Science, medicine, and the future

Alzheimer's disease

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There is a noticeable air of optimism in the research community studying Alzheimer's disease. This because the molecular basis of Alzheimer's disease and other neurodegenerative conditions, such as Parkinson's and Huntington's diseases, is rapidly being elucidated. From these molecular insights, it is likely that effective therapeutic strategies will be developed within the next 10 years. Future treatment will probably be based on combination therapies—such as neurotransmitter replacement combined with a drug to protect against the toxic effect of Aβ amyloid—tailored to the genetic profile of an individual. Assuming proved efficacy and safety, these forms of treatment are likely to be as widespread and acceptable as cholesterol lowering treatment is today.

In the past few years there has been an avalanche of knowledge surrounding the genesis of Aβ amyloid plaques, one of the principal pathological hallmarks of this disease (see fig 1). In the absence of a serious candidate for the pathway leading to the neurofibrillary tangle, the other major pathological lesion in Alzheimer's disease (fig 1), the focus will probably remain on amyloid plaques. Strong genetic risk factors have been identified for Alzheimer's disease, all of which interact directly or indirectly with the Aβ amyloid pathway. Undoubtedly, other genetic factors remain to be discovered, some of which might open the door to the neurofibrillary tangle. More importantly, the major environmental risk factors for Alzheimer's disease remain elusive. This is not surprising, given the relatively few analytical epidemiological studies that have been conducted and the difficulty of case ascertainment, particularly in the early stages of the disease.

We review briefly where the research is heading and give some predictions on where our concepts might lie a decade from now (for more details see recent reviews[1–3]).

Possible futures

- Genotype screening, analysis, and counselling
- Presymptomatic diagnosis
- Rational preventive treatment—drug based or gene based
- Advice on preventive measures—changes to lifestyle to avoid known environmental risk factor(s)
- Drugs targeting the amyloidogenic pathway to modify the course of the disease

Central pathway of disease causation

Many lines of evidence confirm that the generation of Aβ amyloid from the amyloid precursor protein is the central pathway in Alzheimer's disease (see fig 2). The clinching evidence has come from the recognition that rare genetic mutations in the gene encoding amyloid precursor protein actually cause Alzheimer's disease at an early age (onset before 65 years).

Amyloid precursor protein is a normal transmembrane glycoprotein that is widely expressed in the body, but particularly in brain and platelets. Its function remains uncertain: mice lacking the protein seem largely normal but have subtle defects in synaptic function. Expression of the gene for the protein is closely regulated and responds quickly to a wide variety of cellular stresses and exogenous factors (including trauma, oestrogens, and certain metal ions). Generated in the endoplasmic reticulum, amyloid precursor protein is sent to the Golgi apparatus for glycation before export to the cell surface.

At critical points of its biogenesis, amyloid precursor protein is subjected to enzymatic proteolytic cleavages, which in concert generate the Aβ peptides (fig 2). These enzymes, termed secretases, release the amyloid precursor protein from the cell membrane and thereby affect the proportion of the protein that remains on the cell surface or is released into the extracellular milieu. The Aβ peptides encompass part of the hydrophobic transmembrane domain. The exact cleavage sites of the γ-secretases are important, since the length of the hydrophobic tail of the Aβ peptide may be a crucial factor determining its aggregation and toxicity. Thus, the shorter $A\beta_{40}$ is the species most often identified in non-neuronal cells and has less tendency to aggregate than the longer $A\beta_{42}$: it is this longer $A\beta_{42}$ that is found at the centre of amyloid plaques. Neuronal cells have a propensity to make the longer forms, probably in a different cellular compartment

(the endoplasmic reticulum). How either form is released from the cell remains uncertain.

Once released from the cell, Aβ peptides aggregate into amyloid fibrils. The rates of deposition and clearance of Aβ from the brain may be critical determinants in establishing disease. The exact mechanisms by which Aβ exerts its toxicity or adverse "gain of function" is under intense scrutiny.

Other chronic degenerative diseases of aging nervous system

Recent elucidation of a variety of gene mutations causing diverse chronic neurodegenerative diseases point to a common mechanism—the toxic gain of function of small, relatively insoluble, protein polymers (see box). If further research confirms and extends this line of reasoning, Alzheimer's disease may eventually be seen as only one example of a process in which an abnormally shaped molecule accumulates in the brain and causes neuronal damage. In that case, a treatment developed for the toxic effect of the polyglutamine expression of the abnormal gene in Huntington's disease might be relevant for one or all of the other neurodegenerative diseases.

Genetic causes and risk factors

The discovery of the presenilin family of genes has been a major breakthrough for research.[4] Together with mutations in amyloid precursor protein, mutations in these presenilin genes also cause early onset of Alzheimer's disease and probably act directly through the amyloidogenic pathway. We have now identified about half of all the causative genes (responsible for possibly 10-20% of all cases of Alzheimer's disease). Over the next decade, it is highly likely that the remaining genes will be discovered, particularly in view of the rapid progress in mapping and sequencing the human genome.

In contrast with the causative genetic mutations, genetic risk factors are emerging as important contributors to the occurrence of sporadic Alzheimer's disease (responsible for 80-90% of all cases). The first to be identified, the ApoE gene on chromosome 19, has provided clues to the likely size of effect of these "public" genetic polymorphisms in a complex disease. Thus, inheritance of the ApoE-ε4 allele may increase the risk for Alzheimer's disease by up to eightfold. In the near future other genetic loci that act as susceptibility factors for Alzheimer's disease will undoubtedly be discovered. For example, there is much current interest in loci on chromosome 12. These discoveries will bring forward the emerging field of pharmacogenetics, in which treatments and preventive strategies will be tailored to an individual's genetic profile.

Environmental causes and risk factors

Environmental factors might be expected to have a role in causing Alzheimer's disease, in common with all multifactorial complex diseases, but, surprisingly, none has yet been convincingly identified. Estimates of relative risk indicate that factors such as low education, head trauma, smoking, concomitant vascular disease, diabetes, and the menopause have modest or inconsequential effects. Is there a major environmental risk factor still waiting to be discovered by some enterprising epidemiologist? Could some subtle factor in the Western diet or lifestyle be uncovered through a more thorough understanding of the amyloidogenic pathway? For example, we know that metal ions (such as copper and zinc) interact adversely with amyloid precursor protein and Aβ, and evidence is emerging that oxidative stress mediated by hydroxyl radicals could underlie the basis of Aβ toxicity. These clues may provide the impetus for future epidemiological studies.

Neurodegenerative diseases associated with abnormal protein conformations (toxic gain of function)

Disease	Gene product
• Alzheimer's disease	Amyloid precursor protein and Aβ amyloid
• Creutzfeldt-Jakob disease	Prion protein
• Amyotrophic lateral sclerosis	Superoxide dismutase
• Parkinson's disease	α-synuclein
• Huntington's disease	Huntingtin
• Machado-Joseph disease	Ataxin-3

Experimental models

A major impediment to the development of rational treatments has been the lack of an authentic and practical small animal model of Alzheimer's disease. Fortunately, this seems to have been solved by the development of various transgenic mouse models, which are progressively looking more like the human disease. The latest are based on the overexpression of amyloid precursor protein combined with the effects of the causative human mutations.[5] The next step may be to modulate the strain background of the mice or to

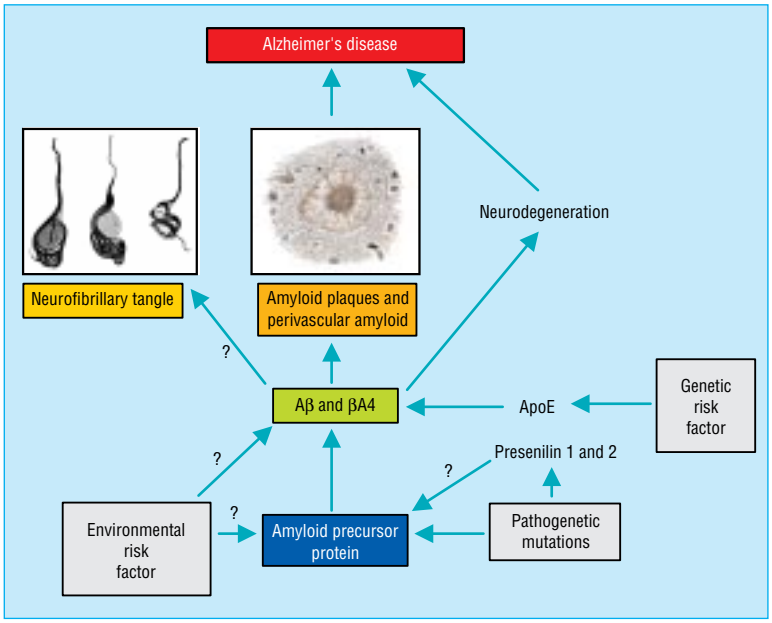

Fig 1 The central pathway leading to Alzheimer's disease involves the processing of amyloid precursor protein into Aβ amyloid, which accumulates as amyloid plaques or perivascular amyloid. Concomitantly, degeneration occurs in neurons and their processes, leading to neurofibrillary tangles. (Images are from Spielmeyer's *Histopathology of the Nervous System*, 1922.) Mutations in the gene encoding amyloid precursor protein can cause Alzheimer's disease, as do mutations in the presenilin genes. Inheritance of particular polymorphisms in genes such as ApoE also can increase susceptibility for Alzheimer's disease. Major environmental risk factors for Alzheimer's disease remain to be determined

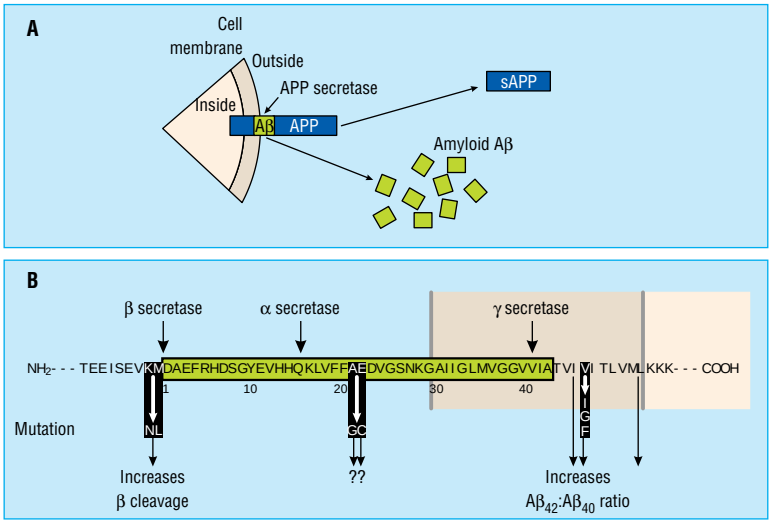

Fig 2 (*a*) Cleavage of amyloid precursor protein (APP) by enzymes (secretases) release the Aβ amyloidogenic fragment. (*b*) The critical region of amyloid precursor protein shown schematically in the one letter amino acid code. The secretases act at three principal sites (α, β, and γ). Mutations in the gene for amyloid precursor protein at these sites can adversely affect the action of secretases: mutations towards the NH_2 terminus increase the absolute rate of β-secretion, while mutations near the COOH terminus affect the ratio of $Aβ_{42}$ to $Aβ_{40}$. The $Aβ_{42}$ forms are more damaging for nerve cells

introduce another transgene to replicate the full human phenotype. Progress in this area has been so rapid that there is every reason to believe that an effective mouse model will soon be available.

Clinical trials and future therapeutics

In the past decade, much has been learned about the conduct of clinical trials by which the efficacy of any proposed treatment for Alzheimer's disease can be assessed. The licensing of compounds such as tacrine, donepezil, and rivastigmin have set standards by which all future drugs will be judged. There are currently four drugs awaiting approval and more than 16 drugs undergoing phase III clinical evaluation. Most are directed at the cholinergic system. Drugs specifically targeting the amyloidogenic pathway (see box) are only now beginning to emerge in a preclinical setting.

Looking ahead to the next decade, it is likely that a comprehensive package of genotypic analysis, presymptomatic diagnosis, and advice on preventive measures will be advocated (see box), with the use of a combination of drugs that effectively modify the course of the disease. Perhaps it is too much to expect any form of curative treatment by the year 2008, but the underlying concepts and principles for preventing the amyloidogenic processes from damaging neurons is straightforward and eminently amenable to intervention. Current estimates of the economic and social costs of Alzheimer's disease vary widely within and between countries, but all agree on the immense size of the problem and that it will increase dramatically over the next decade. The cost effectiveness of any preventive treatment is potentially enormous. In contrast with the small effect of today's symptomatic treatments,[6] future strategies may alleviate a burden that threatens most families; up to 25% of a family's annual income is required to care for a member with Alzheimer's disease.[7] As the average duration of the illness is 10 years, it is relatively easy to derive a rough estimate of the economic impact of the disease. Since a large proportion of the population is at risk of developing Alzheimer's disease (possibly over half), an effective drug based preventive treatment would justify universal screening (probably beginning at ages 40-50 years, when amyloid plaques are starting to appear in the temporal cortex).

Therapeutic targets in the amyloidogenic pathway

- Inhibit Aβ forming enzymes
- Redirect processing of amyloid precursor protein away from $Aβ_{42}$
- Inhibit aggregation or promote dissolution of Aβ
- Ameliorate toxicity of Aβ
- Suppress reactive responses to Aβ toxicity

Managing Alzheimer's disease in the year 2008

- Genotype screening, analysis, and counselling
- Presymptomatic diagnosis
- Rational preventive treatment—drug based or gene based
- Changes to lifestyle—avoiding the environmental risk factor(s)

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One hundred years ago

The doctor as a journalistic attraction

The "medical column" is put forward as an attraction by various newspapers in this country, but not even the most enterprising of our journalistic impresarios has yet as far as we know offered the services of a real live doctor to his subscribers. It is announced that a well-known Paris newspaper has adopted this means of extending its circulation. It has engaged a doctor of medicine at a salary of £480 a year to give gratuitous advice at the office of the paper to all the purchasers of an illustrated weekly supplement which it publishes. Many years ago a firm of cheap tailors in London used to boast that they kept a poet; is the time coming when daily newspapers will advertise the fact that "we keep a doctor?" (*BMJ* 1898;i:1283)

Grand Round

Mycobacterium paratuberculosis cervical lymphadenitis, followed five years later by terminal ileitis similar to Crohn's disease

John Hermon-Taylor, Nick Barnes, Chris Clarke, Caroline Finlayson

Mycobacterium paratuberculosis was first described by Johne and Frothingham in 1895[1] as the cause of a chronic inflammatory disease of the intestine in a German cow. The organism was called Johne's bacillus, and the illness (in which millions of acid fast mycobacteria were visible in the diseased tissues), Johne's disease. In 1901 Thomas Dalziel, a surgeon at the Western Infirmary in Glasgow, operated on a colleague with chronic inflammation of the intestine. He was aware of the description of Johne's disease and of the subsequent bacteriological research in the field. He collected other cases and published his observations in the *BMJ* in 1913.[2] He wrote that the "histological characters" of the disease he had described in humans were so similar to those of Johne's disease that the diseases may be the same. Dalziel's dilemma was that he could not see acid fast mycobacteria in the diseased intestine in humans.[3]

M paratuberculosis belongs to the *M avium-intracellulare* group.[4] The term paratuberculosis suggests close similarity to *M tuberculosis*, but in truth it is very different. Unlike *M tuberculosis*, it can survive in the environment and is highly resistant in vivo to most standard antituberculous drugs. *M paratuberculosis* cannot be reliably detected by culture in the laboratory; different subtypes of the organism with different preferred hosts[5 6] range from very slow growing to unculturable.

M paratuberculosis can cause chronic inflammation of the intestine in many species of animals including primates.[7–9] The disease in animals shows a broad range of histopathological types, from a pluribacillary (lepromatous) form with millions of typical acid fast bacilli visible in the tissues, to a paucimicrobial (tuberculoid) form in which *M paratuberculosis* cannot be seen in the tissues and cannot be detected by culture but in which there is a florid chronic granulomatous inflammatory response.[10] Intermediate paucibacillary forms of the disease, in which only a few acid fast mycobacteria may be visible, also occur.

M paratuberculosis has only rarely been cultured in the laboratory from humans with chronic inflammation of the intestine of the Crohn's disease type.[11–15] When it has been grown, the organism has first appeared in the culture in an unrecognisable non-bacillary form, taking months or years of incubation to alter its phenotype to that of a mature bacillus with an established lipid rich cell wall able to be identified by conventional means. Detection and characterisation of *M paratuberculosis* has been advanced by the advent of DNA based methods that use as their target a 1451bp DNA insertion element IS*900*, of which there are about 16 copies stably integrated into the *M paratuberculosis* genome.[16 17]

The clinical case

In September 1988 a healthy boy aged 7 years 10 months developed enlarged lymph nodes on the right side of his neck. He was asymptomatic and had a negative result on the Mantoux test, and chest radiography was normal. He had not had a BCG vaccination. He was referred to the surgical service at Addenbrooke's Hospital, where the enlarged lymph nodes were removed. The histological picture suggested a mycobacterial infection. Samples of the diseased lymph nodes were incubated on Lowenstein-Jensen slopes with duplicates including pyruvate, but not mycobactin, for 12 weeks at room temperature, 30°C and 37°C. For two months he was treated with rifampicin 450 mg and isoniazid 150 mg once daily and pyrazinamide 250 mg three times daily, but when the cultures were negative drug treatment was stopped. The remaining lymph nodes on the right side of the neck progressively enlarged and were removed by block dissection in November 1989. Mycobacterial culture again proved negative. He remained well until March 1993 when he developed an arthritis affecting both knees, and an anaemia refractory to iron. By the end of 1993, he had daily abdominal pain, anorexia, 2-3 loose bowel motions a day, weight loss, and lethargy.

His weight was 37 kg (on the 30th centile) and his height 1.53 m. He looked pale and ill and had angular stomatitis. Firm lymph nodes were still palpable on the right side of the neck. There was no clubbing. In the

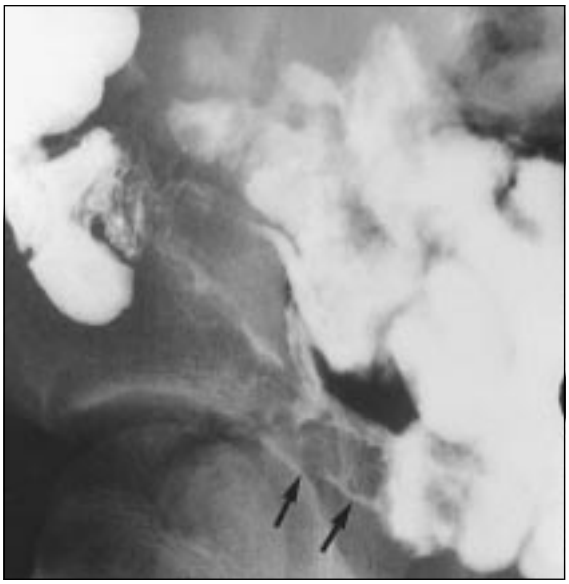

Fig 1 Narrowing and distortion of 10 cm segment of terminal ileum with classic "cobblestoning" of the mucosa (arrowed). The appearances are the same as those seen in Crohn's disease

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abdomen, an enlarged, tender loop of bowel was easily felt in the right iliac fossa. Radiologically, the small gut showed narrowing and distortion of a 10 cm segment of terminal ileum and prominent "cobblestoning" of the mucosa (fig 1). A white cell scan at one hour showed intense uptake of the label over the affected intestine (fig 2 (left)). These findings were considered typical of Crohn's disease.

He was treated daily with rifabutin 300 mg and clarithromycin 500 mg and was advised to have UHT ("long life") milk only. Eight days after the start of treatment lymph nodes on the left side of the neck and residual nodes on the right became enlarged and firm, with surrounding oedema. At the same time he had a transient episode of subacute intestinal obstruction, which subsided on conservative management. With continued rifabutin and clarithromycin treatment the nodes gradually subsided. By mid-1994 after six months' treatment he was virtually asymptomatic, and he was completely so by February 1995, by which time his weight had increased to 49 kg and his height to 1.60 m. White cell scanning then showed a complete resolution of the previously inflamed gut (fig 2 (right)). However, he was beginning to get obstructive symptoms. A barium enema showed no active disease, but there was a tight stricture of the terminal ileum with upstream dilatation. A limited resection was therefore carried out, with removal of the terminal ileum and adjacent ascending colon for a pale fibrotic stricture followed by end to end anastomosis. The histology of the resected stricture will be described. There was no other visible evidence of inflammatory disease throughout the gut.

Treatment with rifabutin and clarithromycin and restriction to UHT milk was continued. The drugs were stopped at the end of October 1996 after a total of 32 months' treatment. By January 1997 his weight was 67 kg (80th centile) and his height was 1.70 m. He was asymptomatic and a well developed adolescent on physical examination.

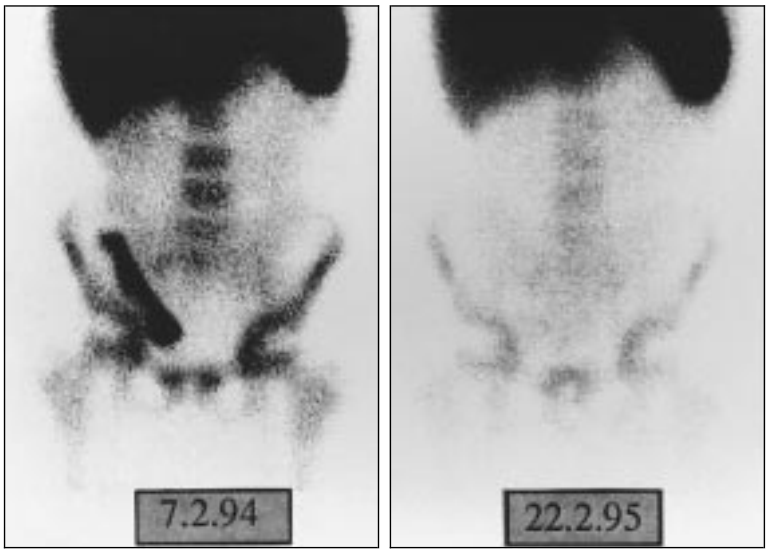

Fig 2 Left: White cell scan before treatment showing uptake of labelled cells by inflamed ileum characteristic of Crohn's disease. Right: White cell scan after one year of chemotherapy using combination of rifabutin and clarithromycin, showing apparent complete resolution

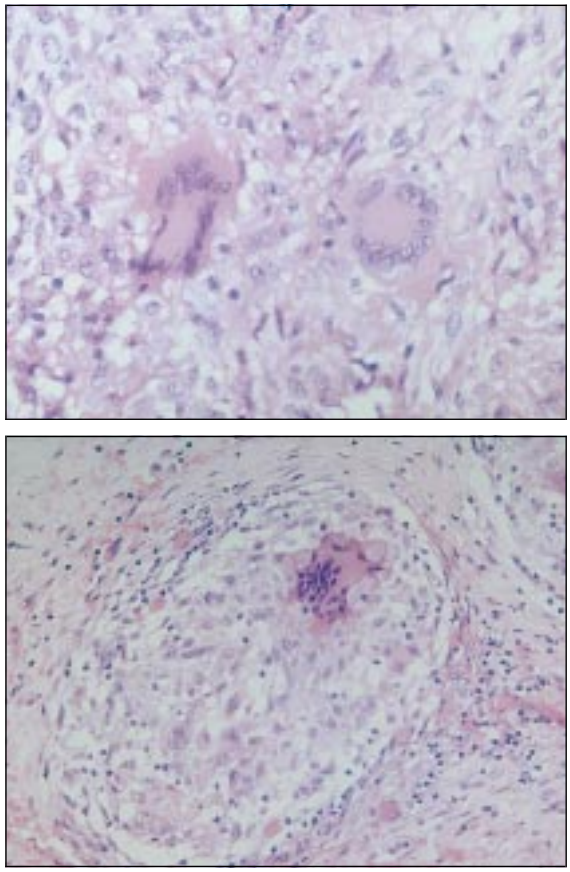

Fig 3 Microscopical appearance (top, ×400; bottom, ×250) of enlarged cervical lymph nodes showing typical Langerhans' giant cells with epithelioid cells, together with a non-caseating granuloma

Histopathology

Sections of the cervical lymph nodes initially removed showed areas of typical caseous necrosis with a only a few acid fast bacilli visible on Ziehl-Neelsen staining. Elsewhere there were marked chronic inflammatory changes with typical Langerhans' giant cells, non-caseating granulomata and an epithelioid cell infiltrate with focal microcalcifications (fig 3). The macroscopical appearances at operation were of a white fibrous stricture 5 mm in diameter and about 20 mm long in the terminal ileum (fig 4 (top), with encroachment of fat over the serosal surface. There was no evidence of chronic inflammatory disease elsewhere in the gut. Microscopically the mucosa over the ileal stricture was intact with little gland distortion and villous atrophy, but the submucosa and gut wall showed extensive transmural fibrosis. There was no evidence of active inflammatory disease or granulomata (fig 4 (bottom)). The mesenteric lymph nodes showed reactive hyperplasia but were otherwise normal.

Identification of *M paratuberculosis* by IS*900* polymerase chain reaction

Two of the paraffin embedded cervical lymph nodes removed in 1988 (fig 5) were subjected to DNA extraction using standard protocols and simultaneous process controls. IS*900* polymerase chain reaction for *M paratuberculosis* was then performed on the DNA extract in triplicate using the primers p90 (5′-GAAGGGTGTTCGGGGCCGTCGCTTAGG-3′) and

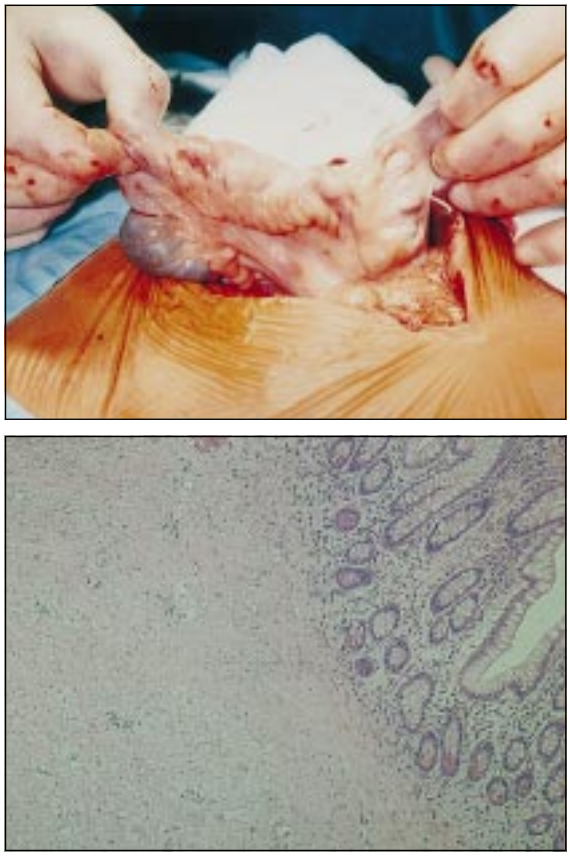

Fig 4 Top: Ileal stricture that developed after one year of treatment with rifabutin and clarithromycin. Bottom: Microscopical appearance (×100) of ileal stricture showing extensive intramural fibrosis

p91 (5′-GGCGTTGAGGTCGATCG CCCACGTGAC-3′).[18] Amplification products were run on agarose gel electrophoresis and identified by hybridisation at high stringency using a ^{32}P labelled 229 bp internal probe followed by autoradiography, as previously described.[18] The DNA extract of the cervical lymph nodes was strongly positive in triplicate for *M paratuberculosis* in the presence of negative process controls and correctly reporting positive and negative internal polymerase chain reaction controls (fig 6). The strongly positive signal from the IS*900* polymerase chain reaction that was obtained on the crude DNA extract from the cervical lymph nodes without hybridisation capture of target DNA[18] probably reflects a relatively high microbial abundance of *M paratuberculosis* in the tissues at an early stage of the infection.

Comment

M paratuberculosis infections in animals

Among animals, Johne's disease, or *M paratuberculosis* infection, occurs in domestic ruminants, especially cattle, sheep, and goats. Studies at the San Diego zoo and elsewhere, however, have shown that a broad range of animals, including subhuman primates, can be affected.[8]

Recently, *M paratuberculosis* has been identified in wild rabbits in Tayside, Scotland.[19] In subclinically infected cows *M paratuberculosis* is secreted abundantly in the milk, so that infection is acquired early in the life of newborn calves, when they are particularly susceptible.[20] The gut is the main target organ; *M paratuberculosis* is shed in the faeces, but it can travel throughout the

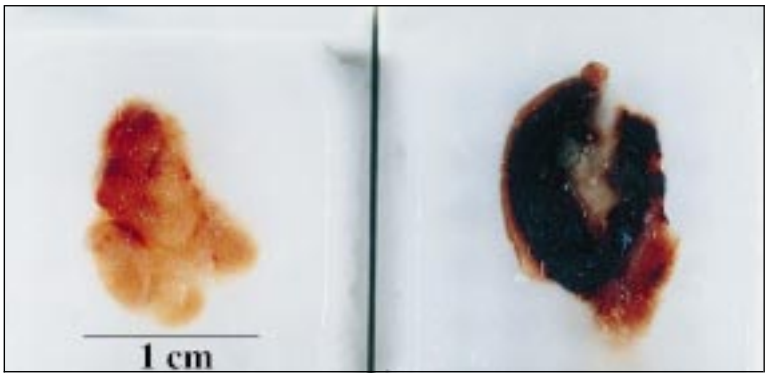

Fig 5 Enlarged paraffin embedded cervical lymph nodes removed in September 1988 from which DNA extract was prepared

animal in macrophages. A long latent interval usually occurs before clinical disease emerges. The signs of the disease in cattle are weight loss and diarrhoea; diarrhoea is not common in sheep. The pathology of the pluribacillary "lepromatous" type of disease is a chronic enteritis with diffuse thickening of the gut wall especially the terminal ileum, but extension to involve the colon is common. Mesenteric lymph nodes are enlarged. The mucosa of the gut is reddened with crevicing and marked oedema but not usually ulceration. Microscopically, there are millions of small acid fast *M paratuberculosis* living in macrophages (fig 7(a)).

We have shown that in about one quarter of cases there is a different form of Johne's disease in sheep.[10] Although the clinical signs and gross pathology are similar, microscopically there is a pronounced chronic granulomatous enteritis with heavy lymphocytic infiltration, typical Langerhans'-type giant cells and scattered granulomata. A few nests of acid fast bacteria can be found in about half of these cases of "tuberculoid" Johne's disease (fig 7 (b)). In the others, no bacillary form *M paratuberculosis* is visible in the tissues at all (fig 7 (c) and (d)).

Transmission of *M paratuberculosis* to humans

In the early part of this century, when milk supplies were at times heavily contaminated with *M tuberculosis* and *M bovis*,[21] tuberculous cervical lymphadenitis was common. The organism entered through not only the

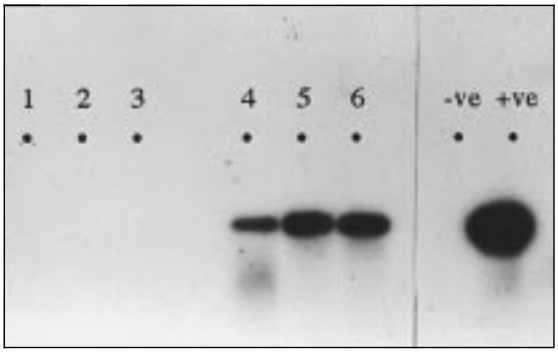

Fig 6 Results of IS*900* polymerase chain reaction with amplification products analysed by agarose gel electrophoresis and autoradiography. Lanes 1, 2, and 3 are simultaneous process controls correctly reporting negative; lanes 4, 5, and 6 are the DNA extract from the enlarged nodes showing strongly positive triplicate reactions for *Mycobacterium paratuberculosis*; and on the right are negative and positive (10fg Mptb target DNA) internal polymerase chain reaction controls

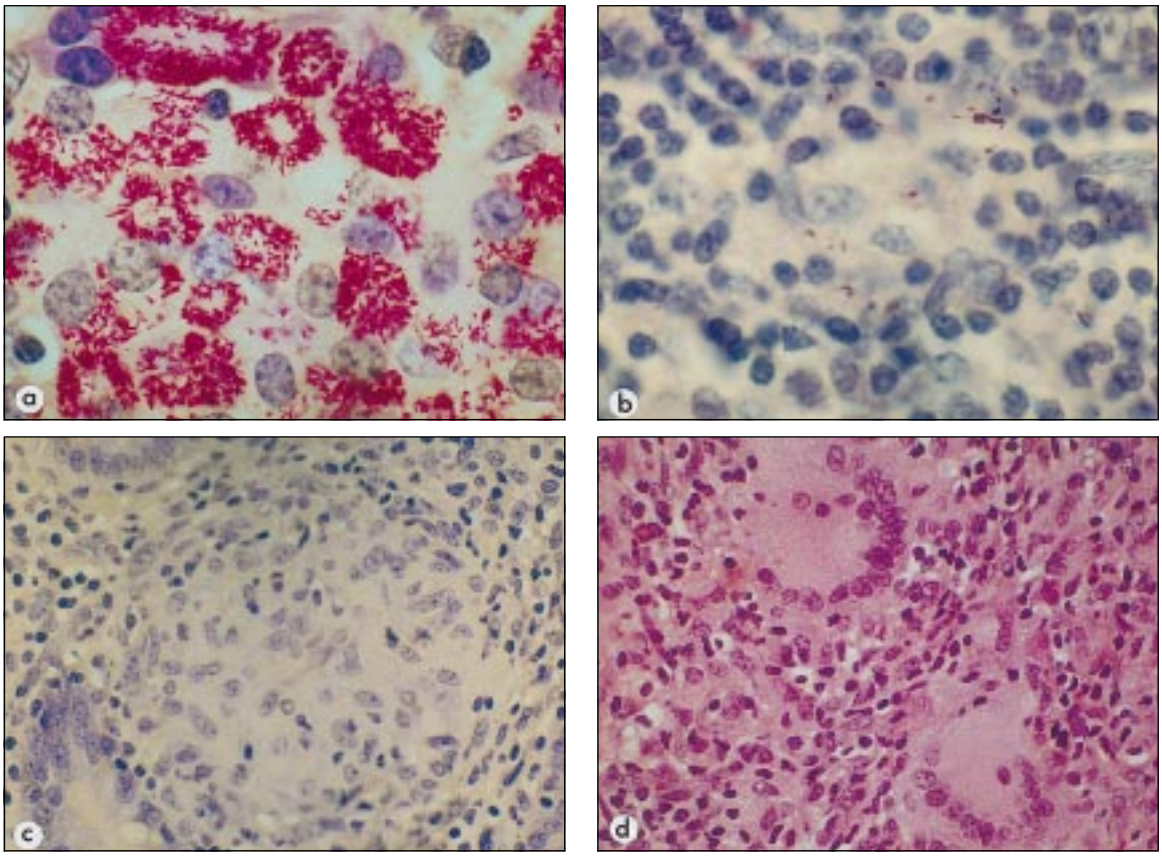

Fig 7 Photomicrographs of sections of intestinal wall, illustrating range of histological appearances and abundance of visible *M paratuberculosis* organisms in infected sheep with different forms of Johne's disease: *(a)* Ziehl-Neelsen stain (×1600) showing classic pluribacillary Johne's disease in which large numbers of small, acid fast *M paratuberculosis* organisms are seen within macrophages, but with little chronic granulomatous response; *(b)* Ziehl-Neelsen stain (×1600) showing paucibacillary form of Johne's disease with scant *M paratuberculosis* visible in tissues and prominent lymphocytic infiltration; *(c)* Ziehl-Neelsen stain (×800) of paucimicrobial form of Johne's disease diagnosed by IS*900* poymerase chain reaction, in which no bacillary form *M paratuberculosis* can be seen; *(d)* haematoxylin and eosin stain (×800) of paucimicrobial Johne's disease in a sheep showing typical Langerhans' giant cells and pronounced chronic granulomatous inflammation.

oropharyngeal lymphoid tissues but also the gut, causing tuberculous ileitis and disease in other parts of the body. The problem was overcome by tuberculin testing of dairy herds and the mandatory introduction of milk pasteurisation, using conditions that ensured the destruction of these well recognised pathogens.

The risk that something similar is happening with *M paratuberculosis* is substantial, although the organism is much less virulent for humans than is *M tuberculosis*.[22] Subclinical infection of dairy cows with *M paratuberculosis* is widespread in Britain and elsewhere in Europe,[23] and such animals shed the organism in their milk.[20 22] Conditions in the laboratory simulating commercial pasteurisation, which demonstrably kill *M bovis* do not always kill the more environmentally robust *M paratuberculosis*.[24 25] Outcomes analysis of the treatment of humans with chronic enteritis of the Crohn's disease type with a combination of drugs predicted to be active against non-bacillary forms of *M paratuberculosis* in vivo suggests that the use of such a drug combination can cause substantial remission in many patients with active Crohn's disease.[26]

The young boy in this case was probably infected by milk contaminated with non-tuberculous mycobacteria including *M paratuberculosis*. The organisms would have entered his cervical lymph nodes and his terminal ileum, as also occurs in animals.[27] After a latent interval of about five years, first an arthritis and then a chronic enteritis emerged. This was similar clinically, radiologically, and on white cell scanning, to Crohn's disease. Shortly after starting to take rifabutin and clarithromycin he had a transient flare of the disease, with widespread enlargement of the cervical lymph nodes, and an episode of subacute intestinal obstruction, similar to disease exacerbations reported in some cases of leprosy and tuberculosis after treatment is started.[28 29] In this case, the gut healed with prolonged treatment, leaving a scar that needed excision.

Discussion

Angus Dalgleish: As *M tuberculosis* is associated with caseation and *M paratuberculosis* is not, could both have been involved?

CF: The involvement of *M tuberculosis* is unlikely as it was not cultured from the lymph nodes, and the boy had a negative result on the Mantoux test. Other organisms such as *M scrofulaceum* can cause caseation in cervical lymph nodes. The information we have on this boy suggests that this was a mixed, non-tuberculous mycobacterial infection, which on this occasion also included *M paratuberculosis* in considerable abundance. We do not of course see caseation in the intestinal lesions of Crohn's disease.

George Griffin: Why do we not all get Crohn's disease?

JH-T: Probably for the same reasons that not everybody got tuberculosis earlier this century. In addition, *M paratuberculosis* has a much lower pathogenicity for humans than *M tuberculosis*. Several susceptibility factors are required for chronic enteritis and clinical disease due to *M paratuberculosis* to develop. These include firstly, an inherited susceptibility,[30] well known in Crohn's disease clinically, especially among Jewish people[31] but possibly also in Celtic races. An understanding of the molecular genetics of susceptibility is just beginning to emerge.[32–34] Secondly, intercurrent microbial infection (particularly gastroenteritis) is well known to be associated with the emergence of Crohn's disease. Lastly, psychological stress factors,[35] which are known to be able to trigger the emergence of Johne's disease in animals, may be associated with both the development of Crohn's disease and its exacerbation.

Devinder Kumar: If *M paratuberculosis* is a ubiquitous environmental organism how do you explain the geographical distribution of Crohn's disease?

JH-T: The environmental distribution of *M paratuberculosis* is not known. Efficient farming methods in industrialised societies, however, may have created localised conditions favouring the amplification of *M paratuberculosis* in our domestic livestock and their controlled habitats over the course of the century. Persistence of the pathogen would be favoured in temperate regions with a high water table.[36] Such conditions may also have increased the mutational frequency of *M paratuberculosis* and resulted in the emergence of a strain with increased pathogenicity for humans.

Robert Boyd: There is clearly a need for a randomised controlled trial of treatment with rifabutin and clarithromycin in patients with Crohn's disease.

NB: This certainly seems to be the case. A trial in children would seem to be particularly appropriate because they present relatively early on during the course of the disease, before they have developed the irreversible tissue damage and the range of complications that often occurs in adults.

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Conflict of interest: JH-T has prosecuted patents on IS*900* and would share with coworkers in a proportion of revenues generated by the successful commercial exploitation and use of this technology.

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Lesson of the week

Pneumatic compression boots for prophylaxis against deep vein thrombosis: beware occult arterial disease

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Patients with occult vascular disease may develop ulcers after treatment with pneumatic compression boots

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Prophylaxis against venous thromboembolism after joint replacement remains contentious.[1] The incidence of deep vein thrombosis is between 45% and 75%.[2–5] The rate of fatal pulmonary embolism is considered to be 1-3%,[2–5] but this is a gross overestimate.[1] Pharmacological techniques for preventing deep vein thrombosis (such as dextran 40, aspirin, warfarin, and heparins) are effective in reducing its incidence but are associated with important complications such as haemorrhage, wound haematoma, and haemarthrosis.[3 6–8]

Mechanical methods such as intermittent pneumatic compression devices have been developed to avoid these problems. These provide similar prophylaxis against deep vein thrombosis[8–10] and are considered free of important complications.[8] We report the occurrence of ulcers in patients using these devices according to approved guidelines.

Guidelines

Consideration of the evidence led the orthopaedic directorate of Glasgow Royal Infirmary and Stobhill NHS Trust to change its policy on prophylaxis against deep vein thrombosis at the end of 1995. Under the previous protocol heparin had been used in combination with graduated compression stockings. All patients having elective surgery who are at moderate or high risk of deep vein thrombosis now wear graduated compression stockings on both legs under pneumatic compression boots (AV Impulse System, Novamedix, Andover) (fig 1). Their design is based on the foot's physiological pumping mechanism.[11] The boot applies pulsatile compression to the sole of the foot (fig 2). Patients receive

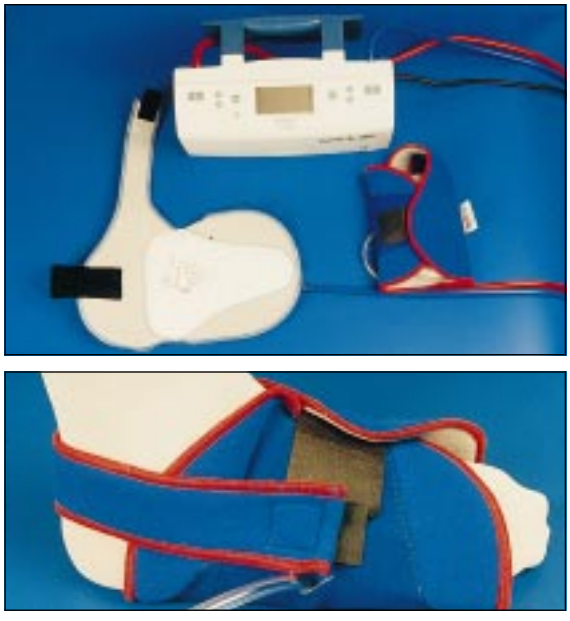

Fig 1 Pneumatic compression boot (top); as worn by patient (bottom)

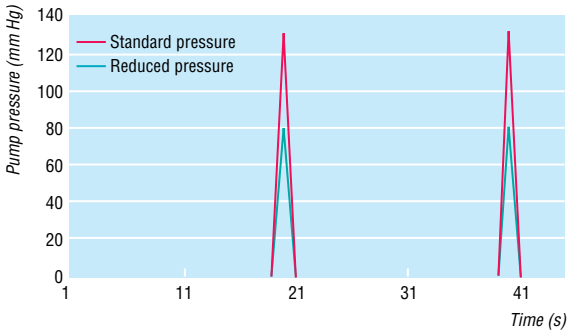

Fig 2 Inflation cycle at two pressure settings of pneumatic compression boot. Boot inflates for 1 s during every full cycle of 20 s

pneumatic compression treatment on the ward until they are fully mobile; treatment is interrupted for physiotherapy. The compression boots do not interfere with the operative site and are effective prophylaxis against deep vein thrombosis.[12–15]

The contraindications described by the manufacturer are congestive heart failure, pre-existing deep vein thrombosis, thrombophlebitis, and pulmonary embolism. The manufacturer also includes advice on care of the patient's skin and advises that extra attention should be given to patients with poor circulation, fragile skin, insensitive extremities, diabetes, and problems with tissue viability, and to those taking anticoagulants. These conditions are not regarded as contraindications.

Case review

The new protocol was implemented in December 1995 and the first problem occurred two months later. The compression boots were used on about 200 patients over the five months analysed. Five patients developed foot ulcers. These were deep and situated on patients' heels or bunions, or both. In four cases the ulcers occurred on one foot and in one case on both feet. The ulcers were not necessarily on the leg that had been operated on. All ulcers healed slowly over 3-10 months.

Causes of ulcers

We considered that the ulcers had four possible causes. Firstly, the ulcers may have been due to poor nursing care. The five patients were, however, operated on by four different consultants working in the two separate trusts with a common orthopaedic directorate. Nursing care was provided by experienced staff in three wards dedicated to elective orthopaedic surgery at the two sites.

Secondly, the patients could have had occult vascular insufficiency, but all of them had palpable foot pulses before surgery and none had a history that suggested vascular claudication. However, one patient (case 1) had a diagnosis of neurogenic claudication secondary to spinal stenosis and another (case 2) took quinine sulphate

for cramps at night. In two cases (3 and 4) postoperative angiography revealed lesions amenable to treatment. We retrospectively reviewed the ratio of ankle to brachial pressure in the other three patients. In one (case 2) it was significantly reduced at 0.55 (normal value is about 1; significant insufficiency is <0.7). Thus three patients (cases 2, 3, and 4) had vascular insufficiency that, because of poor exercise tolerance related to the problem in the joint, was not identified earlier. In cases 1 and 5 the index was inconclusive, and without further investigation vascular insufficiency cannot be proved.

Thirdly, the boots could have caused ulceration through chafing, for example. Two patients (cases 3 and 4) reported discomfort while using them. The skin looked normal on close inspection and they continued to use the boots but with more frequent checking of the skin. Postoperative confusion in two further patients (cases 1 and 5) may have reduced their ability to communicate their discomfort.

Finally, other associated diseases could have resulted in ulcers. The patients had osteoarthritis, rheumatoid arthritis, and avascular necrosis, but no other common factors could be found.

Discussion

We had had no similar postoperative problems before the introduction of the new regimen for prophylaxis against deep vein thrombosis, and we have been unable to find reports of problems with the new protocol in the published work. We believe that these five cases are not an unrelated flurry of unfortunate cases. The review of the cases has led us to conclude that the problem is multifactorial. Occult vascular insufficiency seems to be important but was not detected preoperatively because of the low demand these patients place on their blood system. In two patients (cases 3 and 4) a severely diseased vessel might have become occluded during the manipulation required for joint replacement.

Inflation in the pneumatic compression boot lasts only for 1 second in a cycle of 20 seconds. In some cases this may be sufficient for the stocking to be gradually tightened with each pulse, which may lead to a build up of pressure. This constant pressure could be much more important in the aetiology of the ulcers than the pulsatile pressures generated by the boots themselves.

In retrospect some ulcers could have been avoided by removing the compression boots at the first indication of discomfort. Medical and nursing staff now use a specially designed algorithm to aid decision making about continuing treatment with the pneumatic compression boots when patients experience discomfort (fig 3). This has prevented further cases of ulcers developing in patients.

Both medical and nursing staff have a greater awareness of the problems of occult vascular disease, and there is now a lower threshold for removing the boots and changing management strategies. Because this seems to have controlled the problem we have not implemented routine Doppler ultrasound examinations. We remain committed to this method of prophylaxis against deep vein thrombosis but will continue to monitor our results.

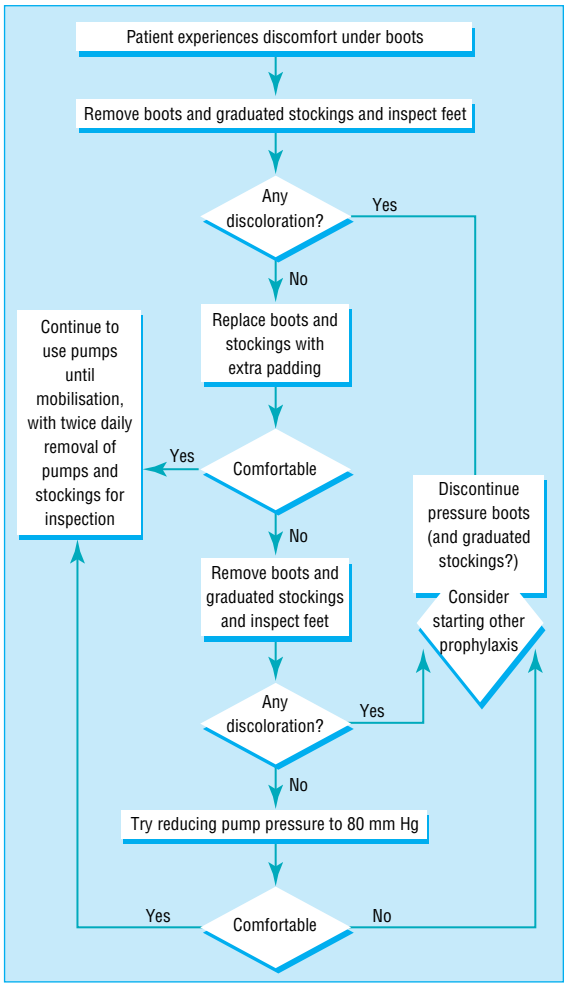

Fig 3 Algorithm used in management of patients experiencing discomfort while using pneumatic compression boots

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