed the infection from a death sentence to a chronic disease.[3] Systematic reviews have shown that HAART or "triple therapy" has undoubtedly been a huge success in halting AIDS progression and suppressing viral load, relative to anti-retroviral therapies only using one or two drugs.[4,5]

By the end of 2003 there had been 3,408 cases of HIV reported in Ireland with 399 new cases in 2003 alone.[6] Even though AIDS mortality rates have declined, it is worrying that from 1994 to 2003 the Irish annual incidence of newly diagnosed HIV infections per year has increased by almost five fold.[6] With HAART, patients who are HIV-positive are living longer and are therefore demanding more from the health services.

CLINICAL ASPECTS

HIV-related oral conditions are numerous, usually prominent and occur early in the disease process. These potentially pathognomonic manifestations have been well classified.[7,8,9,10]

Many reports have focused on the changing spectrum of HIV-related oral lesions in the era of HAART. Oral candidiasis (OC), oral hairy leukoplakia (OHL), HIV-related periodontal disease and Kaposi's sarcoma (KS) have declined in prevalence by varying amounts in different parts of the world. Tappuni and Fleming found that oral manifestations of HIV were found in 30 percent of subjects taking any antiretroviral regimen compared to 46 percent for drug naïve HIV-positive patients.[11] Eyeson et al suggested that substantial differences in prevalence reported for different lesions might be due to inconsistencies between study groups, different study designs and inter-examiner variation.[12] It has been widely observed that oral lesions are associated with CD4+ T cells counts less than 200 /mm^3 and/or plasma HIV RNA greater than 3,000 copies/ml, whether the patient was taking antiretrovirals or not.[11,13,14]

Stage of HIV/AIDS	Possible related oral lesion or infection
Acute seroconversion illness	Aphthous ulceration and oral candidiasis
HIV-infection in Undiagnosed individual	Oral candidiasis, oral hairy leukoplakia, Kaposi's sarcoma, necrotizing ulcerative gingivitis
Clinical disease progression/ predictor of development of AIDS	Oral candidiasis and oral hairy leukoplakia
Immune suppression in HIV	Oral candidiasis, oral hairy leukoplakia, necrotizing periodontal disease, Kaposi's sarcoma, long-standing herpes infection and major aphthous ulcerations

Table 1. *Possible oral lesions in relation to the clinical spectrum of HIV/AIDS.*

Study (year)	OC (%)	OHL (%)	HIV-necrotizing periodontal disease (%)
Margiotta et al. (1999)[15]	9.6	9.6	2.9
Patton et al. (2000)[16]	16.7	11.4	1.7
Schmidt-Westhausen et al. (2000)[13]	0	0	1.6
Eyeson et al. (2002)[14]	4.9	9.9	9.9

Table 2. *Prevalence of strongly associated HIV oral lesions in the era of HAART.*

Candidiasis is often the initial manifestation of HIV.[17,18] The infection may present early in immune dysfunction in two main forms. Initially erythematous candidiasis (EC) presents at higher CD4+ T cell counts (approximately 400 cells/mm^3), followed by pseudomembranous candidiasis (PC) that occurs more commonly when CD4+ T cell counts decrease to around 200 cells/mm^3.[7] Both forms reportedly occurred in 8 percent of patients on HAART compared to 21 percent and 11 percent (for EC and PC respectively) in drug naïve patients.[11] Angular cheilitis and rarely hyperplastic candidiasis are also seen with HIV.[19] It has recently been demonstrated that protease inhibitors (PIs), which are common components in HAART regimens, may directly inhibit a family of candidal virulence enzymes, candida-secreted aspartyl proteinase (Sap). This may explain how the beneficial effect of PIs against OC is independent of the effects of early immune reconstitution.[20] It is interesting to note that a retrospective Spanish study found that HAART influenced a significant reduction in PC but with a compensatory increase in EC thus indicating a partial immune recovery in those affected.[21] Currently, a Cochrane Review is researching the methods of preventing and treating OC in HIV disease.[22] This study will compile the results of relevant, randomised controlled clinical trials and its outcome may be useful to clinicians treating this common infection. Problems encountered in the treatment of OC include the development of resistance and drug-drug interactions. It has been reported that up to 10 percent of candida isolates become azole resistant in HIV-infected individuals.[23] Also, there is a potential risk of reaching toxic plasma azole levels when these anti-fungal agents are co-administered with PIs . Therefore, great caution must be exercised when PIs and azole anti-fungals are used concomitantly; it should be noted that a parallel dilemma occurs with the use of the macrolide antibiotics.[24,25,26,27]

Herpes simplex and zoster infections occur commonly in HIV-disease and are routinely treated with aciclovir. However, both aciclovir and ganciclovir increase HAART toxicity and therefore the combination of these agents demands considerable caution.[27] This difficult issue is worsened by the fact that ganciclovir is the treatment of choice for cytomegalovirus infections which may be severe in HIV disease.

Destructive periodontal disease is a serious dental condition that often renders the patient edentulous and it is widely associated with counts less than 200 CD4+ T cells/mm^3. Necrotizing ulcerative gingivitis (NUG), an acute, severe infection of the gums, is one variant of this disease. In one study, the incidence of NUG was zero percent in those patients taking HAART, compared with six percent of the drug naïve HIV-positive cohort.[11] It is also worth noting that there is a greater incidence of dental caries in patients infected with HIV.[28,29]

OHL is virtually exclusive to HIV-positive patients with low immunocompetence. Therefore it is not surprising that it is now less common with successful antiretroviral therapy. Research has shown that adult smokers are approximately three times more likely than non-smokers to have periodontal disease[30] because tobacco-induced alterations in microbial and host factors have deleterious effects on the periodontium.[14,31] It is thus interesting to note that it has also been reported that smoking increases the prevalence of HIV-specific lesions, namely OHL and condylomata acuminata.[13]

KS has been described as a sinister, opportunistic neoplasm associated with advanced immuno-suppression. The average survival of patients is 21 months (range three to 45 months). In 60 percent of cases of KS, there is oral involvement and this usually involves the hard palate.[19] Oral KS has a prevalence of zero to 38 percent in the HIV-infected population worldwide and is seen predominantly in populations where men have sex with men.[10] With this particular mode of transmission, the receiving partner is most at risk of HIV transmission and at a similar risk to infected needle sharers.[32] It is suggested that KS-associated human herpes virus 8 may be transmitted as a cofactor in these instances. With HAART, oral KS has declined up to four-fold[13,33] and is seen as the mainstay palliative treatment of this cancer.[19]

Conversely, the prevalence of HIV-related salivary gland disease and herpes-virus infections has increased with HAART and human papilloma-virus (HPV)-associated oral warts are reported to have increased up to six-fold.[13,16,34,35] This increase is of concern because some HPV are linked with malignant disease, for instance HPV-16, 18 and 33 have been associated with cervical carcinoma.[36] With HAART, an incompletely reconstituted immune system may vary in functionality against pathogenic microorganisms due to the CD8+ T cell diffuse infiltrative syndrome that is part of

immune reconstitution syndrome, thus the increase of salivary gland disease is expected. [32] This phenomenon has been referred to as the "HAART attack" where recrudescence of latent disease occurs with renewed immune competence.

Antiretroviral therapy is strongly linked with xerostomia, which was reported in up to one-third of patients taking didanosine who had AIDS or AIDS-related complex and had previously demonstrated haematological intolerance to zidovudine.[37] In patients taking HAART, including PIs, up to seven percent have reported xerostomia and/or oral ulceration.[38] Patients taking HAART may also present complaining of facial numbness or tingling which may even have resulted in accidental traumatic injury. Circumoral paraesthesia was reported in 25 to 27 percent of patients on PIs[36,39,40] but in only two percent of patients interviewed by Schmidt-Westhausen *et al*,[13] who proposed that the large difference occurred because their patients did not report this symptom due to its short duration and spontaneous resolution. Taste abnormalities are also linked to the use of PIs[1,25,39] and research shows the prevalence of this adverse effect as being between 10 to 20 percent.[36]

Cross Infection Management Issues

Dentists have a professional duty of care to treat HIV-positive patients without discrimination and HIV-serostatus should not impinge on treatment planning. Regular care is essential in managing HIV-related oral diseases and this treatment should be provided more slowly and carefully if necessary.[41,42,43,44] Asymptomatic patients taking HAART may not necessarily disclose their HIV-serostatus or medication regimen to the dental team and universal precautions should be observed at all times which are designed to safeguard both the patient and healthcare workers against cross-infection. The risk of seroconverting following a needlestick injury from an infected patient is approximately 1:300[30,45] and the availability of post exposure prophylaxis should not cause complacency in healthcare workers.

- The viral load of the carrier (HIV RNA <1,500 copies/ml considered low risk)
- Whether to patient is currently on a HAART regimen
- Dental needles are usually have a small bore unlike those used for venepucture
- Needles may be "cleansed" by passing through a protective rubber glove
- Deep, penetrating wounds usually occur with scalpels in oral surgery or periodontal departments and not in general practice

Table 3. *Factors that influence and potentially lower the risks in the dental setting.*

Successful HAART results in increased numbers of B lymphocytes, T lymphocytes, poly-morphonuclear leukocytes (PMNL) and platelets. However antiretroviral therapy does not dictate whether antibiotic prophylaxis is required. In general, it is not required for routine dental procedures in HIV-infection unless indicated by the patient's medical history. Patients infected with HIV via intravenous drug use are reported to be at increased risk of developing infective endocarditis.[44,46] Apart from the massive transmission risk posed by HIV-infected blood transfusions, this group has the highest "per act risk of acquiring HIV",[30] therefore it logically follows that infected needle sharers have a high risk of being co-infected with other pathogens at every exposure. Table 4 summarises a recent report on the topic in relation to HIV-infection.[44]

- Thorough review of patient's history when count of <200 $CD4^+$ T cells/mm^3
- Antibiotic cover recommended for oral and periodontal surgery if PMNL counts <500 cells/mm^3
- Neutropenia
- Current guidelines should be adhered to in the country of treatment

Table 4. *Protocol for use of antibiotic prophylaxis.*

The use of antibacterial mouthrinse and scaling has been advocated prior to dental and surgical procedures. No scientific evidence exists to suggest an increased risk of post-operative local infection in HIV-positive patients. When it does occur, systemic oral antibiotics may be prescribed, taking into account the antiretroviral therapy the patient may be receiving. However the condition of the immune system in HIV disease may alter symptoms of infection, such as reduced inflammation or lack of purulence in patients with lower immune competence.[44]

CONCLUSIONS

HIV-disease and its treatment is an ever-evolving discipline within medicine. It behoves the dental practitioner to be familiar with the current treatment methods for this expanding subset of patients and the challenges that they bring. Regular oral examination may alert the clinician to a change in the status of HIV-infection and therefore prompt appropriate care, whether it is the curative treatment of opportunistic infections or palliative care in the later stages of the disease. Candidiasis is still seen all too frequently in these patients and its management may not necessarily be as straightforward as it is in HIV-negative patients. HIV-related periodontal disease, Kaposi's sarcoma and oral hairy leukoplakia are

orally-manifesting conditions that can be readily flagged by the attending dentist.

Side effects of HAART regimens will exercise the practitioner, whether it is to reassure patients who develop erythema multiforme or to alert the supervising specialist about more hazardous adverse reactions. HAART-related xerostomia and its inherent problems often require dental intervention and expertise. Drug interactions are particularly important as antiretroviral agents may interfere with relatively commonly prescribed drugs such as metronidazole and antifungal agents and may cause life-threatening respiratory depression when combined with common sedative agents.

It should be noted that HIV-infected CD4+ T cells have been found in follicular dendritic and lymph node mononuclear cells in patients who had undetectable plasma viraemia.[47] This highlights the fact there is no cure for HIV-disease and also reminds the healthcare worker about the serious nature of the infection, even if it is well masked by successful antiretroviral therapies.

REFERENCES

1. Patton LL. HIV disease. *Dent Clin North Am* 2003;47(3):467-92.
2. Ho DD. Time to hit HIV, early and hard. *N Engl J Med* 1995;333(7):450-1.
3. Patton LL, Shugars DC. Immunologic and viral markers of HIV-1 disease progression: implications for dentistry. *J Am Dent Assoc* 1999;130(9):1313-22.
4. Jordan R, Gold L, Cummins C, Hyde C. Systematic review and meta-analysis of evidence for increasing numbers of drugs in antiretroviral combination therapy. *BMJ* 2002;324(7340):757.
5. Rutherford GW, Sangani PR, Kennedy, GE. Three- or four- versus two-drug antiretroviral maintenance regimens for HIV infection (Cochrane Review). *The Cochrane Library* 2004(4).
6. National Disease Surveillance Centre. General information on HIV and AIDS. 2005. Accessed 27 January, 2005 at: http://www.ndsc.ie/DiseaseTopicsA-Z/HIVHumanImmunodeficiencyVirus/)
7. Greenspan JS, Barr CE, Sciubba JJ, Winkler JR. Oral manifestations of HIV infection. Definitions, diagnostic criteria, and principles of therapy. The U.S.A. Oral AIDS Collaborative Group. *Oral Surg Oral Med Oral Pathol* 1992;73(2):142-4.
8. EC-Clearinghouse on Oral Problems Related to HIV Infection and WHO Collaborating Centre on Oral Manifestations of the Immunodeficiency Virus. Classification and diagnostic criteria for oral lesions in HIV infection. *J Oral Pathol Med* 1993;22:289-91.
9. Ramos-Gomez FJ, Flaitz C, Catapano P, Murray PA, Milnes AR, Dorenbaum A et al. Classification, diagnostic criteria, and treatment recommendations for orofacial, manifestations in HIV-infected pediatric patients. *J Clin Paediatr Dent* 1999;23(2):85-96.
10. Patton LL, Phelan JA, Ramos-Gomez FJ, Nittayananta W, Shiboski CH, Mbuguye TL. Prevalence and classification of HIV-associated oral lesions. *Oral Dis* 2002;(8Suppl2):98-109.
11. Tappuni AR, Fleming GJ. The effect of antiretroviral therapy on the prevalence of oral manifestations in HIV-infected patients: a UK study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2001;92(6):623-8.
12. Eyeson JD, Warnakulasuriya KA, Johnson NW. Prevalence and incidence of oral lesions--the changing scene. *Oral Dis* 2000;6(5):267-73.
13. Schmidt-Westhausen AM, Priepke F, Bergmann FJ, Reichart PA. Decline in the rate of oral opportunistic infections following introduction of highly active antiretroviral therapy. *J Oral Pathol Med* 2000;29(7):336-41.
14. Eyeson JD, Tenant-Flowers M, Cooper DJ, Johnson NW, Warnakulasuriya KA. Oral manifestations of an HIV positive cohort in the era of highly active anti-retroviral therapy (HAART) in South London. *J Oral Pathol Med* 2002;31(3):169-74.
15. Margiotta V, Campisi G, Mancuso S, Accurso V, Abbadessa V. HIV infection: oral lesions, CD4+ cell count and viral load in an Italian study population. *Oral Pathol Med* 1999;28(4):173-7.
16. Patton LL, McKaig R, Strauss R, Rogers D, Eron JJ Jr. Changing prevalence of oral manifestations of human immuno-deficiency virus in the era of protease inhibitor therapy. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2000;89(3):299-304.
17. Ferguson FS, Nachman S, Berentsen B. Implications and management of oral diseases in children and adolescents with HIV infection. *N Y State Dent J* 1997;63(2):46-50.
18. Nittayananta W, Chungpanich S. Oral lesions in a group of Thai people with AIDS. *Oral Dis* 1997;(3Suppl1):S41-5.
19. Reichart PA. Oral manifestations in HIV infection: fungal and bacterial infections, Kaposi's sarcoma. *Med Microbiol Immunol* (Berl) 2003;192(3):165-9.
20. De Bernardis F, Tacconelli E, Mondello F, et al. Anti-retroviral therapy with protease inhibitors decreases virulence enzyme expression in vivo by *Candida albicans* without selection of avirulent fungus strains or decreasing their anti-mycotic susceptibility. *FEMS Immunol Med Microbiol* 2004;41(1):27-34.
21. Ceballos-Salobrena A, Gaitain-Cepeda L, Ceballos-Garcia L, Samaranayake LP. The effect of antiretroviral therapy on the prevalence of HIV-associated oral candidiasis in a Spanish cohort. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2004;97(3):345-50.
22. Holmes H, Peinaar ED. Interventions for the prevention and management of oropharyngeal

candidiasis associated with HIV infection in adults and children (Protocol for Cochrane Review). *The Cochrane Library*, 2004(3).
23. Powderly WG, Gallant JE, Ghannoum MA, Mayer KH, Navarro EE, Perfect JR. Oropharyngeal candidiasis in patients with HIV: suggested guidelines for therapy. *AIDS Res Hum Retroviruses* 1999;15(18):1619-23.
24. Flint SR, O'Sullivan C, Arthur N. An update of adverse drug reactions of relevance to general dental practice. *J Ir Dent Assoc* 2000;46(2):67-70.
25. Lewis DA. Antiretroviral combination therapy for HIV infection. *Dent Update* 2003;30:242-47.
26. Monthly Index of Medical Specialties Ireland. p.182, December 2004.
27. British National Formulary. British Medical Association, Royal Pharmaceutical Society of Great Britain editors. Appendix 1; p.629-65. September 2004;48.
28. Madigan A, Murray PA, Houpt M, Catalanotto F, Feuerman M. Caries experience and cariogenic markers in HIV-positive children and their siblings. *Pediatr Dent* 1996;18(2):129-36.
29. Phelan JA, Mulligan R, Nelson E, Brunelle J, Alves MEAF, Navazesh M, Greenspan D. Dental Caries in HIV-seropositive Women. *J Dent Res* 2004;83(11):869-873.
30. Johnson GK, Hill M. Cigarette smoking and the periodontal patient. *J Periodontol* 2004;75(2):196-209.
31. Anonymous. Position paper: tobacco use and the periodontal patient. Research, Science and Therapy Committee of the American Academy of Periodontology. *J Periodontol* 1999;70(11):1419-27.
32. U.S. Department of Health and Human Services. Antiretroviral Postexposure Prophylaxis After Sexual, Injection-Drug Use, or Other Nonoccupational Exposure to HIV in the United States (2005). (Accessed 25 December, 2005 at: http://www.aidsinfo.nih.gov/)
33. Diz Dios P, Ocampo Hermida A, Miralles Alvarez C, Vazquez Garcia E, Martinez Vazquez C. Regression of AIDS-related Kaposi's sarcoma following ritonavir therapy. *Oral Oncol* 1998;34(3):236-8.
34. Greenspan D, Canchola AJ, MacPhail LA, Cheikh B, Greenspan JS. Effect of highly active antiretroviral therapy on frequency of oral warts. *Lancet* 2001;357(9266):1411-2.
35. King MD, Reznik DA, O'Daniels CM, Larsen NM, Osterholt D, Blumberg HM. Human papillomavirus-associated oral warts among human immunodeficiency virus-seropositive patients in the era of highly active antiretroviral therapy: an emerging infection. *Clin Infect Dis* 2002;34(5):641-8.
36. Scully C, Cawson RA. Viral infections. In: Scully C, Cawson RA, eds. Medical problems in dentistry. 5th ed. London: Elsevier, 2005:398-408.
37. Allan JD, Connolly KJ, Fitch H, et al. Long-term follow-up of didanosine administered orally twice daily to patients with advanced human immunodeficiency virus infection and hematologic intolerance of zidovudine. *Clin Infect Dis* 1993;16(Suppl1):S46-51.
38. Scully C, Diz Dios P. Orofacial effects of antiretroviral therapies. *Oral Dis* 2001;7(4):205-10.
39. Greenwood I, Heylen R, Zakrzewska JM. Antiretroviral drugs – implications for dental prescribing. *Br Dent J* 1998;184(10):478-82.
40. Porter SR, Scully C. HIV topic update: protease inhibitor therapy and oral health care. *Oral Dis* 1998;4(3):159-63.
41. Rule J, Veatch R. Ethical Questions in Dentistry. Chicago: Quintessence Publishing Co. Ltd:151-62.
42. Doyal L. Good ethical practice in the dental treatment of patients with HIV/AIDS. *Oral Dis* 1997;3(Suppl1):S214-20.
43. Greene VA, Chu SY, Diaz T, Schable B. Oral health problems and use of dental services among HIV-infected adults. Supplement to HIV/AIDS Surveillance Project Group. *J Am Dent Assoc* 1997;128(10):1417-22.
44. Shirlaw PJ, Chikte U, MacPhail L, Schmidt-Westhausen A, Croser D, Reichart P. Oral and dental care and treatment protocols for the management of HIV-infected patients. Oral Dis 2002;8(Suppl 2):136-43.
45. Flint SR, Power J, Lawlor E. Hepatitis C and the dental practitioner. *J Ir Dent Assoc* 1994;40(2):37-8.
46. Barbaro G, Di Lorenzo G, Grisorio B, Barbarini G. Cardiac involvement in the acquired immunodeficiency syndrome: a multicenter clinical-pathological study. Gruppo Italiano per lo Studio Cardiologico dei pazienti affetti da AIDS Investigators. *AIDS Res Hum Retroviruses* 1998;14(12):1071-7.
47. Ruiz L, van Lunzen J, Arno A, et al. Protease inhibitor-containing regimens compared with nucleoside analogues alone in the suppression of persistent HIV-1 replication in lymphoid tissue. *AIDS* 1999;13(1):F1-8.

Hysterectomy for Benign Gynaecological Disease: A Comparison of Methods

Elaine Donnelly

6th Year Medicine

ABSTRACT

Hysterectomy is the most commonly performed major gynaecological surgery in the United States (US) and the United Kingdom (UK), with 30 percent of American women having undergone the procedure by the age of 60 years. In the UK, 47 percent of hysterectomies are performed by the abdominal route, 30 percent vaginal route and three percent laparoscopically. Vaginal hysterectomy, laparoscopically-assisted vaginal hysterectomy and total laparoscopic hysterectomy are less invasive methods which confer decreased burden of procedure, reduced recovery time along with improved convalescence and cosmesis compared with abdominal hysterectomy. However, overall operative complication rates and severe post-operative complications are greater with the laparoscopic technique compared with abdominal hysterectomy. It has been suggested that improved surgical skill may decrease higher complication rates and thus make laparoscopically-assisted vaginal hysterectomy a worthy alternative to abdominal hysterectomy.

Hysterectomy is the most commonly performed major gynaecological surgery in the United States (US) and the United Kingdom (UK). Thirty percent of American women have undergone the operation by the age of 60 years. In almost 90 percent of women having a hysterectomy the surgery is carried out for benign disorders, particularly fibroids, which alone are the most common indication for hysterectomy in the UK.

Hysterectomy remains the definitive cure for most causes of unacceptable uterine bleeding and rates highest in satisfaction scores compared with other forms of treatment. It is a well-established and extremely safe operation, with an overall visceral damage rate being 0.5 to 2 percent and an overall mortality rate of 0.5 to 2 per 1000.[1] Hysterectomy has developed over the years from a procedure involving an extensive abdominal incision and prolonged convalescence, such as total abdominal hysterectomy (TAH) and subtotal abdominal hysterectomy (STAH), to minimally invasive procedures, including vaginal hysterectomy, laparoscopically-assisted vaginal hysterectomy and laparoscopic hysterectomy. While it may be logical to assume that the minimally invasive approach is the more commonly used operation, less than one-third of women in the UK undergo vaginal or laparoscopic hysterectomy, with 67 percent undergoing TAH.[2] This statistic begs the question as to whether the high rate of TAH is due to an increased rate of complications and post-operative morbidity associated with the other available methods, or whether these methods are similar or superior in terms of outcome and are simply underused at the moment. The research presented in this review will attempt to explore this issue by comparing the available procedures in terms of operative complication rates, recovery times and postoperative outcomes.

The indications for hysterectomy are numerous, but there are several particularly common causes. Uterine leiomyomas or fibroids are a major cause of menorrhagia and intermenstrual bleeding and not uncommonly, pelvic pain and secondary dysmenorrhoea. They are present in up to 25 percent of all women but may be entirely asymptomatic.[3] Another common indication for hysterectomy is dysfunctional uterine bleeding, a diagnosis of exclusion which has no identifiable pathological cause but which results in unacceptable menstrual blood loss for the patient. Other indications include uterine prolapse, endometriosis and neoplasia of the cervix, endometrium and ovary. Hysterectomy may also be used in the surgical management of cancers of the colon, rectum and bladder. The procedure is not purely confined to the realms of gynaecology, but also has a number of obstetrical indications, such as massive post-partum haemorrhage secondary to uterine atony or uterine rupture, septic endometritis with pyometra or the very rare complication of inversion of the uterus after delivery of the placenta. It is also used in the management of some disorders of early pregnancy, such as ectopic pregnancies that have implanted in the cervix or cornual angle and in gestational trophoblastic disease where chemo-therapy has failed to halt the progression of the disease.

Since the first recorded hysterectomy, a subtotal procedure in 1843, there has been considerable

advancement in the types of hysterectomy performed. STAH involves the removal of the uterus only, leaving behind a cervical stump. Further surgical advances and the recognition that cancer occasionally developed in the remaining cervix led to the development of TAH, first attributed to E.H. Richardson in 1929.[1] TAH comprises removal of the body of the uterus and also the entire cervix *en bloc*. A hotly debated topic in gynaecological surgery has been the comparison of risks and benefits of TAH and STAH. A publication from a Finnish research group in 1980 claiming women who underwent STAH had better urinary and sexual function than those undergoing TAH caused further controversy.[4] This research suggested that dis-turbance of the pelvic plexus, which is essential in the coordinated contraction of bladder and bowel and is intimately related to the bladder, cervix and vagina, was at risk of damage during TAH. The interruption of autonomic innervation of the pelvic viscera may cause constipation and urinary problems after a TAH. By extension, disturbance in innervation of the cervix and vagina was thought to interfere with lubrication and orgasm, thus decreasing post-hysterectomy sexual function. This may have been strong evidence in favour of subtotal procedures, but further research from the same group was unable to corroborate the initial findings. Further trials, such as that published in 2003 by Learman *et al.* were also unable to demonstrate a benefit of STAH when compared to TAH, despite numerous factors that seemed to suggest that STAH might be superior to TAH.[5] The risk of developing a cervical cancer in the remaining cervical stump became much less relevant due to the advent of screening and the reduction of cervical cancer incidence by up to two-thirds in countries with a screening programme.[6] In practical terms, STAH is a simpler procedure, requiring little or no mobilisation of the bladder and minimal risk to the ureters as compared to TAH. The belief fostered by the Finnish research that sexual function was increasingly spared by STAH was a popular notion and one that was promoted by the press. However, without conclusive research it was impossible to determine which procedure was superior. Clearly, further rigorous randomised controlled trials were needed to clarify the issue.

The landmark trial that decided the matter for many authorities was published in the New England Journal of Medicine in 2002.[7] It was a randomised double-blinded controlled trial involving 279 women referred for hysterectomy due to benign gynaecological disease. Bladder, bowel and sexual function were all evaluated at 12 months postoperatively, as were postoperative complications. Bladder function was measured by assessment of urinary frequency, reduction in nocturia and reduction in stress incontinence. Bowel symptoms measured included constipation and use of laxatives. Sexual function was determined by ascertaining frequency of intercourse, orgasm and rating of sexual relationship with a partner. Women participating in the trial were randomised to two groups, one receiving TAH, the other undergoing STAH. It was found that neither procedure adversely affected pelvic organ function at 12 months. STAH resulted in fewer short-term complications, such as infection and had more rapid recovery, but caused cyclical bleeding and cervical prolapse long-term. Therefore, the claims that STAH was superior to TAH in terms of organ function were disproved and the presence of some long-term complications associated with the sub-total procedure were highlighted.

A further study published in 2003 suggested that the protective effects on the urinary tract believed to be conveyed by the subtotal procedure were also to be questioned, its results showed that a significantly smaller proportion of women randomised to the TAH group suffered from urinary incontinence one year post-operatively, as compared with the STAH group (9 percent versus 18 percent respectively, p=0.043).[8] This study group also found that 20 percent of the STAH group suffered from vaginal bleeding after the procedure and two of the group had to undergo subsequent procedures to remove the cervix. None of the women who had undergone the abdominal procedure suffered from post-operative vaginal bleeding. In light of this evidence, STAH may be of limited benefit as it may predispose the patient to further procedures if there is excessive vaginal bleeding or malignant change.

An alternative to the abdominal route is vaginal hysterectomy, in which there is no abdominal Pfannenstiel incision, and the procedure is performed entirely *per vaginam*. It was initially thought that it would cause less patient morbidity than the abdominal procedure. This has been shown by various studies, most notably the CREST study which reviewed 1851 hysterectomies performed between 1978 and 1981 in nine hospitals in the US. This study showed that the overall incidence of post-operative complications after antibiotics was 24.5 percent after vaginal

hysterectomy, compared with 42.7 percent after abdominal hysterectomy. It was concluded that the average woman of reproductive age with no significant past medical or surgical history (in particular, previous abdominal surgery) who received antibiotic prophylaxis would benefit more from a vaginal hysterectomy than an abdominal one.[9] The vaginal procedure, though taking slightly longer to perform, is the more cost-effective of the two procedures in terms of patient recovery and convalescence and monetary cost. Ottosen *et al*. demonstrated this in their randomised controlled trial published in 2000, which found that patients undergoing abdominal hysterectomy required on average one day more in hospital and one week more of convalescence than the vaginal cohort.[10]

On the basis of these findings, it would be reasonable to assume that vaginal hysterectomy should be the more commonly performed procedure. However, rates of abdominal hysterectomy in the United Kingdom are 67 percent compared with 30 percent performed vaginally (3 percent being laparoscopic).[2] There are some contraindications to vaginal hysterectomy, namely a large fibroid uterus and widespread endometriosis and/or adhesions. Relative contraindications to vaginal hysterectomy include nulliparity, a non-prolapsed uterus, need for salpingoophorectomy and previous pelvic surgery.[11]

Though there are a significant number of abdominal hysterectomies performed, it has been shown that women with relative contraindications to the vaginal procedure should not be required to undergo the more invasive abdominal procedure. This was demonstrated conclusively by Varma et al in their five-year study in which all hysterectomies were carried out by the vaginal route if technically possible, excluding those women with uterovaginal prolapse, very large leiomyomas (over 16 week size) and malignancy.[12] The rate of abdominal versus vaginal hysterectomies in the study centre at the outset was almost identical to that of the national average, 68 percent and 32 percent respectively. By the end of the study, 95 percent of procedures were performed via the vaginal route with most associated oophorectomies also being performed vaginally by the fifth year. There had been no change in case mix over the years of the study and there was no increase in the rate of complications or patient morbidity. The authors concluded that the major determining factor in the choice of route of hysterectomy was not the clinical scenario, but the attitude or preference of the surgeon. However impressive these results may seem, the clinical implications cannot be implemented unless the appropriate expertise is possessed by the operator. Current training practices do not afford trainee hysterectomists the opportunity to become equally comfortable with the various methods and to become proficient in vaginal hysterectomy, simply due to the continuing high rate of abdominal hysterectomy and a lack of opportunity to watch and participate in a sufficient number of vaginal procedures.[11]

One of the most significant advances in surgical procedure in recent years has been the advent of laparoscopy. This technique has been applied in almost every surgical speciality to great effect and offers a considerably less invasive procedure for the patient with the promise of a more uneventful recovery than if there had been an abdominal wound. As with other forms of major abdominal surgery, hysterectomy has been adapted to allow a laparoscopic approach to the operation. In a total laparoscopic hysterectomy (TLH), the entire procedure is performed under laparoscopic guidance and the uterus is removed through the vagina (either whole or morcellated) with no vaginal incision. In a laparoscopic-assisted vaginal hysterectomy (LAVH), a vaginal hysterectomy is performed after laparoscopic adhesiolysis or oophorectomy and ligation of uterine blood supply. As is the case in vaginal hysterectomy, laparoscopic hysterectomy has a number of relative contraindications including nulliparity, obesity and need for oophorectomy. Research shows that TLH is safe, feasible and results in minimal hospital stay for women irrespective of body mass index, with minimal complication rates in all groups. TLH may extend the possibility of minimally invasive hysterectomy to the very obese, for whom abdominal surgery poses a much greater risk.[13] Rates of complications associated with laparoscopic hysterectomy have been studied intensely.

Reports have varied as to whether the complication rate of laparoscopic hysterectomy differs from that of other methods, such as abdominal hysterectomy.[14,15] One trial which attempted to answer the question definitively was the VALUE study published in 2002, which involved 37,298 patients undergoing hysterectomy in the UK for benign conditions between 1994 and 1995.[2] Overall operative complication rates were found to be highest (6.07

percent) for laparoscopic techniques, compared with an overall complication rate for all procedures of 3.57 percent. Postoperative complications, when considered as an overall figure, were less in the laparoscopic group than the abdominal group (7.98 percent versus 8.31 percent respectively, p=0.01). However, the incidence of more severe post-operative complications was greater in the laparoscopic cohort. Despite this finding, laparoscopic hysterectomy is still a valuable technique due to advantages of shorter hospital stay and recovery time and better cosmesis after the procedure. Furthermore, the disadvantage of a higher complication rate could eventually be minimised by improved surgical skill. In his recent review of laparoscopic hysterectomy, Reich suggests that laparoscopic hysterectomy is an extremely valuable procedure for any surgeon to possess in their "procedural armamentarium" and that advancement of the laparoscopic procedure, as with the vaginal method, will depend primarily on training procedures and availability of experienced personnel in the techniques of laparoscopic hysterectomy.[16]

Despite extensive published research supporting the use of less invasive techniques such as vaginal hysterectomy, LAVH and TLH, there is still a trend amongst practitioners to hysterectomize their patients via the traditional abdominal approach. These procedures have been proven to be superior to abdominal hysterectomy in terms of burden of procedure for the patient, recovery, convalescence and cosmesis. While the risk of intraoperative complication is marginally increased in laparoscopic and possibly in vaginal procedures, it is thought that this can be minimised with operator proficiency and experience. Many intraoperative complications may be repaired laparoscopically without recourse to laparotomy.[16] It is therefore clear that the persistence of an abdominal hysterectomy rate of 67 percent compared to a laparoscopic rate of only 3 percent is a statistic that needs to be addressed by the bodies overseeing surgical training, by practising surgeons and by women themselves. With increased access to information and involvement in the decision-making process, many women may demand a more minimally invasive method of treatment and oblige practitioners to extend their "procedural armamentarium". It is the responsibility of health care professionals to increase teaching and implementation of vaginal and laparoscopic hysterectomies to ensure that women receive the optimal treatment. Treatment options must be supported by the best evidence and also afford maximum satisfaction and quality of life for the patient.

REFERENCES

1. Manyonda I. Hysterectomy for benign gynaecological disease. *Curr Obstet Gynaecol* 2003;13:159-65.
2. Maresh MJ, Metcalfe MA et al The VALUE national hysterectomy study: description of the patients and their surgery. *Int J Obstet Gynaecol* 2002;109:302-312.
3. Impey L. Obstetrics and Gynaecology 2nd Edition Blackwell Publishing 2004.
4. Kilkku P., Gronoos M, Hirvonen T, Rauramo L. Supravaginal uterine amputation versus hysterectomy: Effects on libido and orgasm. *Acta Obstet Gynecol Scand* 1983;62:147-52.
5. Learman LA, Summitt RL, Varner RE, et al. A randomised comparison of total or supracervical hysterectomy: Surgical complications and clinical outcomes. *Obstet Gynecol* 2003;102:453-62.
6. Crum CP, Abbott DW and Quade BJ. Cervical cancer screening: From the Papanicolaou smear to the vaccine era. *J Clin Oncol* 2003;21:224-30.
7. Thakar R, Ayers S, Clarkson P, Stanton S, Manyonda I. Outcomes after total versus subtotal abdominal hysterectomy. *N Engl J Med* 2002;347:1318-25.
8. Gimbel H, Zobbe V, Andersen BM, Filtenborg T, Gluud C, Tabor. A randomised controlled trial of total compared with subtotal hysterectomy with one-year follow up results. BJOG 2003;110:1088-98.
9. Dicker RC, Greenspan JR, Strauss LT et al. Complications of abdominal and vaginal hysterectomy among women of reproductive age in the United States. The Collaborative Review of Sterilization. *Am J Obstet Gynecol* 1982;44:841-48.
10. Ottosen C, Lingman G, Ottosen L. Three methods for hysterectomy: a randomised, prospective study of short-term outcome. *BJOG* 2001;107:1380-5.
11. Batra N, Tuffnell D. Vaginal hysterectomy. *Rev Gynecol Prac* 2004;4:82-8.
12. Varma R, Tahseen S, Lokugamage AU, Kunde D. Vaginal route as the norm when planning hysterectomy for benign conditions: change in practice. *Obstet Gynecol* 2001;97(4) 613-6.
13. O'Hanlan KA, Lopez L, Dibble SL, Garnier AC, Huang GS, Leuchtenberger M. Total laparoscopic hysterectomy: Body mass index and outcomes. *Obstet Gynecol* 2003;102(6):1384-92.
14. Marana R, Busacca M, Zupi E, Garcea N, Paparella P, Catalano GF. Laparoscopically assisted vaginal hysterectomy versus total abdominal hysterectomy: a prospective, randomised multicentre study. *Am J Obstet Gynecol* 1999;180:270-5.
15. Olsson JH, Ellstrom M, Hahlin M. A randomised

prospective trial comparing laparoscopic and abdominal hysterectomy. *BJOG* 1996;103:345-50.
16. Reich H, Roberts L. Laparoscopic hysterectomy in current gynecological practice. *Rev Gynecol Prac* 2003;3:32-40.

The Management of Gastro-oesophageal Reflux in Infants

Yousef El-Gohary, Andrew Karwowski, Gracia Chong, Melissa Kern, Sara Molloy, Boitumelo Pule

6th Year Medicine

ABSTRACT

Objective: To determine the optimal management of gastro-oesophageal reflux in infants. **Methods**: Searches were made on medical databases (Cochrane Library, National Electronic Library for Health, PubMed). Three randomised control studies, a case control study, an intervention study and one systematic review were chosen and appraised according to a critical appraisal checklist. **Results**: Antacids and feed thickeners were found to be effective in decreasing the symptoms, but not the gastric acidity associated with gastro-oesophageal reflux. There was no evidence supporting the use of positioning in decreasing symptoms. No evidence was found to support the use of pharmacological agents (metoclopramide, omeprazole) in the treatment of gastro-oesophageal reflux. **Conclusion**: The optimal management of gastro-oesophageal reflux in infants is parental reassurance and education. If essential, conservative measures such as feed thickeners and antacids may be employed.

INTRODUCTION

Gastro-oesophageal reflux (GOR) is a common self-limiting condition, affecting up to two thirds of infants, that usually resolves by six to 12 months of age (Figure 1).[1] It is usually a normal physiological process involving regurgitation and is described by the lay public as "spitting up". This type of emesis is effortless where there is passive return of gastric contents into the oesophagus, and occurs by three major mechanisms: transient lower oesophageal sphincter relaxation, increased intra-abdominal pressure (for example, sneezing, coughing), or spontaneous free reflux.

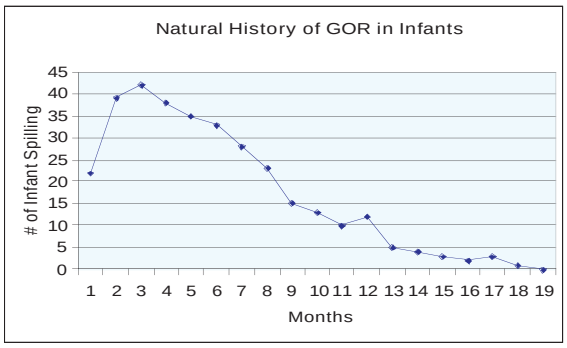


Figure 1. *Natural History of Gastro-Oesophageal Reflux (adapted from Martin et al, 2002).*

Gastro-oesophageal reflux disease (GORD) on the other hand, which affects only 1 in 300 infants, is a pathological process manifested by poor weight gain, persistent respiratory symptoms and signs of oesophagitis (Table 1).[2]

Patients with mild, uncomplicated reflux can be diagnosed clinically and treated without further investigations. The typical clinical features include post-prandial, persistent but effortless non-bilious emesis, and regurgitation with normal weight gain.

GORD	**GOR**
Regurgitation with **poor weight gain**	Regurgitation with **normal weight gain**
Apnoea & Cyanosis Wheezing Recurrent pneumonia Chronic cough Stridor	No significant respiratory symptoms
Irritability Haematemesis Iron deficiency anaemia Failure to thrive	No signs or symptoms of oesophagitis
Neck tilting (Sandifer's syndrome)	No neurobehavioural symptoms

Table 1. *Differences between Gastro-Oesophageal Reflux (GOR) and Gastro-Oesophageal Reflux Disease (GORD) (adapted from Jung, 2001).*

A good history and clinical examination will usually distinguish between GOR and other conditions with similar presentations, such as pyloric stenosis. The 24-hour pH study is considered the "gold standard" for confirming or excluding the presence of abnormal GOR. The reflux index, which is the percentage of total time that oesophageal pH is less than 4.0, provides the most efficient interpretation of the test, with a sensitivity of 96 percent, specificity of 100 percent.[3] Additional parameters that can be measured include number of reflux episodes, number of reflux episodes lasting more than five minutes, the mean duration of each reflux episode, and the duration of the longest reflux episode, within the 24-hour period.

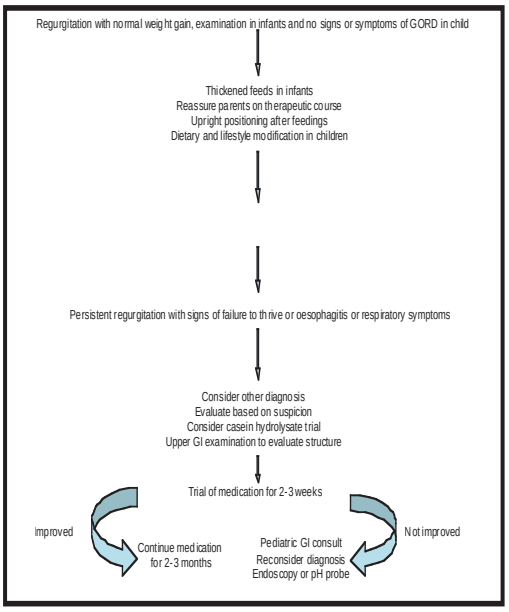


Figure 2. *The American Family of Physicians' Proposed Management in Infants Presenting with Gastro-Oesophageal Reflux and Gastro-Oesophageal Reflux Disease (adapted from Jung, 2001).*

The American Family of Physicians (Figure 2) recommends that parental reassurance and education about the widespread prevalence of physiologic (functional) GOR in infants, especially among those one to four month old, is the most effective management.[2] Other conservative measures recommended include thickening of feeds, dietary changes, and positional changes (the prone position may decrease the incidence of reflux as demonstrated in Figure 3).[4] If the infant does not improve and shows signs of failure to thrive, then various pharmacological interventions are recommended, and these include antacids, H_2 receptor antagonists, proton pump inhibitors and prokinetic agents.

The aim of our study was to determine the optimal

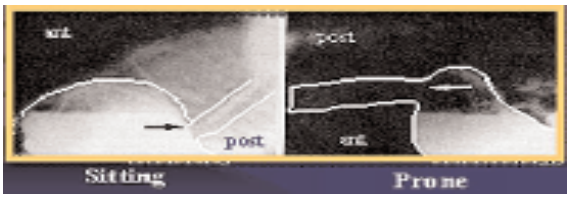


Figure 3. *Barium Study of Infants in the Sitting and Prone Positions, Indicating the Reduced Likelihood of Gastro-Oesophageal Reflux Occurring in the Sitting Position. (adapted from Bishop, 2004).*

management of gastro-oesophageal reflux in infants, through a systematic review of recent studies.

METHODS

Medical databases, such as the Cochrane Library, the National Electronic Library for Health and PubMed were searched for relevant articles using the following keywords: GER, GOR, gastro-oesophageal reflux, infant, treatment, drug names such as antacid, metoclopramide, H_2–receptor antagonist and proton pump inhibitor. Of all articles found, the six most relevant to the clinical question were chosen. They included three randomised control studies (RCT), a case control study, an intervention study and one systematic review. They were then appraised according to a critical appraisal checklist.[5] (Tables 2, 3 and 4)

a. Criteria for SR clearly stated?	√
b. Literature review extensive enough?	√
c. Was quality of studies assessed?	√
d. Results of assessment included?	X
e. Details of studies included in SR?	√
f. Results of individual studies pooled correctly?	Not reported
g. Meta-analysis done?	Not reported
h. Do SR findings add up to more than a sum of individual studies?	X

Table 2. *Appraisal Methodology Checklist for the Systematic Review (SR).*

	Article 1	Article 2	Article 3
Essential Questions			
• All patients accounted for?	√	√	√
• Outcomes assessed blind?	√	√	√
Design			
- Aims clearly stated?	√	√	√
- Measurements valid & reliable?	√	√	√
- Outcomes clinically relevant?	√	√	√
Analysis			
– Treatment groups comparable at baseline	√	√	√
– Were there deviations from planned treatment?	X	X	X
– Results analyzed by Intention to treat?	√	√	√
– Stat. significance assessed?	√	√	√
– Side effects?	√	X	X

Table 3. *Appraisal Methodology Checklist for the Three Randomized Control Trials (RCT).*

	Article 4	Article 5
Essential Questions		
- How were cases obtained?	After diagnosis at research hospital	Referred to research hospital
- Data collected in same way?	√- Questionnaire	√
Design		
• Aims clearly stated?	√	√
• Measurements valid & reliable?	√	√
Analysis		
• Statistical significance assessed?	√	√
Interpretation		
– Biases?	X	Recall, measurement bias
– Confounding variables	Socioeconomic class	X

Table 4. *Appraisal Methodology Checklist for the Article 4 (the Case Control Study) and Article 5 (the Intervention Study).*

RESULTS

The first article under review was a double-blinded RCT assessing the efficacy and safety of aluminium-free alginate versus placebo in 90 infants with recurrent GOR.[6] Infants were assessed at baeline and at seven and 14 days after initiation of treatment. Among 42 patients randomised to receive alginate and 48 patients to receive placebo, alginate was shown to be superior to placebo in reducing the number of reported vomiting or regurgitation episodes (p=0.09) (Figure 4). Although, treatment with placebo was also shown to reduce episodes, this effect did not achieve statistical signifcance. A trend in favour of alginate was also shown to reduce severity of vomiting (p=0.061). The main criticism of this trial was that there was potential for observer bias, since the number of episodes of emesis were subjectively assessed by the parents on a diary card. It was nevertheless concluded that the treatment outcome after active alginate therapy in infants with GOR, was considered superior to placebo by both the investigators and parents.

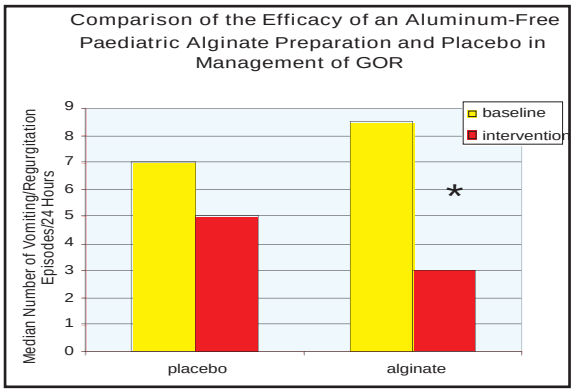


Figure 4. *Comparison of the Efficacy of an Aluminum-Free Paediatric Alginate Preparation and Placebo in the Management of Gastro-Oesophageal Reflux in Infants.*

The second article under review was a randomised, prospective double-blinded crossover trial investigating the efficacy of metoclopramide versus placebo in 30 infants[7]. Several parameters were measured including the average daily occurrence of symptoms and the percentage of time that oesophageal pH was less than 4.0 (the Reflux Index), the total number of episodes in a 24-hour period, and the total number of reflux episodes lasting more than five minutes in each 24-hour period. The results show that metoclopramide produced a decrease in the reflux index (p<0.001) (Figure 5). However, a significant placebo response (p<0.05) was also shown with respect to daily symptom scores.

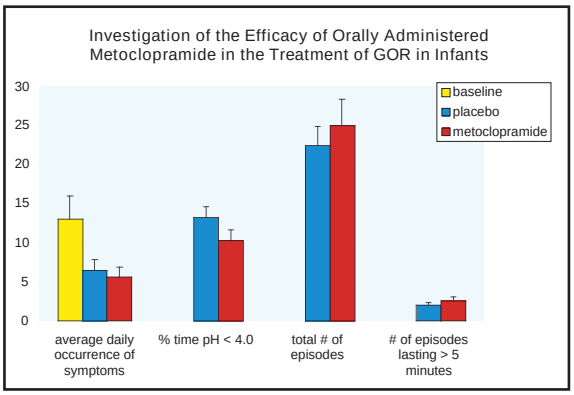


Figure 5. *Investigation of the Efficacy of Orally Administered Metoclopramide in the Treatment of Gastro-Oesophageal Reflux in Infants*

The main criticisms with this study were that even though it stated that the sample size of 30 was justified, there was no description of how that number was arrived at. In addition, the randomization procedures were not described. Even though this was a crossover design, there was no washout period between switching of the

groups; hence there may have been some overlap between the effects of metoclopramide in the two groups. However the investigators concluded that there was no difference between the two treatment groups in any of the parameters except the reflux index.

The third article was a double-blinded RCT assessing the efficacy of omeprazole in 30 irritable infants with GOR.[8] Parameters that were assessed included the reflux index, a cry/fuss diary and a visual analogue scale of infant irritability, as judged by parental impression; these latter two measures were obtained at baseline and at the end of each two-week treatment period. Omeprazole treatment was shown to significantly lower reflux index in comparison with placebo (-8.9 percent +/- 5.6 percent versus -1.9 percent +/- 2.0 percent, p<0.001) (Figure 6). No significant difference was shown in either the cry/fuss time or visual analogue measures. There was potential for observer bias in this study, since the cry/fuss diary and the visual analogue scale of infant irritability were subjectively judged by parental impression. However, it was concluded that compared with placebo, omeprazole significantly reduced oesophageal acid exposure but not infant irritability. Infant irritability improved with time, regardless of treatment.

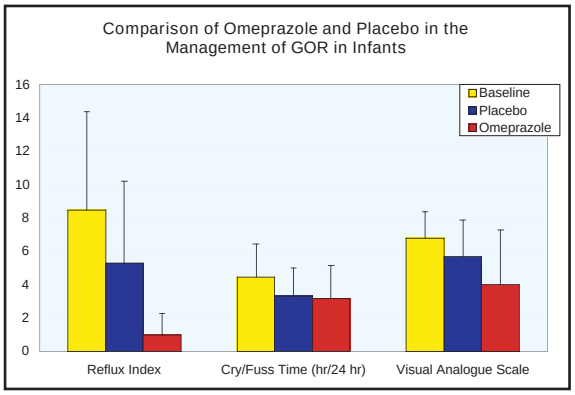


Figure 6. *Comparison of Omeprazole and Placebo in the Management of Gastro-Oesophageal Reflux in Infants.*

The fourth article was a case-control study comparing pre-thickened and home-thickened formulas in 100 infants with GOR.[9] Over a period of three months, infants were randomly selected to receive conventional formula with starch or pre-thickened infant formula. The investigators measured the effect of both pre-thickened and hone-thickened formula and the frequency of regurgitation mad vomiting. the progression of the patients' symptoms were classified as cured, if initial symptoms disappeared or improved, when frequency of symptoms were reduced by 50 percent or greater. Both types of thickened formula relieved reflux. Approximately half of the pre-thickened group and two-fifths of the home-thickened group achieved cure. The small differenc in cure rates observed between groups was not significant (p=0.297). Similarly only a small difference in rates of improvement was found between groups(p=1.00) (Figure 7.). Also, the study was not blinded and the results were collected via a subjective questionnaire.

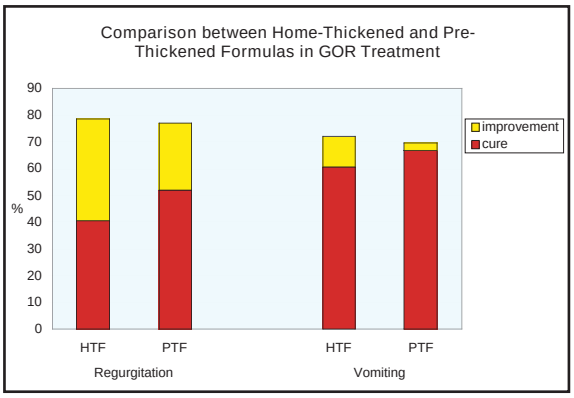


Figure 7. *Comparison between Home-Thickened and Pre-Thickened Formulas in the Treatment of Gastro-Oesophageal Reflux in Infants.*

The fifth article was a prospective intervention study evaluating the effect of smaller volume, thickened formulas on GOR in 12 thriving infants.[10] Two parameters were measured, the number of emesis episodes in each 24-hour period and the reflux index. The study showed reduced vloume of formula decreased the frequency of emesis but not by a significant margin (p>0.05). However, additional modifications by thickening the small-volume feed, further decreased the frequency of emesis to a significant value compared to baseline (p<0.05) (Figure 8). The sample size was justified and the results were consistent with previous reports, however this study was not randomised or blinded, thus introducing the potential for recall and measurement bias. In addition, the trial protocol required feeds that were strictly controlled, which was not applicable to the general population.

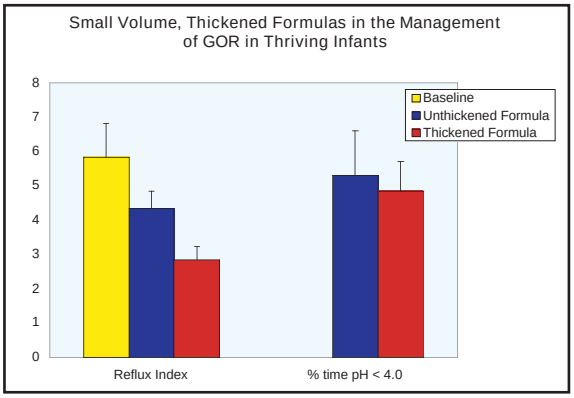


Figure 8. *Small Volume, Thickened Formulas in the Management of Gastro-Oesophageal Reflux in Thriving Infants.*

The sixth article was a systematic review of non-pharmacological and non-surgical therapies in 323 infants with GOR.[11] It examined three main aspects of conservative treatment: positioning, thickened feeds and formula changes. In terms of positioning, three RCTs found that positional changes have no proven efficacy in the treatment of GOR. With regard to thickened feeds, four randomized controlled trials indicated that there was a significant reduction in emesis with thickened feeds vs. unthickened feeds, however no significant difference was found in the amount of reflux. With formula changes, two RCTs showed that whey-based formulas significantly decreased emesis but had no effect on oesophageal pH (Table 5).

While inclusion criteria for this systematic review were provided, the outcome criteria were not. Other limitations of the study were a language and publication bias, since only English articles were considered. The outcomes were not comparable between the various studies as there was no description of a formal statistical method for assessing differences between the studies.

Management	Result
Feed thickeners	↓ emesis
Positioning changes	No effect
Formula change	↓ emesis but no effect on oesophgeal pH
Antacids (alginate)	↓ number & severity of vomiting episodes
Pro-Kinetic agents (metoclopromide)	No ↓ in daily symptoms or number of episodes but _ in time oesophageal pH <4.0
Proton Pump Inhibitors (omeprazole)	↓ oesophageal acid exposure but not irritability
H_2 Receptor Antagonists	No studies found for use in infants

Table 5. *Summary of the Results in the Management of Infants with Gastro-Oesophageal Reflux.*

DISCUSSION

Following the review of current literature on the treatment of gastro-oesophageal reflux, it was determined that the best line of treatment involves parental reassurance and education. No evidence was found to support the use of pharmacological agents in the treatment of GOR. Although both pro-kinetic agents and proton pump inhibitors may reduce oesophageal acid exposure, they do not have any effect on the symptoms of GOR, including infant irritability; which will improve with time, regardless of treatment. In contrast, this review found that antacids and feed thickeners were most effective in decreasing the symptoms, but not gastric acidity associated with GOR. There was no evidence supporting the use of positioning in decreasing the symptoms.

When a parent presents to a doctor with an infant that vomits after every feed, he/she will find it difficult to accept that regurgitation is a normal occurrence. Providing parents with education surrounding this common and normal physiological process is essential to reducing their anxiety and concern. However, if an infant shows features of failure to thrive with persistent respiratory symptoms and signs of oesophagitis, which are manifestations of GORD affecting only 1 in 300 infants, then this would require admission and further evaluation.

Gastro-oesophageal reflux is a self-limiting condition that usually resolves by six to 12 months of age. Infant irritability tends to improve with time, which could be a confounding factor in any study involving therapeutic intervention of GOR. Reassurance and parent education is paramount and should be the first-line of management. If parents insist, conservative treatment may be employed.

REFERENCES

1. Martin AJ, Pratt N, Kennedy JD, et al. Natural history and familial relationships of infant spilling to 9 years of age. *Pediatrics* 2002;109:1061-7.
2. Jung A. Gastroesophageal reflux in infants and children. American Family Physician Dec 1, 2001. (Accessed at: www.aafp.org/afp/20011201/1853.html)
3. Al-Khawari HA, Sinan TS, Seymour H. Diagnosis of gastro-oesophageal reflux in children. Comparison between oesophageal pH and barium examinations. *Pediatr Radiol* 2002;32(11):765-70.
4. Bishop W. Gastroesophageal reflux (GER). Department of Pediatrics: the Vomiting Infant Lecture, University of Iowa, 2004. (Accessed at: http://www.vh.org/pediatric/provider/pediatrics/PedsGIDisease/GER.html)
5. Crombie IK. The pocket guide to critical appraisal: a handbook for health care professionals. London: BMJ Publishing Group, 1996.
6. Miller S. Comparison of the efficacy and safety of a

new aluminium-free paediatric alginate preparation and placebo in infants with recurrent gastro-oesophageal reflux. *Curr Med Res Opin* 1999;15:160-8.

7. Tolia V, Calhoun J, and Kuhns L. Randomized, double-blind trial of metoclopramide and placebo for gastroesophageal reflux. *J Pediatr* 1989;115:141-5.

8. Moore D, Tao B, Lines D, Hirte C, Heddle M, Davidson G. Double-blind placebo-controlled trial of omeprazole in irritable infants with gastroesophageal reflux. *J Pediatr* 2003;143(2):219-23.

9. Penna F, Nortoh R, Carvalh A, et al. Comparison between pre-thickened and home-thickened formulas in gastroesophageal reflux treatment. *J Pediatr* 2003;79(1):49-54.

10. Khoshoo V, Ross G, Brown S, Edell D. Smaller volume, thickened formulas in the management of gastroesophageal reflux in thriving infants. *J Pediatr Gastroenterol Nutr* 2000;31:554-6.

11. Carroll AE, Garrison MM, Christakis DA. A systemic review of non-pharmacological and non-surgical therapies for gastroesophageal reflux in infants. *Arch Pediatr Adolesc Med* 2002;156(2):109-13.

A Review of the Neurodevelopmental Hypothesis of Schizophrenia

Eileen B. Corroon

6th Year Medicine

INTRODUCTION

The complexity and heterogeneity of schizophrenia means that even its definition and diagnostic criteria are cause for debate, therefore the search for a satisfactory aetiological theory of this condition would always be challenging. This essay will introduce the neurodevelopmental theory in the context of other proposed aetiologies, presenting the evidence for neurodevelopmental abnormalities in schizophrenic populations and putative causes for these abnormalities. The models used to explain the development of symptoms will be described with an attempt to integrate other proposed aetiologies with the neurodevelopmental theory.

The genetic component of schizophrenia is undeniable given that having a schizophrenic relative is the biggest risk factor for schizophrenia. However, the fact that the monozygotic twin of a schizophrenic patient has only a 48 percent chance of developing the disease implies that other factors must be involved.[1] Biochemical theories focus on the possibility that localised excesses of one or more neurotransmitters, notably dopamine and serotonin, may account for the symptoms experienced in schizophrenia and the therapeutic effect of neuroleptics blocking these receptors reinforces this idea. Thinking has moved away from Lidz's 1949 'marital skew and schism' theory where the conflict resulting from 'schizophrenogenic' mothering alongside a submissive father-figure led to schizophrenia.[2] Social and familial factors such as low socioeconomic status and unstable parenting are still implicated as precipitants though not necessarily primary causes of the disorder. It seems likely that these influences are effecting change on an already vulnerable psychological makeup.

The neurodevelopmental hypothesis of schizophrenia states that a proportion of schizophrenia begins with impaired foetal or neonatal neurodevelopment rather than in young adulthood when psychotic symptoms first become manifest. The causes for this impairment are not specified but second trimester exposure to influenza, maternal stress and obstetric complications have all been suggested based on epidemiological evidence. Murray and Lewis examined various schizophrenic epiphenomena suggesting that a neurodevelopmental model may serve to connect and elucidate them.[3]

The various neurodevelopmental hypotheses share three main assumptions:

1.The primary pathogenic defect is an early derangement of the orderly development of the central nervous system (CNS).

2.The period of active operation of the causative agent is of short duration, so the process is essentially static.

3.The behavioural consequences of this static process remain relatively latent until long after the primary pathogenetic process has run its course.[4]

There is no direct evidence of a pre- or peri-natal lesion associated with schizophrenia but indirect evidence of impaired development can be seen in macroscopic anatomical variations as well as microscopic immunohistochemical anomalies.

The Evidence

Over the past decade, the most consistently demonstrated phenomenon on CT or MRI, is that schizophrenic patients have larger third and lateral ventricles than controls matched for age and sex, even in the case of monozygotic twins discordant for schizophrenia.[5,6] This phenomenon is not specific for schizophrenia, however, and is only identified in six to 40 percent of subjects.[7] Ventricular enlargement is presumed to take place at the expense of cerebral tissue and may be interpreted as indicating functional disorder in the adjacent brain substance. Widened third ventricles, in particular, may cause damage to diencephalic and limbic structures which are involved in excitatory autonomic functioning. This is evidenced by the relationship to premorbid reduction in electrodermal responses and heart rate in a study of high-risk individuals.[8] This is significant in light of evidence linking diminished autonomic responsiveness with negative symptoms of schizophrenia.

Ventricular enlargement is present at the onset of disease and has been found in most cases to be non-progressive, supporting a neuro-

developmental as opposed to a neurodegenerative aetiology.[6] One serial-MRI study, however, examined patients with schizophrenia, schizo-affective disorder and schizophreniform disorder and found a subgroup of patients whose ventricular expansion rate was significantly greater than that of the controls.[12] A recent investigation found no significant difference between patients and comparison subjects in rates of proportional grey matter reduction with age.[11] Further evidence against neurodegeneration is the lack of reactive gliosis, considered the hallmark of neuronal degeneration, in either immunochemical or Nissl stained post-mortem specimens of the brains of schizophrenic persons. This also casts doubt, however, on the possibility of perinatal damage since gliosis occurs in response to injury from the twentieth week of gestation.[4]

Cytoarchitectural abnormalities are also a feature in neuropathological reports on schizophrenic brains and suggest a defect in the formation of the cortical plate. One study used immunocyto-chemical staining of the frontal lobes of schizophrenic patients for the enzyme nicotinamide-adenine dinucleotide phosphate-diaphorase (NADPH-d). This enzyme is detectable only in a subset of neurons. Akbarian and colleagues found a significant decline in NADPH-d immunoreactive neurons in the cortical grey matter and the superficial white matter. However, rather than merely providing yet another sign of grey matter decay, the authors also documented a significant increase in the number of these neurons, with otherwise normal cellular characteristics in the white matter deeper than 3 mm below the cortical grey matter.[13] In the process of cortical development postmitotic neurons differentiate and migrate to their eventual locations within the grey matter. The cortical cellular lamination patterns emerge in an inside-out pattern; that is, cells destined for the deep layers of cortex emerge first from the ventricular zone, while cells destined for progressively more superficial locations emerge later and later.[14] The findings reported for the frontal cortex by Akbarian *et al.* thus take on added significance in that NADPH-d immunoreactive neurons are normally found in abundance in the early cortical plate. Given their ultimate postmigratory location among the upper cortical layers, they should be among the last cells to pass through the plate. The displacement of the NADPH-d immunoreactive neurons from the superficial cortical white matter to the deep subcortical white matter suggests either abnormal migratory behaviour of this class of neurons or an abnormal degree of survival within their layer of proliferation beyond their normal life cycle in the developing cortex.[15] Based on the timing of this neuronal migration the disturbance of normal development would be expected to occur in the mid to late second trimester.[14] This is in concordance with the proposition that exposure to influenza in the second trimester may play a part in the development of schizophrenia.

Craniofacial dysmorphology may provide another clue to the neurodevelopmental processes in schizophrenia since cerebral morphogenesis is very closely related to craniofacial morpho-genesis. Minor physical anomalies (MPAs) may constitute biological markers of first and early second trimester dysgenesis and have been found to occur in excess in patients with schizophrenia. These MPAs are mainly due to an overall narrowing and elongation of the mid and lower anterior face region and include heightening of the palate and narrowing of the mouth.[16] In schizophrenic subjects there is a correspondence between MPAs and increased third ventricle size. At a symptomatic level, prominence of MPAs has been associated with more severe negative, but not positive, symptoms and with lower premorbid, but not current, intellectual function. Embryological considerations implicate the operation in schizo-phrenia of dysmorphogenic events over a time frame that may have limits of six to 17 weeks, but more likely encompasses nine to 16 weeks of gestation; over this interval, neurogenesis, migration from the ventricular zone, early programmed cell death and early myelination are interacting in the development of brain structure and function.[17]

'Neurological soft signs' (NSS) are present in excess in patients with schizophrenia. These are minor neurological abnormalities in sensory and motor performance identified by clinical examination and are thought to reflect a failure in the integration within, or between, sensory and motor systems. A systematic review by Dazzan and Murray concluded that an excess of NSS is already evident in patients suffering their first episode of psychosis and also in high-risk subjects without psychosis.[18] There was also a particular excess of NSS in those subjects exhibiting mixed-handedness. Crow has suggested that schizophrenia may be attributable to an abnormality in cerebral lateralisation, possibly involving abnormal expression of a cerebral dominance gene on the X chromosome.[20] Another

hypothesis is that the variability observed in schizophrenic patients could be due to some attentional deficit that interferes with a consistent unilateral mode of responding.[21]

One final contributor to the neurodevelopmental theory is the presence of abnormality in the premorbid period. These abnormalities take the form of childhood psychosocial abnormalities[30], for example, aloof social habits, avoidance of social interaction and delay in reaching early developmental milestones.[16] One longitudinal birth cohort study found significant impairments in neuromotor, receptive language and cognitive development only among children later diagnosed as having schizophreniform disorder, as opposed to other psychiatric disorders. These impairments were independent of the effects of socioeconomic, maternal and obstetric factors, providing evidence for an early-childhood pan-developmental impairment that is specifically associated with schizophreniform disorder.[19]

Causative Agents

Aetiological agents for neurodevelopmental abnormalities have been implicated on the basis of epidemiological studies. It is probable that these agents exert influence over a developing CNS that is vulnerable through genetic preloading. There is consistent evidence of a small excess of births of people with schizophrenia in the cold winter months, pointing to some associated environmental factor causing neural damage in the foetus or neonate. Maternal exposure to an infectious agent is a likely candidate and the damage may be immune-mediated rather than a direct consequence of infection. Several studies have suggested that a foetus in its second trimester during an influenza epidemic is at increased risk.[22] A Japanese study reported greater than twice the risk of developing schizophrenia in females exposed to flu epidemic in 1957, while they were at five months gestation.[23] This early damage would correlate with the absence of gliosis on neuropathological examination and would be just inside the timeframe proposed for the action of an agent affecting neuronal migration and formation of the cortical plate.

Maternal dietary insufficiency has also been shown to increase risk. Birth cohorts exposed to early prenatal nutritional deficiency during the Dutch Hunger Winter of 1944/1945 were compared with those unexposed, with regard to the risk of hospitalization for schizophrenia in adulthood. Toward the end of World War II, a Nazi blockade precipitated a severe famine in western Netherlands. The Dutch Hunger Winter was unique in that a famine of brief and clearly defined duration afflicted a population that maintained excellent records on food rations and on health outcomes during the famine and in subsequent decades. The exposed birth cohort had a significant two-fold increase in the risk for schizophrenia.[24]

Considerable evidence shows that schizophrenic patients are more likely than controls to have a history of obstetric complications of any type. A Scottish case control study found that there were highly significant differences between cases and controls for complications of pregnancy and complications of delivery.[25] In this study, pre-eclampsia was the only individual complication of pregnancy for which the case/control difference was significant. Non-spontaneous delivery, Caesarean section, forceps or manipulation, were the only complications of delivery for which the case/control difference was significant. One longitudinal cohort study examined various complications including extreme prematurity and foetal hypoxia and found that pre-eclampsia was the strongest individual risk factor for schizophrenia when the authors controlled for potential confounding factors.[26]

There are difficulties in acknowledging obstetric complication as an aetiological agent for schizophrenia. Firstly, it would be logical to assume that with improved antenatal and obstetric care, the incidence of schizophrenia would drop and that there would be regional variation in schizophrenia rates that could be correlated with the standard of maternity care. There is no evidence of such a relationship. Secondly, the absence of gliosis on post-mortem neuro-pathological examination of schizophrenic brains and the putative critical time period for derailment of cortical development, nine to 16 weeks gestation, do not support the role of obstetric complications in neural damage. Finally, while there is a definite association between schizo-phrenia and obstetric complications, they may just share a common aetiological agent, for example, maternal infection in the second trimester.

Neurodevelopmental Models

The problem with the neurodevelopmental hypothesis of schizophrenia is how to explain the long lag between prenatal damage to the foetal

brain and the onset of psychotic symptoms in adolescence and early adulthood.

Two models have been proposed to explain this latent period:

1. The early neurodevelopmental model.
This is based on the view that a fixed lesion from early life interacts with normal neurodevelopment occurring later, lying dormant until the brain matures sufficiently to call into operation the damaged systems.[3] One problem with this analogy is that the onset of schizophrenia is heralded by an absolute deterioration from previous levels of functioning, not just a failure to keep pace with peers.[4] In addition, this theory is not completely consistent with the results of neuroimaging studies. The ventricles may enlarge with both early and late tissue volume loss but volume measurements that include extracerebral CSF volumes can determine whether or not there has been late volume loss.[4] MRI allows the most reliable measurement of intracranial volume and specific components, extracerebral CSF as well as ventricular CSF. Several studies have demonstrated a significant overall loss of brain tissue with an increase in extracerebral, sulcal, CSF space.[9, 10] Only a diffuse lesion resulting in a loss of brain tissue volume, *after* maximum brain volume expansion has already taken place, will result in an equivalent increase in total CSF space, (extracerebral and ventricular) with no change in intraventricular volume. This is because intracranial cavity expansion is driven by brain growth and is irreversible after the skull sutures fuse. Diffuse loss of tissue limited to the pre- or peri-natal time period will result in a smaller intracranial cavity and persistent enlargement of the lateral ventricles but *not* in an increase in the extracerebral CSF space.[4]

2. The late neurodevelopmental model.
Based on data that indicate substantial changes in brain biology during adolescence, this model proposes that schizophrenia may result from an abnormality in periadolescent synaptic pruning.[27] Synapse density appears to show a rapid rise following birth until a peak at about two years. This is followed by a decline until a plateau is reached during the late teens. The age at which this plateau is reached is close to the greatest acceleration in onset of schizophrenia.[28] A minimum threshold of use exists below which a synapse is pruned.

Genetic predisposition may produce an inappropriately high 'synapse use threshold' and lead to excessive pruning.[28] Also, early developmental injury could cause 'dyspruning' in some areas, for example, in the prefrontal cortex, leading to reduced connectivity and negative symptoms. Compensatory retention or proliferation of some other connections that would normally have been pruned out may also occur. The anomalous persistence of these circuits could lead to 'parasitic foci' that become autonomous, causing positive symptoms.[29]

CONCLUSIONS

It seems that the neurodevelopmental theory, occurring on a background of genetic vulnerability, allows the maximal integration of the epiphenomena and epidemiological evidence associated with schizophrenia. The late developmental model offers a realistic explanation of the timing of onset of the clinical manifestations of the disorder. It seems likely the genes involved in the regulation of pruning are under some element of hormonal control and this may have an influence on the gender difference in age of onset of symptoms. Alterations in fundamental circuitry, especially the anomalous persistence of neurons that would normally be pruned could explain the background of neurotransmitter excess in the dopamine and serotonin theories of schizophrenia. The early developmental model may well characterise a separate subtype of schizophrenia where psychosocial stresses or other environmental factors serve to unmask a deficiency in the neural circuitry, resulting in decompensation.

The heterogeneity of the illness may be evidence of aetiological heterogeneity, however, and the neurodevelopmental theory may only hold true for a subtype of schizophrenia. There is definitely value in clarifying even a small subtype of a disorder that will affect one percent of people in their lifetime, if that in turn elucidates a method of prevention.

REFERENCES

1. Gottesman II. Schizophrenia Genesis: The Origins of Madness. NY: Freeman, 1991.
2. Lidz RW, Lidz T. The family environment of schizophrenic patients. *Am J Psychiatry* 1946;106:332-45.
3. Murray RM, Lewis SW. Is schizophrenia a neurodevelopmental disorder? BMJ 1987;295:6-12
4. Woods BT. Is schizophrenia a progressive neurodevelopmental disorder? Toward a unitary pathogenetic mechanism. *Am J Psychiatry* 1998;155(2): 1661-70.
5. Johnstone EC, Crow TJ, Frith CD, Husband J, Kreel L. Cerebral ventricular size and cognitive impairment in schizophrenia. *Lancet* 1976;2(7992):924-6.
6. Suddath RL, Casanova MF and Goldberg TE. Temporal lobe pathology in schizophrenia: a quantitative magnetic resonance imaging study. *Am J Psychiatry* 1989;146:464-72.
7. Syvalahti EK. Biological Factors in Schizophrenia: Structural and functional aspects. *Br J Psychiatry Suppl* 1994;(23):9-14.
8. Cannon TD, Barr CE, Mednick SA. Genetic and perinatal factors in the etiology of schizophrenia. In: Walker EF, ed. Schizophrenia: A life-course developmental perspective. New York: Academic Press, 1991:9-31.
9. Andreasen NC, Flashman L, Flaum M, et al. Regional brain abnormalities in schizophrenia measured with magnetic resonance imaging. *JAMA* 1994;272:1763-9.
10. Woods BT, Yurgelun-Todd D. Brain volume loss in schizophrenia: when does it occur and is it progressive? *Schizophr Res* 1991;5:202-4.
11. Ananth H, Popescu I, Critchley HD, Good CD, Frackowiak RSJ, Dolan RJ. Cortical and subcortical gray matter abnormalities in schizophrenia determined through structural magnetic resonance imaging with optimized volumetric voxel-based morphometry. *Am J Psych* 2002;159:1497–1505.
12. Garver DL, Nair TR., Christensen JD, Holcomb J, Ramberg J, Kingsbury S. Atrophic and static (neurodevelopmental) schizophrenic psychoses. *Neuropsychopharmacology* 1999;21(1):82-92.
13. Akbarian S, Bunney WE Jr, Potkin SG, et al. Altered distribution of nicotinamide-adenine dinucleotide phosphate-diaphorase cells in frontal lobe of schizophrenics implies disturbances of cortical development. *Arch Gen Psychiatry* 1993;50(3):169-77.
14. Gazzaniga MS, Ivry RB, Mangun GR, eds. Cognitive Neuroscience: The Biology of the Mind. New York: WW Norton & Co, 1998:476-84.
15. Bloom FE. Advancing a neurodevelopmental origin for schizophrenia. *Arch Gen Psychiatry* 1993; 50(3):224-7.
16. Waddington JL, Lane A, Larkin C, O'Callaghan E. The neurodevelopmental basis of schizophrenia: clinical clues from cerebro-craniofacial dysmorphogenesis, and the roots of a lifetime trajectory of disease. *Biol Psychiatry* 1999;46(1):31-9.
17. Waddington JL, Lane A, Scully P, et al. Early cerebro-craniofacial dysmorphogenesis in schizophrenia: a lifetime trajectory model from neurodevelopmental basis to "neuroprogressive" process. *J Psychiatric Res* 1999;33(6):477-89.
18. Dazzan P, Murray RM (2002) Neurological soft signs in first episode psychosis: a systematic review. *Br J Psychiatry* 2002;181(suppl43):50-7.
19. Cannon M, Caspi A, Moffitt TE, et al. Evidence for early-childhood, pan-developmental impairment specific to schizophreniform disorder. *Arch Gen Psychiatry* 2002;59:449-56.
20. Warwick MM, Lawrie SM, Beveridge A, Johnstone EC. Abnormal cerebral asymmetry and schizophrenia in a subject with Klinefelter's syndrome (XXY). *Biol Psychiatry* 2003;53(7):627-9.
21. Green MF, Satz P, Smith C, Nelson L. Is there atypical handedness in schizophrenia? *J Abnorm Psychology* 1989;98(1):57-61
22. McGrath J, Murray RM. 1995. Risk factors for schizophrenia: from conception to birth. In: Hirsch SR, Weinberger DR, eds. Schizophrenia. New York: Blackwell, 1995:187-205.
23. Izumoto Y, Inoue S, Yasuda N. Schizophrenia and the influenza epidemics of 1957 in Japan. *Biol Psychiatry* 1999;46(1):119-24.
24. Susser E, Neugebauer R, Hoek HW, et al. Schizophrenia after prenatal famine: further evidence. *Arch Gen Psychiatry* 1996;53(1):25-31.
25. Kendell RE, Juszczak E, Cole SK. Obstetric complications and schizophrenia: A case control study based on standardised obstetric records. *Br J Psychiatry* 1996;168(5):556-61.
26. Dalman C, Allebeck P, Cullberg J, Grunewald C, Koster M. Obstetric complications and the risk of schizophrenia: A longitudinal study of a national birth cohort. *Arch Gen Psychiatry* 1999;56(3):234-40.
27. Feinberg I. Schizophrenia: caused by a fault in programmed synaptic elimination during adolescence? *J Psychiatry Res* 1983;17:319.
28. Pogue-Geile MF. Developmental aspects of schizophrenia, 1997:137-54.
29. Keshavan MS. Neurodevelopment and schizophrenia: Quo vadis? In: Keshavan MS, Murray RM, eds. Neurodevelopment & Adult Psychopathology. New York: Cambridge University Press, 1997:267-77.
30. McCreadie RG, Connolly MA, Williamson DJ, Athawes RWB, Tilak-Sing D. The Nithsdale schizophrenia surveys XII. Neurodevelopmental schizophrenia: A search for clinical correlates and putative aetiological factors. *Br J Psychiatry* 1994;165:340-6.

Childhood Guillain-Barré Syndrome: Comparing Intravenous Immunoglobulin Treatment with Supportive Care

Sarah McLean and Sheng F. Oon

6th Year Medicine

Abstract

Objectives: Guillain-Barré Syndrome (GBS) is an acute ascending flaccid paralysis that is often preceded by a mild bacterial or viral infection. Management options include supportive care, physiotherapy, intravenous immunoglobulin (IVIG) and plasmapheresis. Our aim was to compare IVIG treatment and supportive care to supportive care alone in cases of moderate to severe GBS in children less than 16 years of age. **Methods:** Using specific keywords, the Cochrane Library and PubMed were searched. Eight relevant articles were found and appraised. The studies compared specific outcome criteria including mortality, days taken to regain independent locomotion, days taken to improve by one disability grade on Hughes' Functional Scale, days of hospitalisation and need for mechanical ventilation. **Results:** Four articles concluded definite benefits from the use of IVIG in GBS and two articles concluded that there was no difference in outcome with IVIG. One study showed statistically significant benefits with IVIG regarding death and need for mechanical ventilation. No studies showed statistically significant differences regarding recovery times or days of hospitalisation. **Conclusion:** The articles concurred with the data on GBS in adults, that timely use of IVIG reduces mortality and morbidity in a paediatric setting. However, IVIG use made no difference in the incidence of long-term sequelae of GBS. Sample sizes were small. Larger studies are needed in order to fully explore the benefits of IVIG with regard to these outcome criteria. Logistically and ethically, this would be difficult and no randomised controlled trials have been done to date for this reason. Acute relapse was a new phenomenon, which had not been previously noted in the pre-IVIG era and warrants further investigation.

INTRODUCTION

In 1916, Guillain, Barré and Strohl described a syndrome of 'radiculoneuritis' with acute flaccid paralysis and an increase in protein in the central nervous system (CNS), but without a cellular reaction. This polyradiculoneuropathy, or Guillain-Barré Syndrome (GBS) is a relatively uncommon condition affecting approximately three in 100,000 each year with an incidence in children of 0.7 to 0.9 in 100,000. It is the commonest peripheral neuropathy seen in children. Two-thirds of GBS cases are associated with an antecedent infection two to three weeks before the onset of the symptoms, most commonly with *Campylobacter jejuni* or cytomegalovirus. These infections are often trivial and may go unnoticed. 'Molecular mimicry' between the microorganism lipopolysaccharides and the host nerve ganglioside components results in an immune-mediated response, which attacks both the infectious antigen and similar epitopes in the host peripheral nervous system. This attack is thought to be mediated by anti-GM-1 and anti-GQ1b antibodies, especially IgG1 and results in demyelination of nerves in the peripheral nervous system, and the clinical syndrome of poly-neuropathy (Figure 1).

Symptoms of distal weakness and/or paraesthesia begin in the fingers and toes and ascend proximally, with progressively worsening weakness and areflexia over several to 21 days. There may also be abnormal sensory symptoms and some cases are complicated by autonomic involvement, which may cause postural hypotension, urinary retention, impaired pupillary responses and cardiac arrhythmias. In the most severe cases, involvement of the bulbar muscles causes difficulty with chewing and swallowing with an increased risk of aspiration. The initial abnormality may be a hoarse voice or a weak cry in an otherwise normal child. Involvement of the respiratory muscles necessitates mechanical ventilation.

GBS is a clinical diagnosis, confirmed by slowed conduction on electromyography and nerve conduction studies. Cerebrospinal fluid (CSF) shows a raised protein level at 1 to 3 g/L (normal = 0.3 to 1.0 g/L), with a normal cell count and glucose levels. The serum creatinine kinase (CK) may also be raised.

The differential diagnosis of GBS includes other paralytic illnesses such as botulism, poliomyelitis, cord compression and primary muscle disease such as Duchenne's Muscular Dystrophy. Other causes of polyneuropathy include chronic inflammatory demyelinating polyneuropathy,

hereditary sensory neuropathies such as Charcot-Marie-Tooth syndrome and neuropathies secondary to vitamin deficiencies, for example, vitamin B_{12} deficiency and toxins (alcohol, vincristine, and lead).[2] Variants of GBS include Miller-Fisher syndrome, characterised by ataxia, areflexia and ophthalmoplegia. Acute motor axonal neuropathy (AMAN) is another variant which involves axonal damage in addition to demyelination and carries a worse prognosis.[1]

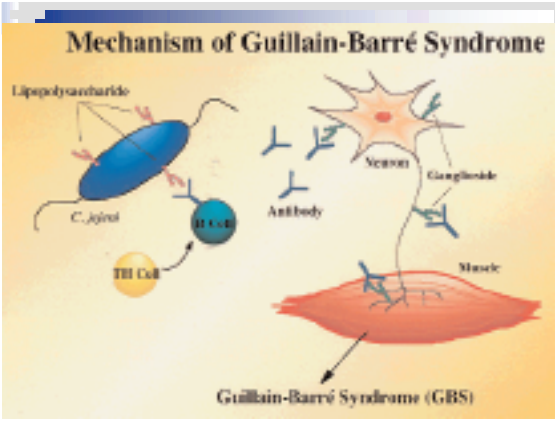


Figure 1. *Mechanism of Guillain-Barré Syndrome: Adapted from the Gifu University Website: http://www.gifu-u.ac.jp/~kassei/guillain.html.*

Current management centres on supportive care, provided by a multidisciplinary team of doctors, nurses, physiotherapists, speech and language therapists, occupational therapists and clinical psychologists. Vital signs, respiratory capacity and urine output of the patient should be monitored. Physiotherapy and splints are used to maintain muscle strength and flexibility, both in the acute and recovery stages and to clear respiratory secretions. As much activity as can be tolerated should be encouraged. Adequate hydration is maintained with intravenous fluids and nutrition with nasogastric feeding or total parenteral nutrition, if there is bulbar muscle involvement. Bladder and bowel problems may be managed using laxatives and urinary catheterisation, whether suprapubic or intermittent. Corneal exposure should be avoided. Low molecular weight heparin and thromboembolic deterrent stockings are used to avoid venous thrombo-embolism. Neuropathic pain, muscle cramps and backache can be very distressing and can be treated with analgesics and muscle relaxants. Light sedation may also be used if children are distressed. Complications such as urinary tract infections and pneumonia are detected and treated. Monitoring of pulse oximetry and peak expiratory flow rate will detect the development of respiratory involvement and the need for mechanical ventilation.[3] If mechanical ventilation is required for more than a week, a tracheostomy may be conducted.

Children may be managed at home with outpatient physiotherapy in the recovery stages or in mild GBS. Indications for admission would include difficulty with mobility, significant paralysis, difficulty breathing and complications such as pneumonia or cardiac arrythmias. Parents must be educated regarding warning signs, such as shortness of breath, gagging or coughing on eating. [3]

Reassurance and encouragement to both the children and their parents is also vital as the overall prognosis is generally very good. Clearly, the illness may be extremely frightening for children who, though paralysed, are still aware of their surroundings. Familiar voices and objects are reassuring and devices to aid communication will help the most severely affected. Children may continue to be moody and tearful for some time after resolution of the acute symptoms. Parents may find contact with a support association such as the GBS Support Group helpful.[4]

The prognosis of childhood GBS is excellent, with 95 percent of children making a complete recovery, though this may take up to two years. There is a three to five percent mortality rate and up to 10 percent of children have a permanent disability, ranging from subtle weakness of the lower extremities to being wheelchair dependant.

While the vast majority of children make a full recovery with only supportive care, a number of interventions have been shown to hasten recovery and improve outcome in children with more severe GBS. Corticosteroids had been used for many years but have been shown to be of no value. More recently, intravenous immunoglobulin (IVIG) and plasmapharesis have been shown to be of benefit in shortening the course of the illness and decreasing morbidity.

Specific indications for IVIG include a diarrhoeal prodrome, Miller Fisher Syndrome, autonomic instability and poor venous access.[5]

Generally, IVIG is well tolerated and there were no reports of adverse effects in the cases described below from Our Lady's Hospital for Sick Children, Crumlin, Dublin, Ireland (OLHSC). Mild adverse effects such as headache, flushing, low backache, nausea and wheeze, are often associated with a fast infusion rate and respond rapidly on slowing the infusion. Rare episodes of

life-threatening anaphylaxis may occur, especially in IgA deficient patients. Patients should therefore be screened for IgA deficiency before treatment and if deficient should receive IgA depleted preparations of IVIG. IVIG is a blood product and there is anxiety about transmission of viruses such as hepatitis C and HIV, however there have been no reports of HIV transmission with IVIG and the introduction of specific anti-viral measures in the manufacture of IVIG should relieve concern. Where very high doses of IVIG are administered, for example for immunomodulatory purposes, Coomb's test positive haemolytic anaemia, aseptic meningitis and renal profile disturbances have been reported.[6]

The most common adverse effect associated with plasmapharesis is hypotension, which may cause syncope, blurred vision, diaphoresis or abdominal cramps. Bleeding secondary to anti-coagulant drugs used may also occur. Perioral and distal paraesthesia, muscle cramps, seizures and cardiac arrhythmias can also occur. Anaphylaxis with pruritis, wheeze and rash is the most serious adverse consequence of this treatment.

Practically, plasmapharesis is difficult. Each session takes several hours during which the patient is restriced to the bedside due to the large bore cannulae in each arm. Several sessions may be needed before improvement is seen. It is generally performed only in the intensive care unit.

The role of immunotherapy in GBS is accepted and it has been shown that IVIG and plasmapharesis are equally effective in reducing morbidity and mortality in adult GBS.[7] However, there is a higher rate of relapse in adults treated with plasmapharesis compared to those treated with IVIG.[1] Though studies have compared each with placebo in childhood GBS, no studies have compared IVIG with plasmaphoresis in childhood disease. Due to practical reasons, notably the ease of administration, IVIG is currently the first line immunotherapy in childhood GBS.

This study aims to determine if there is a benefit in the use of IVIG in children with moderate to severe GBS in addition to standard supportive care, by analysing the data on previous similar studies according to a predetermined set of criteria.

REVIEW OF AVAILABLE LITERATURE ON THE TREATMENT OF GBS

This research was conducted during a two-month attachment in the National Children's Hospital attached to the Adelaide and Meath Hospital (AMNCH), Dublin, Ireland. Seven members of the group combined to collect research data and articles. Some recent case histories of childhood GBS were also obtained from the OLHSC, three of which were presented in June 2004 as part of a final year paediatrics project.[8]

The study population included all children with moderate or severe GBS under the age of 16 years. The severity of the GBS was graded according to internationally accepted diagnostic criteria: grade three, four and five in the American National Institute of Neurological Disease and Stroke (ANINDS) criteria for GBS, as well as those children over grade five on the Asbury Cornblath Criteria for GBS. The study also restricted the population to those children who received treatment within one month of the onset of symptoms. Children who had had prior treatment with plasmapheresis or who had a contraindication to IVIG were excluded from the study.

The intervention studied was IVIG at a standard dose of 400 mg/kg over five days or at a single dose of IVIG 2g/kg, with supportive care. This was compared to supportive care alone. The cases receiving only supportive care (the controls) included cases from the pre-IVIG era, late referrals and in one, controls from their own study. The outcomes measured and compared were days to regain independent locomotion, days taken to improve by one grade on Hughes Function Disability Scale, days of hospitalisation needed, need for mechanical ventilation and deaths.

Search engines used included the Cochrane Library, PubMed, Medline, the Evidence Based Medicine website with its links to the British Medical Journal, Lancet and various other paediatric journals.

The keywords used were Guillain Barré Syndrome, polyradiculoneuropathy, polyradiculoneuritis, children, paediatric, paediatric, treatment, flaccid paralysis, intravenous immunoglobulins.

Trials and studies of children under the age of 16 years, with moderate to severe GBS were included. Moderate to severe GBS was defined as grades three to five on the ANINDS criteria or grade five or higher on the Asbury Cornblath criteria for GBS. Exclusions were children who

had received prior treatment with plasmapharesis, who had a contraindication to IVIG or those who presented more than one month after the onset of symptoms.

Hughes Functional Disability Scale (HFDS)
O=normal
1=minor symptoms, capable of running
2=able to walk up to 10 meters without assistance but unable to run
3=able to walk 10 meters with assistance of one person, a walker or a cane
4=unable to walk
5=requires assisted ventilation
6=death

Figure 2. *Table to show the Hughes Functional Disability Scale.*[9]

Eight articles that fulfilled these criteria were found and all were included. Follow-up varied from one month to five years after the onset of symptoms. Each article investigated different potential benefits of IVIG compared with supportive therapy and other variables, including early IVIG treatment compared with late treatment.

Pre-IVIG era compared to post-IVIG era

A prospective study by Koul *et al.* followed 42 cases of GBS for 10 years from 1990 and compared the outcomes of the patients with one retrospective study and 10 other case studies from the pre-IVIG period (Figure 3).[10] The study concluded a reduced time to recovery and a favourable duration of hospital stay with IVIG treatment, compared to outcomes described during the pre-IVIG era. There were several deaths in the pre-IVIG era studies, but none in the post-IVIG era.

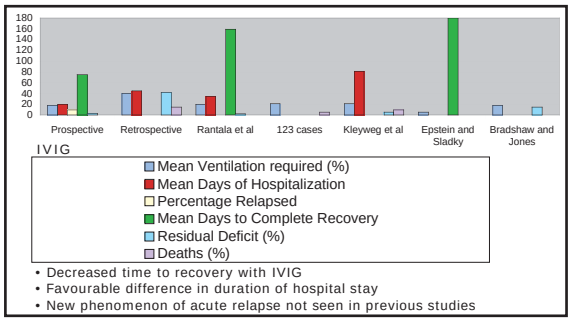


Figure 3. *Comparison of patient outcome with GBS Pre-IVIG and Post IVIG.*[10]

Long-term benefits of IVIG

Another recent article by Vajsar *et al.* assessed 31 children for evidence of motor and functional deficits on an average of five years after the onset of GBS symptoms.[11] Twenty-four of these children had moderate or severe GBS and were given IVIG. Of the children who received IVIG, 27 percent reported fatigue and 46 percent had mild motor or sensory abnormalities on examination. Findings included foot drop, pes cavus and tremor. The findings were compared to a pre-IVIG 1978 study, which found that 24 percent of children had long-term motor deficits five years after the acute episode of GBS. The conclusion was that there was no significant difference in the long-term motor sequelae of childhood GBS treated with IVIG compared to supportive care alone.

IVIG in early GBS and late GBS

A study performed in India, by Shanbag *et a1.*, also reported definite benefits with the use of IVIG.[2] The study compared IVIG use in 17 elective early admissions with IVIG use in eight children whose presentation to hospital was delayed and who, in some cases, were already in respiratory distress.

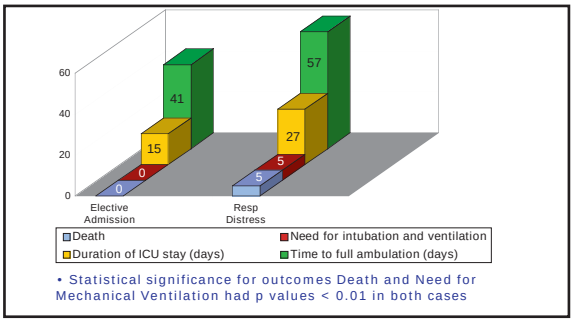


Figure 4. *Comparison of IVIG use in early severe GBS to late severe GBS.*[12]

There were five deaths (62.5 percent) in the late admission group, all of whom had needed artificial ventilation. The duration of stay in the intensive care unit (ICU) was 27 days, compared with 15 days in the children who received IVIG early. The surviving three children also took a longer time to regain full ambulation, indicating that timely use of IVIG significantly improved outcome.

Single dose IVIG compared with a five-day infusion of IVIG

A 1997 study followed nine consecutive cases where a single dose of IVIG 2g/kg was given within 10 days of onset of paralysis.[13] This was found to be as effective as a five-day infusion in preventing further progression of symptoms, thus, shortening the clinical course of GBS. Though the study involved only nine children and was intended to be a pilot study for a more comprehensive prospective controlled trial, single-dose treatment, with possibly a shorter hospital stay, has obvious benefits from an economic and resource point of view.

IVIG with supportive care compared to supportive care alone

Another study by Graf *et al.* of 20 children with moderate or severe GBS found no evidence of IVIG improving outcome or shortening the duration of illness when compared with supportive care alone (Figure 5).[14] This was a well designed study, comparing outcome in 12 children who received only supportive care with eight who received supportive care and IVIG at the standard dose of 0.4g/kg/day for five days. Three children were lost to follow-up and one who had received plasma exchange was excluded from the study. As seen in Figure 5, the addition of IVIG to supportive care did not impact substantially on the time taken to recover from the highest severity score to a score of two on the Hughes Functional Disability Scale, though some benefit was seen. However, the authors pointed out several potential sources of confounding bias, including the timing of the immunotherapy and the more benign natural history of the illness in children.

Acute relapses

Several other studies remarked on the recurrence of symptoms in children who had previously shown clinical improvement, usually two to three

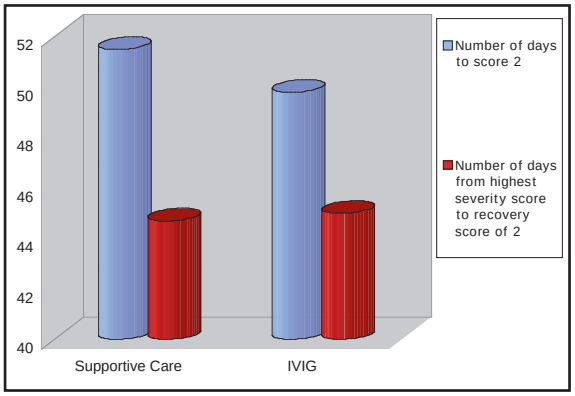


Figure 5. *Outcome in severe childhood GBS after IVIG with supportive care or supportive care alone.* [15]

weeks after IVIG administration. This is a seemingly new phenomenon, which was not described in the pre-IVIG era. One study of nine consecutive cases saw one child relapse after five months.[13] Another Japanese study found that three patients out of a group of 11 previously treated with IVIG had recovered and subsequently deteriorated, but who subsequently made a full recovery with re-treatment with IVIG.[15]

CLINICAL CASES

Three recent cases that presented to OLHSC are illustrated below. These cases demonstrate varying clinical presentations of GBS, all at the severe end of the spectrum with varying progression and prognosis of the illness. This also allows comparison of cases in which treatment with IVIG was commenced immediately on presentation (Case 1), in which treatment of IVIG was commenced only when no clinical improvement was seen with supportive care alone (Case 2), and one case which was managed with supportive care alone (Case 3).

These examples illustrate the relatively benign course of GBS in children and the excellent prognosis enjoyed by the vast majority of children affected by GBS. They also demonstrate the inherent variation in the prognosis and duration of illness in childhood GBS that is such a confounding factor when objective comparisons of treatments are being made: is a fast recovery the result of a specific intervention or would recovery have occurred even without the treatment?

Case 1

S.F. is a 14 year-old girl who was transferred to OLHSC from Louth County Hospital, Ireland, with an eight-day history of discomfort and weakness in her legs and a one-day history of left-sided facial weakness. She had a history of a flu-like illness and otitis media three weeks prior to the onset of symptoms, which had been treated by her general practitioner with oral antibiotics.

On examination S.F. was well and her vital signs were normal. There was marked unsteadiness of gait, proximal myopathy in both upper and lower limbs and a left-sided facial weakness that spared the forehead. Reflexes in both upper and lower limbs, including plantar reflexes, were absent. Sensation was normal. A lumbar puncture was performed showing raised protein in her CSF. There was also a raised CK and liver transaminases. Nerve conduction studies confirmed a demyelinating neuropathy.

S.F. was treated with a five-day course of IVIG 0.4g/kg/day and physiotherapy. Her CK levels returned to normal and she was discharged back to Louth County Hospital 10 days later. She had improvement in her facial weakness, but still retained significant disability. She was scheduled for follow-up nerve conduction studies six to eight weeks later.

Case 2

K.P. is an 18-month-old girl who was referred

from Portiuncula Hospital, Ballinasloe, Ireland, where she presented with a sudden onset of reduced lower limb movement, inability to bear weight or walk. Her birth history, motor and cognitive development had all been normal up to this point.

On examination K.P. was alert but irritable on handling. There was truncal weakness and she was unable to sit without support. There was bilateral facial weakness and upper and lower limb power and tone were reduced bilaterally. Her reflexes were absent. Sensation appeared intact in the upper and lower limbs and she had no sphincter disturbances or other signs of autonomic neuropathy.

A diagnosis of GBS was made on clinical grounds, confirmed by nerve conduction studies and a CSF protein level of 1.54mmols/L.

She was admitted for supportive care and physiotherapy, but no change was observed. On day three of admission, a five-day course of IVIG 0.4g/kg/day was started. K.P. showed rapid improvement and was discharged home when the course of IVIG was finished. She was scheduled for ongoing physiotherapy and follow-up two months later.

Case 3

J.A. is a four-year-old boy who was admitted to OLHSC with a one-month history of muscle weakness. The weakness began with difficulty climbing stairs, which progressed to an inability to run and then to walk. At presentation he was dragging himself with his arms in order to move around. J.A. is a known asthmatic and uses Becotide and Ventolin inhalers daily. He had a history of an upper respiratory illness two weeks before the onset of his symptoms. He had no significant medical or family history.

On examination J.A. had grade one to two out of five power in the upper and lower limbs bilaterally and Gower's sign was positive. There was also a tremor in his hands and reflexes were absent in all his limbs. The muscle bulk and sensation was normal and there were no signs of autonomic neuropathy. There was no abnormality detected on cranial nerve examination.

A CT scan of J.A.'s brain was normal. The diagnosis of GBS was confirmed after CSF results revealed an increased CSF protein and nerve conduction studies showed delayed nerve conduction, along with a raised CK. J.A. was treated with physiotherapy and had improved significantly on his discharge 10 days after admission.

DISCUSSION

There was a paucity of data on childhood GBS. Some articles found were inaccessible or were not in English. In the end, a relatively small number of articles were available for appraisal that fit the inclusion criteria for the review. Two of these articles concluded that IVIG made no difference to outcome and four studies concluded a definite benefit with IVIG. Only one study provided statistically significant evidence for deaths and the need for ventilation within the clinical criteria.[12] A study by Graf *et al.* indicated that a sample size of 370 patients would be needed to show statistical significance in improvement in time to recovery with IVIG. There were no randomised controlled trials or systematic reviews available and overall sample sizes were small. Childhood GBS is an uncommon condition and ethical and logistic barriers prevent the study of a large sample size.

While the commonly accepted benefits of IVIG in adult GBS cannot be automatically extrapolated to the paediatric population it has been shown that IVIG has the potential to significantly reduce the mortality and morbidity arising from this syndrome. Koul *et al.* found that IVIG treatment reduced the time taken to recovery and the time spent in hospital. Shanbag *et al.* reported statistically significant benefits with IVIG regarding time to recovery, the need for mechanical ventilation, and reduced mortality. Though Graf *et al.* found no convincing evidence of the benefits of IVIG the authors enumerated several confounding factors that may have affected this data. IVIG has not been found to have an impact on the long term sequelae of GBS, for example, the presence of fatigue and motor or sensory deficits. Acute relapse after apparent recovery is a new phenomenon which had not been previously noted in the pre-IVIG era and warrants further investigation. The administration of IVIG as a single dose of 2g/kg merits further study also. This approach could enhance the usefulness of IVIG as a treatment for GBS, which not only reduces its morbidity and mortality, but also enables discharge from hospital earlier, benefiting both the patient and the hospital from an economic point of view.

IVIG and plasmaphoresis are the two most

commonly used immunotherapies in GBS. The practicalities and expense of administering plasmaphoresis makes IVIG the immunotherapy most used in practice. Though expensive, IVIG is simple to administer, whether over five days or a single day. As demonstrated in the case studies above, IVIG is not in routine use as a first-line therapy in clinical practice in Ireland today and would generally be administered only in a tertiary care centre, such as OLHSC. In the case of J.A., recovery occurred with supportive care alone and in the case of K.P., IVIG was only commenced when no clinical improvement was seen with supportive care alone. It is well recognised that GBS in children runs a more benign course than in adults. Recovery usually occurs spontaneously even without specific intervention. As mentioned, this is a major confounding factor in the study of its management.

The natural history of GBS in children has a gradual onset associated with milder symptoms and a faster recovery compared with the disease in adults. GBS has the potential, however, to cause significant disability and disruption to children's lives, education and development for up to and beyond five years after an acute episode. Its acute phase can be extremely traumatic for both child and parents. In particular, pain is more characteristic of GBS in children than in adults and may be severe. Therapies that shorten the course of the illness not only allow the child to return to normal life sooner, but also reduce the risk of complications of immobility, for example, pneumonia and allow the inevitable trauma experienced to be minimised. In addition, it cannot be forgotten that the illness carries a mortality rate of three to five percent, which has been shown to be reduced by the adequate and timely use of IVIG. The use of IVIG can be seen to have considerable benefits in this context and with time the full potential of this treatment in the management of childhood GBS will undoubtedly come to light.

ACKNOWLEDGEMENTS

The authors would like to acknowledge with gratitude the assistance of Dr Webb and Dr McMenamin, consultant neurologists at OLHSC, in carrying out this project. The authors would also like to thank Dr Sharon Condon, Department of Paediatrics, AMNCH for her collaboration during this study.

REFERENCES

1. Ariga T, Yu RK. Antiglycolipid antibodies in Guillain-Barré Syndrome and related diseases: Review of clinical features and antibody specificities. *J Neurosci Res* 2005;[epublication ahead of print].
2. Kumar P and Clark M, eds. Clinical medicine. 5th ed. United Kingdom: Bath Press Ltd., 2002.
3. Healthwise Medical Database. Guillain-Barré Syndrome. (Accessed at http://www.caminomedical.org.)
4. A collection of resources on Guillain-Barré, Miller-Fisher and other related syndromes. (Accessed at http://www.gbs.org.)
5. Dalakas MC. Intravenous immunoglobulin in the treatment of autoimmune neuromuscular diseases: present status and practical therapeutic guidelines. *Muscle Nerve* 1999;22(11):1479-97.
6. Misbah SA, Chapel HM. Adverse effects of intravenous immunoglobulin. *Drug Saf* 1993;9(4):254-62.
7. Van Doorn PA. Guillain-Barré Syndrome. (http://www.orpha.net, Updated Sep 2004.)
8. McLean S, Oon SF, Conran S, et al. Childhood Guillain-Barré Syndrome: Comparing intravenous immunoglobulin treatment with supportive care.

Presentation by S McLean, June 2003, Adelaide and Meath Hospital, Incorporating the National Children's Hospital, Tallaght, Dublin, Ireland.
9. Sharrack B, Hughes RAC, Soudain S, Dunn G. The psychometric properties of clinical rating scales used in multiple sclerosis. *Brain* 1999;122(1):141-59.
10. Koul R, Chacko A, Ahmed R, et al. Ten year prospective study (Clinical Spectrum) of childhood Guillain-Barré Syndrome in the Arabian Peninsula: Comparison of outcome in patients in pre- and post-intravenous immunoglobulin eras. *J Child Neurol* 2003;18(11):767-71.
11. Vajsar J, Fehlings D, Stephens D. Long term outcome in children with Guillain-Barré Syndrome. *J Paediatr* 2003;142(3):305-9.
12. Shanbag P, Amirtharaj C, Pathak A. Intravenous immunoglobulins in severe Guillain-Barré Syndrome in childhood. *Indian J Pediatr* 2003;70(7):541-3.
13. Zafeiriou DI, Kontopoulos EE, Katzos GS, Gombakis NP, Kanakoudi FG. Single dose immunoglobulin therapy for childhood Guillain-Barré Syndrome. *Brain Dev* 1997;19:323-5.
14. Graf WD, Katz JS, Eder DN, et al. Outcome in severe paediatric Guillain-Barre syndrome after immunotherapy or supportive care. *Neurology* 1999; 52:1494.
15. Yata J, Nihei K, Ohya T, et al. Study group for pediatric Guillain-Barré Syndrome. High dose immunoglobulin therapy for Guillain-Barré Syndrome in Japanese children. *Pediatr Int* 2003;45(5):543-9.

Chronic Myeloid Leukaemia: Molecular Abnormalities and Treatment Options

Niamh Appleby*, Eimear Burke*, Terry-Ann Curran* and Elaine Neary*

*4^{th} *Year Medicine*

ABSTRACT

Chronic myeloid leukaemia (CML) is a malignant, myeloproliferative disorder of haemopoietic stem cells. It arises from a stem cell acquiring a specific translocation t(9;22) which results in the formation of a hybrid oncogene, BCR-ABL. Selecting the most appropriate therapy for a patient with CML remains difficult. Currently, stem cell transplantation is generally accepted as offering the best prospect of a cure. However, advances in the study of tyrosine kinase inhibitors and immunological treatments may direct the future of CML treatment.

INTRODUCTION

Chronic myeloid leukaemia (CML) is a malignant myeloproliferative disorder of haemopoietic stem cells, giving rise to abnormally elevated numbers of myeloid cells in peripheral blood and myeloid hyperplasia in bone marrow. Uncontrolled production of myeloid precursors results in increased levels of maturing granulocytes, mainly neutrophils but also eosinophils and basophils. Elevated numbers of erythoid cells and platelets are also found.

Clinical Features of CML

Peripheral blood abnormalities account for many of the clinical features of CML (Table 1). Systemically the excess of neutrophils is accompanied by splenomegaly, and occasionally hepatomegaly or adenopathy, and varying degrees of myelofibrosis and extramedullary haemato-poiesis. Symptoms related to leukostasis arising from high white cell count include blurred vision, headaches and rarely in males, priapism. Some 40 percent of patients are asymptomatic, with diagnosis following a routine blood test.

EPIDEMIOLOGY

CML occurs with an incidence of one to two cases per 100,000 people per year and accounts for 15 percent of adult leukaemias.[1] CML may affect any age group, though it is unusual in children. The peak incidence is between 40 and 60 years with the median age at diagnoses being 53 years.[2]

CML: A Progressive Triphasic Disease

The clinical course of CML is divided into three distinct phases, characterised by progressive changes in leukocytes and accumulation of genetic abnormalities. The stage of CML is an important consideration in deciding on treatment course.

The chronic phase (present at time of diagnosis in approximately 85 percent of patients) is associated with large increases in the pool of committed myeloid progenitors, leading to peripheral blood

PRESENTATION OF CML
Clinical Findings • Fatigue • Anorexia • Weight Loss • Splenomegaly • Hepatomegaly 40 percent of patients asymptomatic at diagnosis
Peripheral Blood: • White Cell Count > 25,000/mm^3 • 30-50 percent have elevated platelet count • Basophilia • Reduced leukocyte alkaline phosphatase activity • All stages of granulocyte differentiation visible on peripheral smear
Bone Marrow • Hypercellularity, with reduced fat content • Increased ratio of myeloid to erythoid cells • Increased numbers of megakaryocytes

leukocytosis and often thrombocytosis with a prominent left shift in the differential count and basophilia.[1] Left untreated the chronic phase lasts three to five years before developing into the accelerated phase.

In the accelerated phase of CML neutrophil differentiation becomes progressively impaired and leukocyte counts are more difficult to control with myelosuppressive medications.

Finally CML culminates in blast crisis. The blast phase is defined by the presence of 30 percent or more leukaemic cells in peripheral blood or marrow or the presence of extramedullary infiltrates of blast cells. The blast phase of CML resembles acute leukaemia in which myeloid or lymphoid blasts fail to differentiate.

In one third of cases, the blasts have a lymphoid morphology and express lymphoid markers such as terminal deoxynucleotidyl transferase or CD10 (common acute lymphoblastic leukaemia antigen). The remaining two-thirds of cases have a phenotype similar to that of acute myeloblastic leukaemia and form a heterogeneous group.

The distinction is important because CML in the lymphoid blast phase may respond to treatment with regimens that are active against acute lymphoid leukaemia. However, the median survival after progression to blast crises remains approximately six months.

Occasionally, CML does not follow this pattern but transforms into myelofibrosis, in which case death results from bone marrow failure.

Molecular Abnormalities

CML is characterised by the presence of a distinct molecular abnormality. First described by Nowell and Hungerford in the 1960s who dubbed it the "Philadelphia chromosome," the molecular abnormality has since been identified as a reciprocal translocation between chromosomes 9 and 22.

Now designated t(9;22)(q34;q11) this translocation involves the BCR gene on chromosome 9 and the ABL gene on chromosome 22. The resultant BCR-ACL fusion gene, directs synthesis of a protein with tyrosine kinase activity. This abnormal tyrosine kinase protein product is unique to the leukaemia cells and is the fundamental cause of all the abnormalities observed in Philadelphia chromosome-positive leukaemias.

How this translocation initially occurs is not fully understood although radiation has been implicated. It has also been suggested that the close proximity of the BCR and ABL genes during interphase may facilitate the mutation.[3]

BCR and ABL in Normal Cells

In a normal cell the BCR gene codes for a 1271-amino acid cytoplasmic phosphoprotein of 160 kDa that has several known domains:

Amino acids 1-427: contains a coiled domain that mediates homo-oligomerisation and a novel serine kinase activity.

Amino acids 490-690: contains a region of homology to the DBL oncoprotein that functions as a guanine nucleotide exchanger

C-terminus: contains a domain with GTPase activating protein homology and GAP activity toward RAC and Cdc42 proteins. Mice lacking BCR show an increased respiratory burst in their neutrophils.

There is less known about the c-ABL gene. It may be involved in cell response to genotoxicity (damage to genetic material) and oxidative stress, and in integrin and platelet-derived growth factor (PDGF) signalling. c-ABL tyrosine kinase activity is tightly controlled in the normal cell, and appears to be regulated at multiple levels by serine and tyrosine phosphorylation, by NH2-terminal sequences and by a cellular inhibitor that binds to Src homology 3 domain on c-ABL.

BCR-ABL Cell Transformation

Although BCR and c-ABL have no intrinsic oncogenic properties themselves, BCR-ABL has the ability to transform cell lines and primary cells *in vitro*.

The abnormal BCR-ABL hybrid encodes for an abnormal, constituently active tyrosine kinase receptor. Simply, BCR-ABL can be thought of as always "switched on." BCR-ABL has primary mitogenic activity and can stimulate cell cycle entry of haematopoietic cell lines and primary cells in the absence of growth factors. It activates multiple signal transduction cascades including the RAS, MYC and PI3K pathways thereby allowing the growth and survival of haemoto-

poetic stem cells to continue independent of their regulatory cytokines.[2, 3] BCR-ABL reduces the expression of cell surface adhesion molecules, facilitating the dissemination of leukaemic cells in the peripheral blood.[1] In addition, the BCR-ABL allows leukaemic cells to evade apoptosis. Philadelphia chromosome positive cells are protected from apoptosis upon cytokine withdrawal, radiation and cytotoxic chemotherapeutic agents.

The malignant clone in CML is genetically unstable and acquires multiple genetic abnormalities during the progression from chronic phase to blast crisis. As the disease progresses, leukaemia cells acquire further mutations including trisomy 8 and alterations in RAS and RB1 genes.[1] Mouse studies have suggested that BCR-ABL may directly induce karyotypic instability.

CML Histopathology

In contrast to normal marrow, which is approximately 50 percent cellular and 50 percent fat, CML marrow is 100 percent cellular, predominantly composed of maturing granulocytic precursors. Seen on microscopic investigation are increased numbers of megakarocytes, including small dysplastic forms, erythroid progenitors which are usually in normal or decreased numbers, scattered storage histiocytes with wrinkled, sea-blue cytoplasm, increased deposition of reticulin fibres (but overt marrow fibrosis is rare at presentation) and marked leukocytosis often exceeding 100,000 cells/mm^3.

BCR-ABL: Three Forms

There are three principal forms of the BCR-ABL mutation: p190, p210 and p230. They all encode the c-ABL gene sequence (except the first exon) and the entire ABL tyrosine kinase catalytic domain, but differ in the length of the BCR transcription sequence at the N-terminus.

Briefly, all three have increased tyrosine kinase activity *in vivo* and *in vitro* relative to c-ABL with p190 exhibiting the highest intrinsic kinase activity. The mechanism of activation is unknown but may involve oligomerisation of BCR-ABL via the coiled cell domain, and interaction of BCR with the ABL src homology 2 domain, blocking the inhibition of an ABL inhibitor.

TREATMENT OF CML

CML treatment options can broadly be divided into stem cell transplantation and non-transplant based therapies, which include oral chemotherapeutic agents interferon-alfa (IFNα) and oral tyrosine kinase inhibitors. Treatment choice depends on the phase of the disease (chronic, accelerated or blast), the age of the patient and the availability of a suitable stem cell donor.

Given the growing range of therapeutic choices, the patient and disease factors which must be taken into account, the question of selecting the correct treatment for the patient who will benefit most is quite complicated.

Unfortunately, less than 20 percent of CML patients are eligible for transplantation either due do age limitations or lack of a suitable donor.[2] For the majority of cases of CML, drug treatment remains the mainstay.

Imatinib

With the discovery that the product of the Philadelphia chromosome accounted for cell transformation in CML, inhibition of BCR-ABL soon became the focus of research efforts in the search for a cure. BCR-ABL was an ideal molecular therapeutic target because it is unique to leukaemic cells, is expressed at high levels and its tyrosine kinase activity is essential for the induction of leukaemia.

STI-571, later renamed imatinib mesylate (Glivec, Gleevec, Novartis, Basel, Switzerland), was the most promising tyrosine kinase inhibitor discovered. It binds to the ATP domain of BCR-ABL preventing the phosphorylation of its substrates and thereby blocking the downstream signal cascades. Imatinib stimulates apoptosis of BCR-ABL positive cells without affecting normal stem cells.

In vitro studies found that imatinib produced a 92 to 98 percent reduction in CML colony growth without inhibiting growth of normal cells.[4] Phase I trials began using imatinib in patients with chronic phase CML who had failed to respond to IFNα therapy or were intolerant to it. Complete haematological response, defined as reduction in white-cell count to less than 10,000/mm^3 and in the platelet count to less than 450,000/mm^3, was achieved in 53 of 54 patients treated.[5]

In a multicentre phase II trial using similar patient criteria, complete haematological response was obtained in 95 percent of patients and a major cytogenetic response in 60 percent.[6] Major cytogenetic response is defined by reduction in Philadelphia chromosome-positive cells in metaphase to less than 35 percent of cells in bone marrow. Ninety-five percent of complete responders remained in remission after two years.

In the International Randomised Interferon-alfa versus STI-571 (IRIS) Study, a phase III trial comparing imatinib versus IFN plus cytarabine (Ara-C), imatinib was shown to be a more effective treatment in patients with newly diagnosed chronic phase CML. Imatinib showed greater cytogenetic and haematologic responses and also reduced the incidence of progression to accelerated or blast phase. Imatinib was also tolerated better than the IFNα and Ara-C regimen.[7] A follow up study to the phase III trial measured the amount of BCR-ABL transcripts in the blood of patients who had a complete cytogenetic response. Results from the study showed that the proportion of patients that had at least three log kill at 12 months was greater in the imatinib group compared with controls receiving IFNα plus Ara-C (39 percent vs. 2 percent, $p < 0.001$).[8]

Imatinib is also effective in the accelerated phase of CML. It has produced a major cytogenetic response (less than 35 percent Philadelphia chromosome-positive cells in the bone marrow) in 24 percent of patients in the accelerated phase, short remissions of up to six months and has been used to control the leukaemia before transplantation.

In the blast phase of CML, imatinib induced a major cytogenetic response in 16 percent of patients and therapeutic response was associated with prolonged survival. Imatinib was also shown to be less toxic and it increased median survival when compared with Ara-C.[10]

Imatinib is licensed for first line treatment of CML in patients unsuitable for transplantation, as a second line treatment in patients unresponsive to interferon therapy and has been used to induce remission in the accelerated and blast phases of the disease.[11] It may also be used in the treatment of gastrointestinal stromal tumours, inhibiting the c-kit tyrosine kinase dependent signalling in this cancer.[12]

Limitations of Imatinib Therapy

Although the development of imatinib has significantly improved the outlook for patients diagnosed with CML, there remains two major hurdles to successful therapy.

Firstly, imatinib suppresses CML but does not eradicate all Philadelphia chromosome-positive cells. The persistence of detectable BCR-ABL transcripts in patients treated with imatinib is known as "residual disease." CML cells which fail to respond to imatinib therapy act as a reservoir for disease.[13] Imatinib therapy suppresses disease and induces remission but does not cure the leukaemia and it remains a life-long treatment.

Secondly, the emergence of resistance to imatinib therapy limits the effeciacy of the drug. Resistance to imatinib is common in advanced phases of disease. Patients may relapse during treatment but resistance can occur in previously untreated patients.

Several mechanisms of resistance have been demonstrated, the most common of which is a point mutation in the BCR-ABL gene, impairing binding of the drug to the tyrosine kinase receptor. Mechanisms of resistance include amino acid substitution that changes conformation of the ATP binding site, reactivation of BCR-ABL signal transduction and amplification of BCR-ABL gene.[12]

CML progenitor cells are relatively insensitive to imatinib, using an efflux pump to reduce the intracellular concentration of the drug.[13]

Another possibility is that BCR-ABL may not tell the full story of CML. If this is true, it has significant implications for the development of future tyrosine kinase inhibitors. If the ABL tyrosine kinase is not solely responsible for CML cell survival and proliferation, then no matter how powerful an inhibitor may be, it will still fail to eradicate residual disease. There is some evidence from studies in mouse cell lines to support this idea. Imatinib did not inhibit the growth of mouse myeloid cells in the presence of interleukin-3.[14]

It has been posulated that inhibition of the ABL kinase does not directly stimulate apoptosis, but allows the leukaemic cells to return to their normal cytokine dependent growth and proliferation. Thus, imatinib causes the apoptosis of the excess cells which lack the cytokines to support them.[13]

Interferon-alfa

Interferons are glycoproteins produced by cells in response to antigenic stimuli, for example, viral infection or malignancy. IFNα has a range of immunomodulatory effects including activation of cytotoxic T cells. While its precise mechanism of action in CML remains unclear, it has been shown to reduce the survival and inhibit proliferation of CML cells *in vitro*.

IFNα induces haematological remission in the chronic phase of CML. Complete cytogenetic response is achieved in between 20 to 30 percent of patients with the sustained disappearance of CML cells reported in less than 10 percent of those treated.

The combination of IFNα and Ara-C, as compared with IFNα alone, has been shown to increase the rate of major cytogenetic response and prolongs survival in patients with the chronic phase of CML. Guilhot *et al.* demonstrated significant advantages of combination therapy. The survival rate was 85.7 percent with IFNα and Ara-C and 79.1 percent with IFNα alone.[15]

Prior to the introduction of imatinib, IFNα therapy was the treatment of choice in patients unsuitable for transplantation. CML was among the first diseases to be treated with a biological agent. IFNα remains an important tool in the management of the disease.

Chemotherapeutic Agents

Hydroxyurea and busulfan are the most commonly used chemotherapeutic agents in the treatment of CML. Both reduce the number of circulating neutrophils and allow patients to avoid the thrombotic complications of a high white cell count. However, neither alters the course of the disease and, as such, are considered palliative treatments. Hydroxyurea may be preferable to busulfan as it has less toxic side effects, particularly in patients who may undergo transplantation.[2]

Haematopoietic Stem Cell Transplantation

High-dose chemotherapy with or without total body irradiation followed by allogeneic stem cell transplantation has been shown for many years to be curative in chronic phase of CML.

However, successful transplantation depends on the availability of suitable donors and the condition of the recipient. Transplantation should ideally occur with a HLA-matched, related donor to a recipient under 40 years of age who has been diagnosed with chronic phase CML within the last year (Table 1). Unfortunately less than 20 percent of patients with CML are suitable for bone marrow transplantation, due to a combination of age and lack of availability of donors.[2]

Transplantation during the chronic phase increases the likelihood of survival. Patients transplanted within one year of diagnosis do best. This suggests that CML cells may develop some degree of resistance to treatment even before progression to the accelerated phase. Although different centres have different criteria for eligibility, patients greater than 40 years of age have been shown to suffer a greater incidence of transplantation-related mortality. HLA-matched donors have been shown to reduce some of the risks of stem cell transplantation.[16,17,18]

Prognostic Factors for Chronic Myeloid Leukaemia Patients considering Stem Cell Transplantation (European Group for Blood and Marrow Transplantation)
Disease Phase
• Chronic • Accelerated • Blast - Give chemotherapy prior to transplant to achieve second chronic phase of transplant in blast phase or use tyrosine kinase inhibitor
Disease duration
• Transplant within the first year of diagnosis
Age
• Younger age (<40 years) improves survival

Table 1. *Prognostic factors for stem cell transplantation.*

Stem cell transplantation can cure CML but it carries serious risks of infections and graft-versus-host-disease (GVHD). Therefore, a clinician must balance the prospects of a cure against the morbidity and mortality associated with the treatment.

Graft-Versus-Leukaemia Effect

The relationship between the incidence of GVHD, a life-threatening complication of transplantation and the long-term disease free survival of patients are termed the graft-versus-leukaemia (GVL) effect. This correlation illustrates the importance of the immune system in the treatment of CML and may direct future therapies.

The key role of the T cell in GVL is highlighted by a series of transplants performed using T cell depleted marrow, aiming to reduce the incidence of GVHD. Goldman *et al.* demonstrated the the probability of relapse was higher for recipients of T cell-depleted bone marrow compared with recipients of non-T cell-depleted bone marrow and for patients who did not develop chronic GVHD compared with patients who did.[19]

T cells are also significant in inducing remission of relapsed CML. Patients who have undergone stem cell transplant and subsequently relapse have been effectively treated by infusion of donor lymphocytes.[20]

Future Therapies

Finding a way to reduce the danger of the GVHD while retaining the benefits of the GVL is a challenge for the future. One approach has been to remove the T cells for transplantation and reinfuse them at a later date. Another possibility is that two different populations of T cells may mediate the GVHD and GVL effect.

Vaccination

With the T cell widely acknowledged as playing a vital role in the success of bone marrow tranplantation, it is no surprise to find researchers questioning the possibility of a vaccination against the disease. Work is underway to determine if T lymphocytes may be activated specifically against CML cells. Cytotoxic T cells that recognise the PR1 component of proteinase 1 or Wilms Tumour 1 antigen, both over expressed in leukaemia cells, have been shown to kill CML cells *in vitro*.

Trials at the University of Texas of a vaccine for CML based on the PR1 peptide have already met with some success, with half of the subjects remaining disease free three years after receiving the vaccine. The vaccine induces cytotoxic T cells to preferentially kill leukaemic instead of normal progenitor cells.[21]

Researchers at Siena University, Italy have gone further and developed a p210 multipeptide vaccine against CML. Sixteen patients with stable residual disease who had been treated with imatinib for a minimum of 12 months or interferon alfa therapy for 24 months underwent a course of vaccination. Fifteen of 16 patients demonstrated improved cytogenetic responses, with half of the patients achieving complete cytogenetic remission in three months.[22]

These studies lend support to the argument for the development of a vaccine in addition to existing therapies.

Further Targets for Molecular Therapy

The second generation of tyrosine kinase inhibitors are also under development. BMS-354825 is a dual inhibitor of SRC and ABL kinase with a 100-fold greater potency than imatinib. It has been shown to inhibit the kinase activity of 14 out of 15 imatinib resistant forms of BCR-ABL.[22]

Other potential therapeutic targets include treatments directed at the downstream targets of BCR-ABL are under development. Inhibitors of heat shock protein 90, a protein that stabilises BCR-ABL and other oncoproteins as well as inhibitors of the RAS and PI3K pathways may offer prospects for future therapies.[12]

REFERENCES

1. Faderl S, Talpaz M, Estrov Z, O'Brien S, Kurzrock R, Kantarjian HM. The biology of chronic myeloid leukemia. *N Engl J Med* 1999;341:164-72.
2. Sawyers C. Chronic myeloid leukemia. *N Engl J Med* 1999; 340:1330-40.
3. Goldman JM. Chronic myeloid leukemia - advances in biology and new approaches to treatment. *N Engl J Med* 2003; 349:1452-64.
4. Druker BJ, Tamura S, Buchdunger E, et al. Effects of a selective inhibitor of the ABL tyrosine kinase on the growth of BCR-ABL positive cells. Nat Med 1996;2:561-6.
5. Druker BJ, Talpaz M, Resta DJ, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med* 2001;344:1031-7.
6. Kantarjian, H, Sawyers, C, Hochhaus, A, et al. Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. *N Engl J Med* 2002;346:645-52.
7. O'Brien SG, Guilhot F, Larson RA, et al. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N Eng J Med* 2003;348:994-1004.
8. Hughes TP, Kaeda J, Branford S, et al. Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. *N Engl J Med* 2003;349:1423-32.
9. Talpaz, M, Silver, RT, Druker, BJ, et al. Imatinib

induces durable hematologic and cytogenetic responses in patients with accelerated phase chronic myeloid leukemia: results of a phase 2 study. *Blood* 2002;99:1928-37.
10. Kantarjian HM, Cortes J, O'Brien S, et al. Imatinib mesylate (STI571) therapy for Philadelphia chromosome-positive chronic myelogenous leukemia in blast phase. *Blood* 2002;99:3547-53.
11. British National Formulary 48, September 2004.
12. Guilhot F. Indications for imatinib mesylate therapy and clinical management. *Oncologist* 2004;9:271-81.
13. Ren R. Mechanism of BCR-ABL in the pathogenesis of chronic myelogenous leukaemia. *Nature Reviews Cancer* 2005;5:172-82.
14. Gambacorti-Passerini CB, Gunby RH, Piazza R, et al. Molecular mechanisms of resistance to imatinib in Philadelphia-chromosome-positive leukaemias. *Lancet Oncol* 2003;4:75.
15. Guilhot F, Chastang C, Michallet M, et al. Interferon alfa-2b combined with cytarabine versus interferon alone in chronic myelogenous leukemia. *N Engl J Med* 1997;337(4):223-9.
16. Druker BJ, Sawyers CL, Capdeville R, Ford JM, Baccarani M, Goldman JM. Chronic myelogenous leukemia. *Hematology* 2001;2001:87-112.
17. Negrin R. Treatment of chronic myeloid leukemia. (Accessed 11 March, 2005 at: http://www.uptodate.com)
18. Negrin R. Hematopoietic cell transplantation in chronic myeloid leukaemia. (Accessed 11 March, 2005 at: http://www.uptodate.com)
19. Goldman JM, Gale RP, Horowitz MM, et al. Bone marrow transplantation for chronic myelogenous leukemia in chronic phase: increased risk for relapse associated with T-cell depletion. *Ann Intern Med* 1988;108:806-14.
20. Kolb HJ, Mittermüller J, Clemm C, et al. Donor leukocyte transfusions for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. *Blood* 1990;76:2462-5.
21. Researchers at MD Anderson. Vaccination with the PR1 Leukemia-Associated Antigen Can Induce Complete Remission in Patients with Myeloid Leukemia. 46th Annual Meeting of the American Society of Hematology. Abstract 259.
22. Bocchia M, Gentili S, Abruzzese E, et al. Effect of a p210 multipeptide vaccine associated with imatinib or interferon in patients with chronic myeloid leukaemia and persistent residual disease: a multicentre observational trail. *Lancet* 2005;365:657-62.