Recent advances

Gastroenterology

G O Barbezat

Gastroenterology has developed rapidly as a scientific and clinical discipline. Advances have resulted from simple, painstaking clinical observations on one hand and highly sophisticated scientific methods on the other. Some of the important developments in gastroenterology are reviewed here.

Methods

Topics were selected after formal discussion with colleagues, and priority was given to areas of current interest. Reference material was chosen after a literature search in the medical library and included abstracts from proceedings of recent conferences. Attendance at conferences allowed discussions with some experts; communication in writing was made with others.

Haemochromatosis—gene mutation

Haemochromatosis usually becomes symptomatic between the ages of 40 and 70 years, by which time the liver, pancreas, heart, joints, skin, and endocrine systems may have been damaged severely and often irreversibly. Deposition of iron in vital organs may be prevented by depleting the body of iron. Venesection, common in the 18th century, remains an effective treatment for haemochromatosis today.

Hereditary haemochromatosis is a recessive single gene disorder that occurs in at least 1 in 400 white people, reflecting a very high carrier frequency of 1 in 10.¹ The problem has been to establish the diagnosis at an early stage in this common disease. A genetic key to this would not only prevent complications but allow patients to enjoy a normal life expectancy.

A 20 year search

The increased frequency of the HLA-A3 allele in patients with hereditary haemochromatosis was the first indication that the gene for this disorder might be situated near the major histocompatibility complex on chromosome 6p.² In the 20 years since that discovery, the search for the gene has been challenging. Research groups from Australia to California joined the race to discover this elusive gene.^{3,4} Wolff's group in California seem to have the answer—a novel major histocompatibility class 1-like gene mutation.⁴ They have identified a 250 kilobase region more than 3 megabases telomeric of the major histocompatibility class which is identical by descent in 85% of chromosomes from patients with this

Summary points

Genetic screening for hereditary haemochromatosis is now a reality

New treatments for Barrett's oesophagus can remove gastric and intestinal metaplasia

Helicobacter pylori can be eradicated effectively in 1 week by a regimen of drugs given twice daily

Progress is being made in developing an oral vaccine against *H pylori*

Endoscopic screening reduces mortality from colon cancer, but issues of social cost need further evaluation

Although the cause of Crohn's disease remains elusive, response to some antibiotic regimens suggests an infective link involving intestinal bacteria in susceptible people

disorder. The gene related to the class 1 family contains two missense alterations, and this has been termed HLA-H. Cys282Tyr was the most prevalent mutation (linked to a disruption of a putative β_2 microglobulin binding domain) in 85% of 356 chromosomes from 178 patients; it was found in only 5% of controls. The other substitution, His63Asp, seems to be a weaker determinant; it may only act in compound heterozygotes. In all, 87% of patients with hereditary haemochromatosis were homozygous for Cys282Tyr or compound (Tyr282/Asp63) heterozygotes.⁴ Environmental or other genetic factors may explain the disorder in the 13% of patients without this mutation

Simple tests based on polymerase chain reaction methods can now detect homozygosity for hereditary haemochromatosis. The ability to diagnose and treat this disorder before symptoms occur and complications have developed is a most important advance.

Barrett's oesophagus—a new treatment

Barrett's oesophagus is common. It occurs in about 10-20% of patients who present with gastro-oesophageal reflux. Recent debate has centred on the

Dunedin School of Medicine,
University of Otago,
PO Box 913,
Dunedin,
New Zealand
G O Barbezat,
Mary Glendinning
professor of medicine
gil.barbezat@stonebow.otago.ac.nz

BMJ 1998;316:125-8

definition of Barrett's oesophagus.⁵ The key diagnostic criterion is the finding of at least 3 cm of columnar lined oesophagus (the extent is often difficult to measure), with or without intestinal metaplasia. Where shorter segments of the lower oesophagus are affected, the diagnosis can still be made if intestinal metaplasia is present. However, the clinical importance of "short segment" Barrett's oesophagus has yet to be established. Barrett's oesophagus is regarded as a premalignant condition, particularly if it is associated with intestinal metaplasia and with ulceration. Malignancy is heralded by dysplastic changes.^{5 6}

The incidence of oesophageal adenocarcinoma has increased in the West, and attention has been focused on prevention or early treatment. Although oesophageal resection carries considerable morbidity and mortality, carcinoma has been found in an appreciable proportion of resected specimens from patients with high grade dysplasia. The efficacy of endoscopic surveillance programmes has yet to be established, and the cost (in human and human resource terms) has been the subject of much debate. Decision analysis suggests that endoscopy every 2-3 years might provide the greatest benefit in quality adjusted life expectancy.⁷ More effective management of Barrett's oesophagus would clearly be a major advance.

Developments

Modern treatments have offered some hope. While long term treatment with proton pump inhibiting drugs often relieves symptoms, controls inflammation, prevents extension of the affected area, and even restores islands of squamous epithelium in the Barrett's mucosa, no satisfactory evidence exists that this reduces the risk of malignancy. However, studies showed that ablating an affected area with an argon laser and then inhibiting acid secretion by long term treatment with proton pump inhibitor drugs led to regrowth of squamous epithelium in the affected area.^{8 9} This treatment had limitations—several treatments were required and success was not uniform. The approach was refined by using low dose photodynamic treatment incorporating a photosensitising substance, in this case a haematoporphyrin derivative.¹⁰ The haematoporphyrin derivative was injected 48 hours before laser treatment, which was given via an optical fibre. Although the activated photosensitiser destroyed dysplastic and cancer cells to which it had been bound, this treatment resulted in systemic photosensitisation, adverse side effects, and sometimes stricture formation because of deep oesophageal ulceration.

Photodynamic treatment with protoporphyrin IX

The evolution of photodynamic treatment using endogenously generated protoporphyrin IX is an exciting development.^{11 12} Endogenous photosensitisation with 5-aminolaevulinic acid given orally targets the photosensitiser directly at the dysplastic mucosa and limits systemic photosensitisation. Accumulation occurs mostly in mucosal cells of the gastrointestinal tract, and photosensitisation is limited to 24-48 hours. Although only five patients were treated, all showed squamous re-epithelialisation in treated areas, and this persisted for 26-44 months of follow up. In two patients, non-dysplastic Barrett's mucosa remained beneath the normal regenerated squamous epithelium.

No relevant side effects were reported, but patients had to be given maintenance treatment with omeprazole (40 mg daily).

Clearly, larger studies and longer follow up are needed. Whether this regimen will prevent dysplasia and cancer in the long term is not yet established, but initial evidence is encouraging.

Helicobacter pylori—continuing evolution

Since the revolutionary discovery that duodenal ulcers were related to gastric colonisation with *Helicobacter pylori*, evidence that this fascinating organism has many pathophysiological and other relationships has been accumulating.

Gastric acid secretion is important

Recent studies have shown a positive correlation between the extent of gastric metaplasia and gastric acid output. Neither the prevalence nor the extent of gastric metaplasia was affected by *H pylori* status.¹³ These findings emphasise the importance of acidity in the duodenal bulb in the development of metaplasia, and eventually ulceration, in this area. Metaplasia does not regress after *H pylori* eradication.^{14 15} The association of *H pylori* with duodenal metaplasia seems more relevant to the pathogenesis of duodenal ulcer than does the presence of metaplasia alone.

Pathological importance of subtypes of *H pylori*

Uncertainty remains over the relative roles of host and organism in explaining why some people develop peptic ulceration when infected with *H pylori* while others do not. Recent studies have determined that some strains of *H pylori*—those producing the vacuolating cytotoxin encoding gene (*vacA*)—are more likely than others to cause inflammation of the gastric antrum and, eventually, duodenal ulceration.¹⁶ The *sla* strains of *H pylori* were related to more antral inflammation than *slb* and *s2* types; and infection was associated with duodenal ulcer disease in 89% of 18 patients with the *sla* strain of *H pylori*, 29% of 14 with *slb*, and 20% of 10 with *s2*.¹⁶ Unfortunately, these differences cannot be measured serologically. Further studies are needed to improve understanding of the host-organism relation and of any diagnostic role it may have.

Treatment

Classic triple treatment is generally effective for 80%-85% of patients, but it is complex and has too many side effects. Newer, 1 week regimens using twice daily dosage of three drugs have better results and fewer side effects. These regimens include a proton pump inhibiting drug (omeprazole or lansoprazole) and two antibiotics (clarithromycin (250 mg or 500 mg), amoxycillin (1 g), or metronidazole (400 mg or 800 mg) twice daily), and eradicate *H pylori* in 90%-95% of patients.^{17 18} In one study, more than 95% of patients were still negative for *H pylori* 6 months after treatment.¹⁹ A new combination drug, ranitidine bismuth citrate (Pylorid), has shown promising results when used with clarithromycin.²⁰

Links with malignancy

Although *H pylori* is linked causally to gastric cancer, debate continues on the role (if any) of its eradication in

preventing gastric cancer. Recent studies have clearly confirmed that eradication of *H pylori* is effective treatment for mucosa associated lymphoid tissue lymphoma of the stomach. A study of 68 patients with this rare subtype of gastric lymphoma showed that histological regression can be expected but may take up to 3 years. Disappearance of monoclonal antibodies, however, occurred in only two thirds of patients within 3 years. Close observation of patients is still required, however, since recurrence of lymphoma has been recorded after reinfection with *H pylori*.²¹

Effective universal elimination generally requires successful immunisation

Progress is being made in developing oral vaccines against *H pylori*. Although these are still at the experimental animal stage, some aspects of human immunisation are being initiated.²²⁻²³ One of the most exciting findings in several animal studies is that successful oral immunisation also resulted in eradication of *H pylori*.²² Simultaneous success in immunisation and treatment may well be achievable.

Colon cancer screening—random or selective?

Cancer of the colon is common and an important cause of death in industrialised societies. Since most colon cancers develop from polyps, which can now be removed relatively easily at colonoscopy, detection of polyps or early cancers should reduce mortality. Studies have been in progress for many years to determine whether random faecal occult blood testing in the asymptomatic population can lead to earlier diagnosis of treatable cancer and save lives. Long awaited results of large “definitive” studies from Britain and Denmark have now been published.²⁴⁻²⁵ These have shown that colorectal cancer can be diagnosed at an earlier stage by faecal occult blood testing every 2 years, and that mortality from colonic cancer can be reduced. However, this reduction was modest (15%-18%), and only about 25% of cancers in the screened groups were detected at screening. Furthermore, total mortality (from all causes) was not reduced in either of these studies, and the cost for each prevented death was high £126 000. An American study of patient volunteers also showed a greater reduction in colorectal cancer mortality, but at considerably greater cost since the colonoscopy rate was nearly six times that of the European studies and faecal occult blood testing was performed yearly.²⁶ Mortality from all causes was not reduced by screening in the American study.

Gene defects

A big advance has been made in detecting the gene defect in familial adenomatous polyposis. The mutation (on 5q21) is found in everyone with familial adenomatous polyposis. Unfortunately, this cancer accounts for fewer than 0.5% of all colorectal cancers.

Patients with hereditary non-polypoid colorectal cancer syndrome (only 5%-10% of those with colorectal cancer) can also be targeted for screening.²⁷ Interesting advances have been made in identifying the gene defects in this syndrome, particularly the finding of four missense mutations, but routine testing is not yet

available. This will be an important advance for people with a relevant family history (colorectal cancer in at least three family members spanning two generations, with one or more cases diagnosed before the age of 50 years). In patients who fulfil the risk criteria, colonoscopic screening should begin at age 25 years, or 5 years earlier than the youngest age at which colorectal cancer was found in that family. The optimum screening interval is still contentious—recommendations vary from every 6 months to every 1-2 years.

The cost of screening programmes needs to be considered carefully in these days of rationing of healthcare funding. In the meantime, targeted screening, usually with colonoscopy, is still advocated for those with first degree relatives or several second degree relatives, or both, with colorectal cancer, particularly if the cancers were diagnosed before the age of 45 years.²⁸

Crohn's colitis—is it an infectious disease?

Although much is known about the physiological abnormalities in Crohn's disease and the immunological links in the inflammatory process, its precise cause remains unknown. An infectious origin has long been suspected but has not yet been proved. Viruses have come under scrutiny, and recent attention has been focused on the measles virus as a possible pathogenetic agent.²⁹ However, studies using validated, sensitive molecular biology methods have found no evidence of measles genomic material in Crohn's disease tissues.³⁰

Crohn's disease and *Mycobacterium paratuberculosis*

The close resemblance to tuberculosis in humans and Johne's disease in sheep and goats is striking, but reproducible acid fast bacilli have not been found in affected human tissues.³¹ Many thorough clinical trials of antimycobacterial treatment have failed to show any effect.³²

The relevance of detecting mycobacterial fragments in Crohn's tissues using molecular biology techniques such as the polymer chain reaction is controversial. Recent studies have focused on *Mycobacterium paratuberculosis*. A strong relation has been found between a DNA sequence specific to *M paratuberculosis* (IS900) and Crohn's tissue, at least in

Targeted screening, usually with colonoscopy, is still advocated for people with a familial risk of colorectal cancer

ST BARTHOLOMEWS HOSPITAL/SPL

some clinical manifestations of the disease.³³ This sequence has also been found in whole pasteurised cows' milk in England and Wales, confirming its prevalence in the community.³⁴ In one Japanese study, the IS900 sequence was detected in all the 10 patients with Crohn's disease, but it was also detected in 61% of eight patients with ulcerative colitis and 88% of 16 control subjects.³⁵ Another study from Leeds failed to detect any such DNA fragments in their patients with Crohn's disease.³⁶ Perhaps various forms of Crohn's disease exist, or previous clinical trials of antimycobacterial treatment may have concentrated on the wrong spectrum of antibiotics.

Treatment with clarithromycin

Treatment directed more specifically at *M paratuberculosis* has produced encouraging results. A small controlled trial showed that five of seven patients treated with clarithromycin (shown to be effective against *M paratuberculosis*) achieved clinical remission in 3 months, while the indices of clinical activity were considerably improved in the remainder. Only one of the eight controls responded. Furthermore, 1 year after the study, all those who achieved remission remained in remission.³⁷ Two further studies of open treatment with clarithromycin have shown promising results.^{38, 39} In a British study there were statistically significant improvements in ability to stop corticosteroid treatment (17 of 19 patients), disease activity index, measures of inflammation, and serum albumin concentrations. Furthermore, the results seemed to be sustained, and were more striking in severely affected patients.³⁹

Extension of existing studies and new randomised double blind trials are clearly indicated, as are studies to link *M paratuberculosis* or other bacteria with the wealth of immunologically related data already available. Other luminal antigens of endogenous or exogenous bacterial origin may also be trigger factors for the immunological cascade that has now been clearly shown in Crohn's disease. Immunocytochemical evidence of listeria, *Escherichia coli*, and streptococcus antigens has also been found in Crohn's tissue.⁴⁰ These studies do not yet explain the aetiology of Crohn's disease but may be important in directing attention to a new approach to treatment—appropriate antibiotic drugs.

Funding: None.

Conflict of interest: None.

- Haemochromatosis ... definite maybe! *Nature Genetics* 1995;13:375-6.
- Simon M, Bourel M, Fauchet R, Genetet B. Association of HLA-A3 and HLA-B14 antigens with idiopathic haemochromatosis. *Gut* 1976; 17:332-4.
- Burt MJ, Smit DJ, Pyper WR, Powell LW, Jazwinska EC. Megabase YAC contig and physical map over the haemochromatosis gene region. *Genomics* 1996;33:153-8.
- Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nature Genetics* 1996;13:399-408.
- Spechler SJ, Goyal RK. The columnar-lined esophagus, intestinal metaplasia, and Norman Barrett. *Gastroenterology* 1966;110:614-21.
- Van der Burgh A, Dees J, Hop WCJ, van Blankenstein M. Oesophageal cancer is an uncommon cause of death in patients with Barrett's oesophagus. *Gut* 1996;39:5-8.
- Provenzale D, Kemp JA, Arora S, Wong JB. A guide for surveillance of patients with Barrett's esophagus. *Am J Gastroenterol* 1994;89:670-80.
- Berenson MM, Johnson TD, Markowitz NR, Buchi KN, Samowitz WS. Restoration of squamous mucosa after ablation of Barrett's esophageal epithelium. *Gastroenterology* 1993;104:1686-91.
- Spechler JJ. Laser photoablation of Barrett's epithelium: burning issues about burning tissues. *Gastroenterology* 1993;104:1855-8.
- Overholt BF, Panjehpour M. Barrett's esophagus: photodynamic therapy for ablation of dysplasia, reduction of specialised mucosa, and treatment of superficial esophageal cancer. *Gastrointest Endosc* 1995;42:64-70.

- Regula J, MacRobert AJ, Gorchein A, Buonaccorsi GA, Thorpe SM, Spencer GM, et al. Photosensitisation and photodynamic therapy of oesophageal, duodenal, and colorectal tumours using 5 aminolevulinic acid induced protoporphyrin IX—a pilot study. *Gut* 1995;36:67-75.
- Barr H, Shepherd NA, Dix A, Roberts DJH, Tan WC, Krasner N. Eradication of high-grade dysplasia in columnar-lined (Barrett's) oesophagus by photodynamic therapy with endogenously generated protoporphyrin IX. *Lancet* 1996;348:584-5.
- Harris AW, Gummert PA, Walker MM, Misiewicz JJ, Baron JH. Relation between gastric acid output, *Helicobacter pylori*, and gastric metaplasia in the duodenal bulb. *Gut* 1996;39:513-20.
- Noach LA, Rolf TM, Bosma NB, Schwartz MP, Oosting J, Rauwe AJ, Tytgat GNJ. Gastric metaplasia and *Helicobacter pylori* infection. *Gut* 1993;34:1510-4.
- Fossati D, Alvisi C, Frego R, Perego M, Luinetti O, Fiocca R. No change in intestinal metaplasia after H pylori eradication. *Gut* 1996;39(suppl 2):A104.
- Atherton JC, Peek RM, Tham KT, Cover TL, Blaser MJ. Clinical and pathological importance of heterogeneity in vacA, the vacuolating cytotoxin gene of *Helicobacter pylori*. *Gastroenterology* 1997;112:92-9.
- Bazzoli F, Zagari RM, Fossi S, Pozzato P, Alampi G, Sottili AS, et al. Short-term low-dose triple therapy for the eradication of *Helicobacter pylori*: a randomised, double-blind, controlled study. *Gut* 1996;39(suppl 2):A33.
- Misiewicz JJ, Harris AW, Bardhan KD, Levi S, Langworthy H. One week low dose H pylori eradication therapy heals 90% of duodenal ulcers. *Gut* 1996;39(suppl 2):A34.
- Misiewicz JJ, Harris AW, Bardhan KD, Levi S, Langworthy H. Over 95% of patients remain H pylori negative 6 months after one week low-dose eradication therapy. *Gut* 1996;39(suppl 2):A34.
- Axon ATR, Ireland A, Lancaster Smith MJ, Rooprams PD. Ranitidine bismuth citrate and clarithromycin twice daily in the eradication of *Helicobacter pylori*. *Aliment Pharmacol Ther* 1997;11:81-7.
- Franzin G, Zamboni G, Savio A, Scarpa A, Capelli P, Mombello A, et al. Gastric MALT low-grade lymphoma: follow-up study after eradication of *Helicobacter pylori*. *Gastroenterology* 1996;110:A109.
- Corthesy-Theulaz I, Porta N, Glauser M, Saraga E, Vaney AC, Haas R, et al. Oral immunisation with *Helicobacter pylori* urease B subunit as a treatment against *helicobacter* infection in mice. *Gastroenterology* 1995;109:115-21.
- Kreiss C, Budin T, Cosma M, Biollaz J, Corthesy-Theulaz I, Porta N, et al. Safety of oral immunisation with recombinant urease in H pylori infected humans. *Gastroenterology* 1996;110:A162.
- Hardcastle JD, Chamberlain JO, Robinson MHE, Moss SM, Amar SS, Balfour TW, et al. Randomised controlled trial of faecal-occult-blood screening for colorectal cancer. *Lancet* 1996;348:1472-7.
- Kronborg O, Fenger C, Olsen J, Jorgensen OD, Sondergaard O. Randomised study of screening for colorectal cancer with faecal-occult-blood test. *Lancet* 1996;348:1467-71.
- Mandel JS, Bond JH, Church JR, Snover DC, Bradley GM, Schuman LM, et al. Reducing mortality from colorectal cancer by screening for faecal occult blood. *N Engl J Med* 1993;328:1365-71.
- Lynch HT, Smyrk TC, Watson P, Lanspa SJ, Lynch JF, Lynch PM, et al. Genetics, natural history, tumor spectrum and pathology of hereditary nonpolyposis colorectal cancer: an updated review. *Gastroenterology* 1993;104:1535-49.
- Mulcahy HE, Farthing MJG, O'Donoghue DP. Screening for asymptomatic colorectal cancer. *BMJ* 1997;314:285-91.
- Wakefield AT, Ekblom A, Dhillon AR, Pittilo RM, Pounder RE. Crohn's disease: pathogenesis and persistent measles virus infection. *Gastroenterology* 1995;108:911-6.
- Chadwick N, Bruce I, Davies M, Pounder R, Wakefield A. Hybrid capture followed by RT-PCR does not detect measles RNA in intestinal tissue. *Gastroenterology* 1997;112:A946.
- Morgan KL. John's and Crohn's: chronic inflammatory bowel diseases of infectious aetiology? *Lancet* 1987;ii:1017-9.
- Swift GL, Srivastava ED, Stone R, Pullan RD, Newcombe RG, Rhodes J, et al. Controlled trial of anti-tuberculous chemotherapy for two years in Crohn's disease. *Gut* 1994;35:363-8.
- Mishina D, Katsel P, Brown ST, Gilberts ECAM, Greenstein RJ. On the etiology of Crohn disease. *Proc Natl Acad Sci USA* 1996;93:9816-20.
- Millar D, Ford J, Sanderson J, Withey S, Tizard M, Doran, et al. IS900 PCR to detect *Mycobacterium paratuberculosis* in retail supplies of whole pasteurised cows' milk in England and Wales. *Appl Environ Microbiol* 1996;62:3446-52.
- Suenaga K, Yokoyama Y, Okazaki K, Yamamoto Y. *Mycobacteria* in the intestine of Japanese patients with inflammatory bowel disease. *Am J Gastroenterol* 1995;90:76-80.
- Rowbotham DS, Mapstone NP, Trejdosiewicz LK, Howdle PD, Quirke P. *Mycobacterium paratuberculosis* DNA not detected in Crohn's disease tissue by fluorescent polymerase chain reaction. *Gut* 1995;37:660-7.
- Graham DY, Al-Assi MT, Robinson M. Prolonged remission in Crohn's disease following therapy for *Mycobacterium paratuberculosis* infection. *Gastroenterology* 1995;108:A826.
- Rubin PH, Chapman ML, Scherl E, Sachar DB, Stamaty C, Present DH. Clarithromycin in active Crohn's disease (CD): preliminary results of open label pilot study. *Gastroenterology* 1996;110:A1005.
- Gui GPH, Thomas PRS, Tizard MLV, Lake J, Sanderson JD, Hermon-Taylor J. Two-year-outcomes analysis of Crohn's disease treated with rifabutin and macrolide antibiotics. *Br Soc Antimicrob Chemother* 1997;39:393-400.
- Liu Y, van Kruiningen J, West AB, Cartun RW, Cortot A, Columbel J-F. Immunocytochemical evidence of listeria, *Escherichia coli*, and streptococcus antigens in Crohn's disease. *Gastroenterology* 1995;108:1396-404.

(Accepted 28 August 1997)

Statistics Notes

Weighted comparison of means

J Martin Bland, Sally M Kerry

In a recent Statistics Note¹ we referred to a weighted two sample *t* test. Here we describe how it is done. The data were the percentage of requests from general practitioners for x ray examinations which were judged appropriate (table 1), where general practitioners had been randomised to intervention or control groups.²

If we compare the two sets of percentages by the usual two sample *t* method, each observation (practice) has an equal impact on the result. As some practices contributed fewer requests than others, we wish these practices to have a lesser effect on the estimate of the difference. We can do this by weighting the practices by the number of requests.

To calculate the mean percentage in each group, we simply add the observations together and divide by the number of observations. To calculate the weighted mean, we multiply each observation by the weight, add, then divide by the sum of the weights:

$$\frac{20 \times 100 + 7 \times 100 + 16 \times 94 + \dots + 18 \times 56}{20 + 7 + 16 + \dots + 18} = \frac{34\,105}{429} = 79.50$$

If the weights are all the same this gives the usual, unweighted, mean. Note that the weighted mean, 79.50, is not the same as the unweighted mean in the table, 81.6. There is a slight (but not significant) tendency for general practitioners who make more referrals to have a lower proportion conforming to the guidelines, which explains this. For the second group the weighted mean is $51050/704 = 72.51$.

The weighted standard deviation is found in a similar way. Firstly, we need a weighted sum of squares. To calculate an unweighted sum of squares about the mean, we square and add the observations, then we subtract a correction term, the number of observations times the mean squared. Here we calculate the weighted sum of the observations squared, then subtract the number of observations times the weighted mean squared. For the first group, the weighted sum of the observations squared is:

$$\frac{20 \times 100^2 + 7 \times 100^2 + 16 \times 94^2 + \dots + 18 \times 56^2}{(20 + 7 + 16 + \dots + 18)/17} = \frac{2755709}{429/17} = 109\,200.59$$

To get the weighted mean we divided by the sum of the weights; to get a weighted sum we divide by the mean of the weights.) To get the sum of squares about the mean we subtract the correction term, $17 \times 79.50^2 = 107\,444.25$, giving $109\,200.59 - 107\,444.25 = 1756.34$. Dividing this by the degrees of freedom, $17 - 1 = 16$, gives the weighted estimate of the variance, $1756.34/16 = 109.77$, and the square root is the standard deviation, $\sqrt{109.77} = 10.48$. For the second group, the weighted sum of the observations squared is $3\,751\,934/(704/17) = 90\,600.68$ and the sum of squares about the mean is $90\,600.68 - 17 \times 72.51^2 =$

Table 1 Number of requests conforming to guidelines for each practice in the intervention and control groups

Practices	Intervention group		Control group	
	No of requests	% Conforming	No of requests	% Conforming
1	20	100	7	100
2	7	100	37	89
3	16	94	38	84
4	31	90	28	82
5	20	90	20	80
6	24	88	19	79
7	7	86	9	78
8	6	83	25	76
9	30	83	120	75
10	66	80	88	73
11	5	80	22	68
12	43	77	76	68
13	43	74	21	67
14	23	70	126	66
15	64	69	22	64
16	6	67	34	62
17	18	56	10	40
Total	429		702	
Mean (SD)		81.6 (11.9)		73.6 (13.1)

1219.78. Hence the estimated variance is $1219.78/(17-1) = 76.24$ and the standard deviation $\sqrt{76.24} = 8.73$.

We find the pooled sum of squares by adding the sums of squares within the two groups, $1756.34 + 1219.78 = 2976.12$ and the common variance estimate for the two groups by dividing by the combined degrees of freedom, $2976.12/(17 + 17 - 2) = 93.00$. We can now use the weighted estimates of the means and common variance in the usual two sample *t* formulas. The standard error of the difference between the means is $\sqrt{93.00 \times (1/17 + 1/17)} = 3.31$ and the difference is $79.50 - 72.51 = 6.99$. The 95% confidence interval is therefore $6.99 - 2.04 \times 3.31 = 0.2$ to $6.99 + 2.04 \times 3.31 = 13.7$. The test of significance is $t = 6.99/3.31 = 2.11$. With 32 degrees of freedom this gives $P = 0.04$.

For comparison, the unweighted difference is 8.00 and the pooled variance estimate is 157.81. The standard error of the difference is $\sqrt{157.81 \times (1/17 + 1/17)} = 4.3$. The 95% confidence interval is -1 to 17 percentage points. In this example the number of requests varies greatly between practices. This must lead to some deviation from the assumptions of the *t* test, as the variance will not be uniform. The weighted analysis meets the assumptions better and produces a worthwhile reduction in the size of the confidence interval. Some statistical software will do these calculations very simply. The same basic principle is used in meta-analysis to combine studies of varying size.

1 Kerry SM, Bland JM. Statistics Notes: analysis of a trial randomised in clusters. *BMJ* 1997;316:54.

2 Oakeshott P, Kerry SM, Williams JE. Randomised controlled trial of the effect of the Royal College of Radiologists' guidelines on general practitioners' referral for radiographic examination. *Br J Gen Pract* 1994;44:197-200.

Department of Public Health Sciences, St George's Hospital Medical School, London SW17 0RE

J Martin Bland, professor of medical statistics

Division of General Practice and Primary Care, St George's Hospital Medical School, London SW17 0RE

Sally M Kerry, lecturer in medical statistics

Correspondence to: Professor Bland.

BMJ 1998;316:129