Population based study of prevalence of islet cell autoantibodies in monozygotic and dizygotic Danish twin pairs with insulin dependent diabetes mellitus

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Abstract

Objective: To study the comparative importance of environment and genes in the development of islet cell autoimmunity associated with insulin dependent diabetes mellitus.
Design: Population based study of diabetic twins.
Setting: Danish population.
Subjects: 18 monozygotic and 36 dizygotic twin pairs with one or both partners having insulin dependent diabetes.
Main outcome measures: Presence of islet cell antibodies, insulin autoantibodies, and autoantibodies to glutamic acid decarboxylase (GAD65) in serum samples from twin pairs 10 years (range 0-30 years) and 9.5 years (2-30 years) after onset of disease.
Results: In those with diabetes the prevalence of islet cell antibodies, insulin autoantibodies, and autoantibodies to glutamic acid decarboxylase in the 26 monozygotic twins was 38%, 85%, and 92%, respectively, and in the dizygotic twins was 57%, 70%, and 57%, respectively. In those without diabetes the proportions were 20%, 50%, and 40% in the 10 monozygotic twins and 26%, 49%, and 40% in the 35 dizygotic twins.
Conclusion: There is no difference between the prevalence of islet cell autoantibodies in dizygotic and monozygotic twins without diabetes, suggesting that islet cell autoimmunity is environmentally rather than genetically determined. Furthermore, the prevalence of islet cell antibodies was higher in the non-diabetic twins than in other first degree relatives of patients with insulin dependent diabetes. This implies that the prenatal or early postnatal period during which twins are exposed to the same environment, in contrast with that experienced by first degree relatives, is of aetiological importance.

Introduction

Much is known about the autoimmune phenomena associated with insulin dependent diabetes mellitus, but little is known about the nature or the time of initiating aetiological events. The crude concordance rates of insulin dependent diabetes are no higher than 23-53% in monozygotic twin pairs,[1-4] indicating that exogenous factors are important in the pathogenesis of the disease. Such putative exogenous factors could have considerable impact during fetal life, when the immune system is immature and tolerance to various antigens is induced.[5] Several findings suggest that exposure to a virus during fetal life may stimulate the development of islet cell autoimmunity.[6-8] Thus, the rubella embryopathy syndrome is associated with an increased risk for later development of insulin dependent diabetes, and maternal enteroviral infection during pregnancy is also a risk factor for development of childhood insulin dependent diabetes.[8] The risk of developing insulin dependent diabetes later in life also seems to be influenced by diet in infancy, especially early exposure to cows' milk products.[9-11] Several environmental factors present early in life thus seem to be potentially associated with an increased risk of developing insulin dependent diabetes.

Studies in first degree relatives of patients with insulin dependent diabetes have shown that islet cell antibodies—that is, insulin autoantibodies, islet cell antibodies, and glutamic acid decarboxylase autoantibodies—can be detected several years before clinical insulin dependent diabetes,[12-16] suggesting a long disease prodrome. Patients become positive for islet cell antibodies before 5 years of age in 85% of cases,[17] suggesting that the initial lesion could occur early in life.

To investigate the contribution of environmental and genetic factors we analysed the presence and concentration of the three antibodies in a Danish population based cohort of dizygotic and monozygotic twins in which at least one twin had insulin dependent diabetes.

Patients and methods

Twin cohort

The diabetic twin pairs were identified through the population based Danish twin register. This cohort consists of 20 888 twin pairs born from 1 January 1953 to 31 December 1982, representing 74.4% of all twin pairs born in Denmark during 1953-67 and 97.4% of those born during 1968-82.[18]

The diabetic twin pairs (table 1) were identified by means of a questionnaire to the total cohort, with an

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Table 1 Characteristics of twin pairs investigated for presence of autoantibodies associated with insulin dependent diabetes

Detail	Monozygotic twins	Dizygotic twins
No of pairs	18	36
No of female pairs	6	7
No of male pairs	12	9
No of opposite sexed pairs	-	19
No of concordant pairs for diabetes	8	1
No of discordant pairs for diabetes	10	35
Mean (range) age at diabetes diagnosis (years)	18.3 (2-38)	15.1 (1-35)
Mean (range) duration of diabetes (years)	10.0 (0-30)	9.5 (2-30)

individual response rate of 92.6%, and through record linkage with the Danish register of causes of death. The total number of twin pairs in which one or both partner had insulin dependent diabetes according to criteria of the World Health Organisation was 95: 26 monozygotic and 69 dizygotic pairs. The cumulative concordance, which is comparable with risk of recurrence in other relatives, estimated by life table analysis from birth to age 40 was 70% in monozygotic and 13% in dizygotic twins.[1] A total of 54 pairs (18 monozygotic and 36 dizygotic) were available for detailed studies; the remainder did not participate because of death or emigration of one of the twins, disability in the diabetic twin, or unwillingness to participate. The absence of diabetes in the unaffected twin was confirmed by means of an oral glucose tolerance test performed immediately before or after the clinical examination.[1]

For the twin pairs participating in the clinical investigation, zygosity was established by serological analysis of 11 blood and enzyme type systems by using the same approach as in paternity testing. Twin pairs with complete concordance for all systems were regarded as monozygotic; the incidence of misclassification is below 1% for dizygotic twin pairs.[19]

Glutamic acid decarboxylase autoantibodies

Glutamic acid decarboxylase antibodies were measured as previously described.[20] Briefly, the human islet glutamic acid decarboxylase cDNA[21 22] was transcribed and translated in vitro according to the manufacturer's instructions (Promega, Madison, United States) in the presence of methionine labelled with sulphur-35 (Amersham). Serum samples were incubated with the tracer overnight at 4°C, with or without the addition of 1 µg of unlabelled, affinity purified, recombinant human glutamic acid decarboxylase as competitor. The immune complexes were isolated on protein A Sepharose (Pharmacia, Sweden), and the amount of immunoprecipitated glutamic acid decarboxylase was measured by scintillation counting. All samples were tested in duplicate. Concentrations of glutamic acid decarboxylase antibody were expressed as index values: (cpm of sample – cpm of negative control sample)/(cpm of positive control sample – cpm of negative control sample), where cpm is counts per minute.

Serum samples were regarded as positive when index values exceeded the mean plus 3 SD of glutamic acid decarboxylase antibody indices in 50 healthy controls (data not shown).

Islet cell antibodies

Undiluted serum samples were screened for conventional IgG islet cell antibody by means of indirect immunofluorescence on 4 µm cryostat sections of blood group O human pancreas.[14] Positive samples were then titred by doubling dilutions in phosphate buffered saline on tissue obtained from a single pancreas under standard incubation conditions.[23] Local standard serum samples calibrated to 2, 4, 8, 16, 32, and 80 JDF units were included in each assay. End point titres were converted to JDF units, which are based on a standard curve of known serum samples from patients with insulin dependent diabetes mellitus who are positive for islet cell antibodies. The threshold of islet cell antibody detection was 4 JDF units; thus values reported as 0 indicate <5 JDF units.

Insulin autoantibodies

Insulin autoantibodies were assayed as previously described.[14] Serum samples were extracted by using acid washed, dextran coated charcoal to remove endogenous insulin; 80 µl of serum was then incubated for 48 hours at 4°C with 80 µl of 40 mmol/1 phosphate buffer and 5.3×10^{-3} pmol radiolabelled human insulin (specific activity 2000 Ci/mmol; Amersham), with and without excess (2.55 pmol/tube) non-labelled insulin (Actrapid, Novo-Nordisk, Bagsvaerd, Denmark). The immunoglobulin fraction was precipitated with polyethylene glycol 6000 (12.9% weight/volume) and washed. The specific binding was calculated by subtracting the counts in the presence of cold insulin from the counts without the cold insulin. Results were expressed as percentage displaced binding. Subjects were classified as positive for insulin autoantibody if the corrected binding was >3SD above the mean of 172 adult blood donors.

Statistical analysis

Statistical analyses included the Mann-Whitney, χ^2, and Fisher's exact tests; and 5% was chosen as the level of significance.

Results

Antibodies in monozygotic and dizygotic diabetic twins

Of the individual diabetic monozygotic twins 85% (22/26) were positive for insulin autoantibodies, 38% (10/26) were positive for islet cell antibodies, and 92% (24/26) were positive for glutamic acid decarboxylase autoantibodies, comparable with prevalences found in individual diabetic dizygotic twins—that is, 70% (26/37), 57% (21/37), and 60% (21/37), respectively (table 2 and figure 1).

The level of insulin autoantibodies in both the diabetic monozygotic and dizygotic twins was, as expected, high—median 4.4 (95% confidence interval 4.4 to 13.7) and 3.9 (6.2 to 19.0) units, respectively—as all had been treated with insulin, sometimes for many years (fig 1). The levels of islet cell antibodies and glutamic acid decarboxylase autoantibodies were also comparable between the diabetic monozygotic and dizygotic twins—that is, the median titre was 13.5 (6.0 to 49.1) and 7 (7.6 to 29.0) JDF units, respectively, and the median glutamic acid decarboxylase autoantibody titre

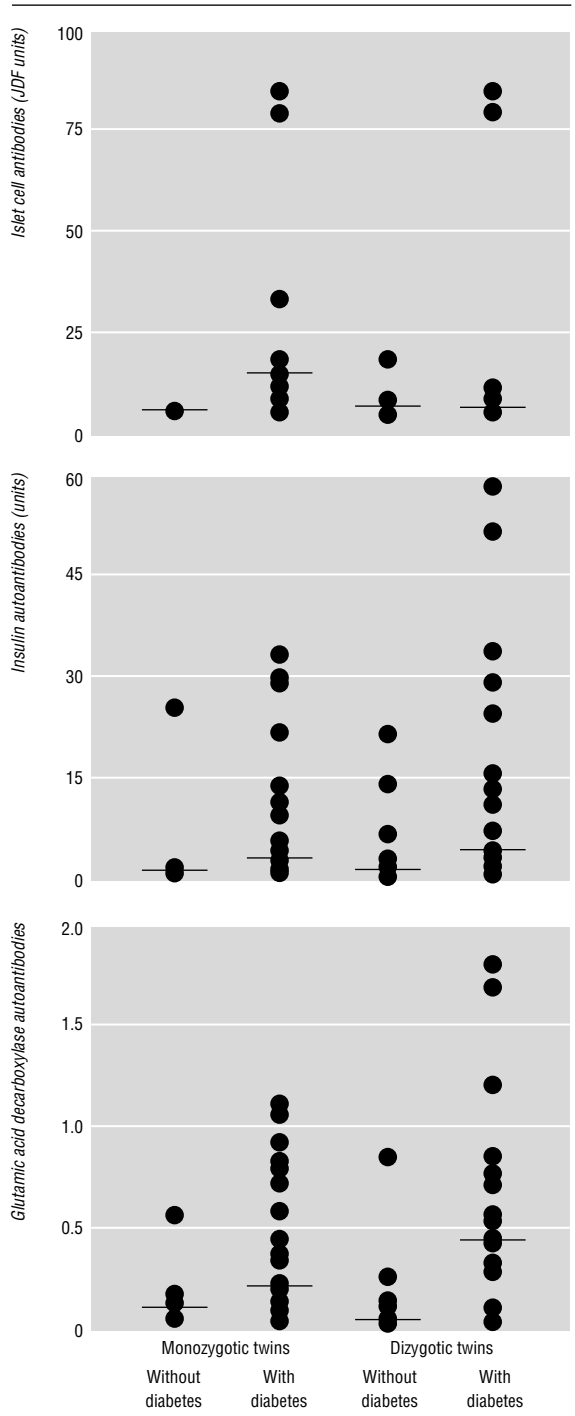

Fig 1 Levels of islet cell antibodies, insulin autoantibodies, and glutamic acid decarboxylase autoantibodies in monozygotic and dizygotic twins with or without insulin dependent diabetes. Solid line shows median of values in each group. Only twins who screened positive for autoantibodies are shown. Difference in levels of glutamic acid decarboxylase autoantibodies in dizygotic twins with and without diabetes was significant at P=0.004

was 0.22 (0.07 to 0.23) and 0.47 (0.11 to 0.33), respectively (fig l).

Antibodies in monozygotic and dizygotic non-diabetic twins

Of the non-diabetic monozygotic twins, 5 out of 10 were positive for insulin autoantibodies, 2 out of 10 were positive for islet cell antibodies, and 4 out of 10 were positive for glutamic acid decarboxylase autoantibodies (table 2 and fig 1). Similar high prevalences were also found in the non-diabetic dizygotic twins—that is, 17 out of 35, 9 out of 35, and 14 out of 35, respectively (table 2 and fig 1).

Table 2 Prevalences of islet cell, insulin, and glutamic acid decarboxylase antibodies in dizygotic and monozygotic twins with or without diabetes. Values are numbers (percentages) of subjects who screened positive for each antibody

	Islet cell antibody	Insulin autoantibody	Glutamic acid decarboxylase autoantibody
Monozygotic twins:			
With diabetes (n=26)	10 (38)	22 (85)	24 (92)
Without diabetes (n=10)	2 (20)	5 (50)	4 (40)
Dizygotic twins:			
With diabetes (n=37)	21 (57)	26 (70)	21 (57)
Without diabetes (n=35)	9 (26)	17 (49)	14 (40)

The levels of the three antibodies in non-diabetic monozygotic and dizygotic twins were also similar—that is, the median concentrations of insulin autoantibodies were 1.30 (–7.44 to 19.80) and 1.28 (1.23 to 7.71) units; median islet cell titres were 6.5 (0.14 to 12.85) and 7 (4.56 to 12.19); and median levels of glutamic acid decarboxylase autoantibody were 0.15 (–0.14 to 0.60) and 0.06 (0.1 to 0.26), respectively (fig 1).

Combinations of the three antibodies in monozygotic and dizygotic twins

The presence of more than one kind of islet cell autoantibody was more common in the diabetic monozygotic and dizygotic twins than in non-diabetic twins (fig 2). Both glutamic acid decarboxylase and islet cell antibodies were present in 38% (14/37) and 38% (10/26) in the diabetic twins, respectively, compared with 12% (4/35) (P=0.014) and 10% (1/10) (P=0.13) in non-diabetic twins (fig 2). In non-diabetic twins 31% (11/35) and 40% (4/10), respectively, were positive for more than one autoantibody (fig 2). A similar calculation for the diabetic twins was not done because they had been treated with insulin for many years. Overall, 77% (27/35) and 70% (7/10) of the non-diabetic dizygotic and monozygotic twins were positive for at least one type of islet cell autoantibodies, respectively, compared with 87% (32/37) (P=0.367) and 100% (26/26) (P=0.0168) of the diabetic dizygotic and monozygotic twins, respectively (fig 2).

Discussion

Twin studies are a powerful tool to investigate the environmental and genetic contribution to the development of islet cell autoimmunity. Most twin studies conducted to date comprise mostly monozygotic twin pairs and have not been population based.[2-4] Furthermore, non-population based studies are subject to potential selection bias in terms of clinical symptoms or pairwise concordance. In contrast, the twins in this study were ascertained from a population based twin register and independently of zygosity and diabetes status in the twin partner.[1] Selection bias should thus have been avoided, and this is supported by the fact that the distribution of the monozygotic and dizygotic twin pairs was as expected within the general population.[1]

In the general population the prevalence of autoantibodies to islet cell antoantigens is only a low percentage[20 24]—for instance, the prevalences in a

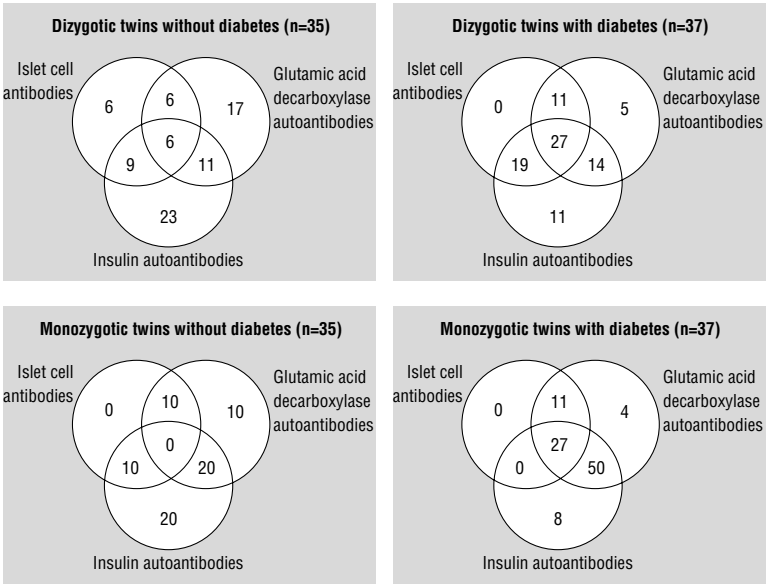

Fig 2 Concordance (%) of islet cell antibodies, insulin autoantibodies, and glutamic acid decarboxylase autoantibodies in monozygotic and dizygotic twins with or without insulin dependent diabetes. Each circle represent prevalence (%) of three types of autoantibodies; shared parts between circles represent prevalence (%) of subjects positive for two or three antibodies

recent study that analysed a large number of children (n = 415) were 4%, 3%, and 4% for islet cell antibodies, insulin autoantibodies, and glutamic acid decarboxylase autoantibodies, respectively.[24] In this study, however, as many as 70% (7/10) of the non-diabetic monozygotic twins had at least one of the three autoantibodies (fig 2), despite a rather long onset of disease in the affected twin, thus confirming the results from a recent report.[4] In this study 11 monozygotic twins discordant for insulin dependent diabetes (discordance for 8-39 years) with normal β cell function were analysed for islet cell autoantibodies—that is, islet cell antibodies, insulin autoantibodies, glutamic acid decarboxylase autoantibodies, and ICA512. Thirty six per cent were persistently positive for one or more autoantibodies in addition to 27% being transiently positive, thus overall 63% showed sign of islet cell autoimmunity. The prevalence of autoantibodies is thus much higher than that in first degree relatives of patients with insulin dependent diabetes, in whom these autoantibodies are found in prevalences of about 5%[12-16]; even combined they will be less than 15%,[16] suggesting that the presence of islet cell autoimmunity might be genetically determined. Surprisingly, our new finding that the non-diabetic dizygotic twins have a similar high prevalence of islet cell autoantibodies (77% (27/35), fig 2) indicates that the presence of islet cell autoimmunity is probably determined by environmental rather than genetic factors. The observation that the presence of antibodies is high in unaffected twin partners and independent of zygosity suggests that shared environment rather than shared genes initiates autoimmunity. Accordingly, the period over which the twin partners within a pair are equally exposed to the same environment—that is, during fetal life or soon after birth—may be very important for the subsequent risk of developing insulin dependent diabetes.

Environmental exposure leading to islet cell autoimmunity can take place during fetal life or soon after birth. If the environmental exposure takes place after birth a negative correlation would be expected between the presence of islet cell autoantibodies and the time between the delivery of siblings who develop diabetes and their non-affected siblings. Thus, the longer the siblings are exposed to the same environment (because of a short time interval between deliveries) the higher the prevalence of autoantibodies. In a large study of first degree relatives of patients with insulin dependent diabetes (n = 752) less than 15% were positive for islet cell autoantibodies, but there was no correlation between the time interval between deliveries and the presence of these autoantibodies (M Knip, personal communication). This might suggest that the period of shared environment after birth is less important for the development of islet cell autoimmunity and that the events resulting in the high prevalence of islet cell autoantibodies in both diabetic and non-diabetic twins probably take place during fetal life. Not only is the prevalence of islet cell autoantibodies higher in the non-diabetic dizygotic twins than in first degree relatives of diabetic patients[12-16] but the cumulative concordance or recurrence risk of insulin dependent diabetes up to the age of 40 years in dizygotic twin pairs is twice as high as in ordinary first degree relatives of patients up to the same age.[24] The prevalence of islet cell autoantibodies is thus much higher than the prevalence of insulin dependent diabetes,[17 20 21 25-27] indicating that islet cell autoimmunity may occur without progression to clinical diabetes. We suggest that a continuum of events is required for islet cell autoimmunity to progress to β cell destruction and clinical insulin dependent diabetes, such that a non-pathogenic immune response against islet cell antigens could be promoted by, for example, a viral infection, to a more pathogenic cellular immune response against islet cell antigens. This speculation is supported in humans by epidemiological studies showing that viral infections are correlated to subsequent development of insulin dependent diabetes.[28 29] As the initiation of autoimmunity seems to be environmentally determined, promotion of the autoimmune process leading to clinical insulin dependent diabetes must be genetically controlled to account for the well established strong associations with markers from the HLA complex and other loci.

It should be noted that, although the non-diabetic twins had a high prevalence of islet cell autoimmunity, the levels were generally lower than in the diabetic twins (fig 1). Combinations of more than one autoantibody—for instance, glutamic acid decarboxylase autoantibodies and islet cell antibodies—were also more common in twins with insulin dependent diabetes than in those without (fig 2), an observation similar to that in studies in siblings, in which the presence of multiple autoantibodies greatly increases the risk of developing insulin dependent diabetes.[14 15]

We conclude that the development of autoantibodies is determined by environmental rather than genetic factors. Furthermore, the higher prevalence of autoantibodies in non-diabetic monozygotic and dizygotic twins compared with other first degree relatives of patients with insulin dependent diabetes suggests that the period over which the twins are exposed to the

Key messages

- Autoantibodies against several islet cell antigens greatly increase the risk of developing insulin dependent diabetes
- Development of islet cell autoimmunity is determined by enviromental rather than genetic factors
- Fetal life is aetiologically important for induction of islet cell autoimmunity
- Progression to clinical insulin dependent diabetes depends on genetically controlled responses to enviromental exposures after fetal life

same environment—that is, during fetal life—is aetiologically important for induction of islet cell autoimmunity. Progression to clinical insulin dependent diabetes must, however, depend on genetically controlled responses to further exposures.

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Conflict of interest: None.

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One hundred years ago

Unqualified practice and the Medical Acts

The encroachment of chemists into the area of medical practice is a matter which is continually being commented upon in the columns of the *British Medical Journal*, but this special form of irregular practice is by no means the only one which imperatively calls for legislation in the shape of an amended Medical Act.

We have lately had our attention called to a circular issued from a nurse residing at one of the health resorts, in which it is announced that the establishment which she conducts is for the "treatment of gout, rheumatism, sciatica, lumbago, neuralgia, dyspepsia, insomnia, uterine and ovarian disturbances, torpid liver, deafness, etc. Patients received by appointment. Patients reduced from 5 to 10 inches in a fortnight."

There is not a word in the circular to indicate that the "treatment" is under the supervision of any medical practitioner; apparently the practice is carried on entirely by the unqualified owner of the establishment, and patients may come and patients may go without being seen by a medical man. This is only one of many instances in which nurses, either obstetrical or medical, are performing in various parts of the country duties for which neither their education nor training has fitted them. (*BMJ* 1897;ii:105.)

Doctors and patients don't agree: cross sectional study of patients' and doctors' perceptions and assessments of disability in multiple sclerosis

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Abstract

Objectives: To compare the judgments of clinicians on which domains of health in the short form questionnaire (SF-36) would be most important to patients with multiple sclerosis with the opinions of patients themselves; to compare assessment of physical disability in multiple sclerosis by a clinician using Kurtzke's expanded disability status scale and a non-clinically qualified assistant using the Office of Population Census and Surveys' (OPCS) disability scale with self assessment of disability and other domains of health related quality of life by patients using the SF-36 and the EuroQol questionnaire; and to compare the scores of patients for each domain of the SF-36 with control data matched for age and sex.
Design: Cross sectional study.
Setting: Clinical department of neurology, Edinburgh.
Subjects: 42 consecutive patients with multiple sclerosis attending a neurology outpatient clinic for review or a neurology ward for rehabilitation.
Main outcome measures: Scores on the SF-36; EuroQol; Kurtzke's expanded disability status scale; the OPCS disability scale.
Results: Patients and clinicians disagreed on which domains of health status were most important ($\chi^2 = 21$, df = 7, $P = 0.003$). Patients' assessment of their physical disability using the physical functioning domain of the SF-36 was highly correlated with the clinicians' assessment ($r = -0.87$, $P < 0.001$) and the non-clinical assessment ($r = -0.90$, $P < 0.001$). However, none of the measures of physical disability correlated with overall health related quality of life measured with EuroQol. Quality of life correlated with vitality, general health, and mental health in the SF-36, each of which patients rated as more important than clinicians and for each of which patients scored lower than the controls.
Conclusions: Patients with multiple sclerosis, and possibly those with other chronic diseases, are less concerned than their clinicians about physical disability in their illness. Clinical trials in multiple sclerosis should assess the effect of treatment on the other elements of health status that patients consider important, which are also affected by the disease process, are more closely related to overall health related quality of life, and may well be adversely affected by side effects of treatment.

Introduction

Optimal assessment of the efficacy of a clinical intervention depends on the natural course of the disease under study. Sometimes it may be reasonable simply to measure the effect of treatment on case fatality or the risk of major sequelae, such as stroke or myocardial infarction. Many conditions, however, are neither fatal nor characterised by acute sequelae. In these circumstances assessment of outcome usually entails either direct measurement of the extent and severity of the disease or assessment by a physician of the physical impairment or disability caused by the disease. However, both these approaches tend to be expensive, time consuming, and prone to bias if blinding of assessors to treatment allocation is imperfect,[1] and neither takes account of the potential adverse effects of treatment on other aspects of health. Self assessment by patients avoids bias by an external assessor, allows measurement of the effect of treatment on overall health related quality of life, and may not necessarily be less informative than clinical assessment with regard to physical disability.

Recent trials of interferon beta have highlighted the uncertainty about how best to measure outcome in clinical trials in multiple sclerosis.[2-5] Treatment reduces the frequency of new lesions on magnetic resonance brain scans and perhaps the number of relapses in patients,[2 3] and it may reduce neurological impairment and the rate of progression of disability as measured by neurologists.[4 5] However, the effect of treatment on other domains of health, which may be as or more important to patients, has not been measured. Indeed, little information exists on which aspects of health are considered important by patients with multiple sclerosis or other disabling conditions and, consequently, whether it is necessary to measure the effect of treatment on more than just the physical manifestations of the disease. Moreover, it is unclear whether assessment of physical disability requires a neurologist or whether self assessment by patients is adequate.

We therefore compared the perceptions of patients with multiple sclerosis and clinicians as to the relative importance of the eight different domains of health related quality of life measured by the short form 36 (SF-36),[6 7] and for each domain we compared the observed quality of life of patients with that expected for general population controls matched for age, sex, and locality. We further assessed the correlation between patients' self assessment of physical disability using the physical function domain of the SF-36 and physical disability measured by a neurologist and a non-medically qualified assistant (using different scales), and we examined the correlation between each measure of physical disability and the overall health related quality of life estimated by patients.

Methods

Over eight weeks we studied all patients with multiple sclerosis, either admitted to the neurology ward for rehabilitation or attending the neurology outpatient

clinics at the Western General Hospital, Edinburgh. Patients were eligible if they gave informed consent and fulfilled the following criteria: had a clinically definite or laboratory supported clinically probable diagnosis of multiple sclerosis according to Poser *et al*'s criteria[8]; had had no acute neurological relapse during the previous month; were resident in Lothian region; and knew that they had multiple sclerosis. A neurologist measured neurological impairment by using Kurtzke's expanded disability status scale.[9 10] An independent non-clinically qualified assistant administered the disability questionnaire of the Office of Population Censuses and Surveys (OPCS),[11] and patients completed the SF-36[6 7] and the EuroQol health related quality of life questionnaire.[12] The order of clinical assessment and questionnaire administration was not specified, and both investigators were blind to each other's findings.

After completion of the questionnaires, patients were given a standard written description of each of the eight elements of health related quality of life assessed in the SF-36 and were asked which three elements were the most important determinants of their overall quality of life. Using the same descriptions, clinicians working in the department of clinical neurosciences (senior and junior neurologists or neurosurgeons) were asked which three domains of the SF-36 they thought were the most important determinants of health related quality of life for patients with multiple sclerosis.

Age and sex stratified tabular control data for each of the domains of the SF-36 were obtained from a survey of health related quality of life in over 6000 people resident in Lothian in 1993.[13] The data were in the form of mean scores for men and women in five year age bands. The expected score for each case was taken as the corresponding age and sex matched mean control value.

Statistical analysis

Kurtzke's scale was treated as an ordinal scale, and scores on this scale were correlated with other scores by using the Spearman rank method with a two tailed test of significance. The OPCS scale and the SF-36 were treated as interval scales. All analyses were performed with the statistical software package SPSS for Windows (version 3.0).

Results

Of the 47 eligible patients who attended the department during the study period, 42 (89%) agreed to participate. Median age was 41 (range 28-68) years; 28 patients were women, and 33 were outpatients. The median score on Kurtzke's scale was 5.5 (range 1-8). The frequency distribution of domains of the SF-36 reported to be important by patients and clinicians (fig 1) differed from those expected by chance alone (patients: $\chi^2 = 16.2$, df = 7, $P = 0.02$; clinicians: $\chi^2 = 44.7$, df = 7, $P < 0.0001$) and from each other ($\chi^2 = 21.4$, df = 7, $P = 0.003$). The clinicians were significantly more likely than the patients to rate physical functioning and physical role limitations as important and significantly less likely to be concerned with mental health and emotional role limitations. The mean score among the patients was less than that expected on the basis of the age and sex matched control data from the general population for physical functioning, physical role limitations, general health, mental health, and vitality in the SF-36 (table 1).

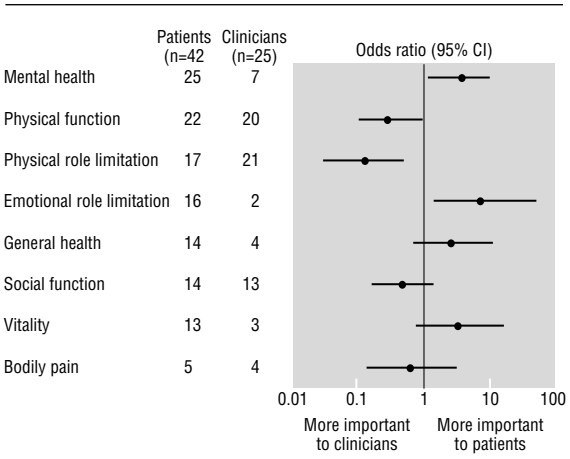

Fig 1 Frequency with which each of eight domains of health related quality of life in SF-36 were said to be among the three most important determinants of overall quality of life by 42 patients with multiple sclerosis compared with frequency expected by 25 clinicians working in clinical neurosciences department

Physical disability measured by the non-medically qualified assistant using the OPCS disability scale was correlated with the score on Kurtzke's scale obtained by the neurologist ($r = 0.84$, $P < 0.0001$). Physical disability measured by the patients using the physical functioning domain of the SF-36 was highly correlated with both the score on Kurtzke's scale and the overall OPCS disability score (fig 2). However, none of the scores of the other domains of the SF-36 were

Table 1 Median scores of 42 patients with multiple sclerosis for each domain of SF-36 compared with expected scores derived from general population controls matched for age and sex

Domain	Expected mean score*	Patients with multiple sclerosis: Median (range) score†	Patients with multiple sclerosis: % Below expected score	P value
Physical functioning	87	20 (0-90)	93	<0.0001
Physical role limitations	81	25 (0-100)	93	<0.0001
Vitality	60	35 (0-95)	83	<0.0001
General health	72	47 (10-90)	76	0.001
Mental health	74	68 (4-96)	69	0.02
Emotional role limitations	82	100 (0-100)	48	0.88
Social functioning	82	75 (0-100)	55	0.64
Bodily pain	76	92 (0-100)	40	0.28

*Population control data were normally distributed and are therefore expressed as means.
†The distribution of scores for patients with multiple sclerosis was skewed, and the scores are therefore expressed as the median and range.
‡Sign test.

Table 2 Correlation between each of eight domains of SF-36 and scores on Kurtzke's expanded disability status scale, OPCS disability scale, and patients' estimate of their overall health related quality of life using EuroQol questionnaire

Domain	Kurtzke's score: *r*	Kurtzke's score: P value	OPCS disability score: *r*	OPCS disability score: P value	EuroQol score: *r*	EuroQol score: P value
Physical functioning	–0.87	<0.0001	–0.90	<0.0001	0.26	0.11
Physical role limitations	–0.29	0.07	–0.30	0.06	0.42	0.006
Vitality	–0.22	0.10	–0.47	0.002	0.57	<0.0001
General health	–0.24	0.09	–0.22	0.10	0.49	0.001
Mental health	–0.05	0.77	–0.27	0.08	0.44	0.004
Emotional role limitations	–0.08	0.62	–0.14	0.38	–0.02	0.92
Social functioning	–0.27	0.08	–0.26	0.09	0.26	0.11
Bodily pain	–0.04	0.80	–0.15	0.34	0.20	0.21

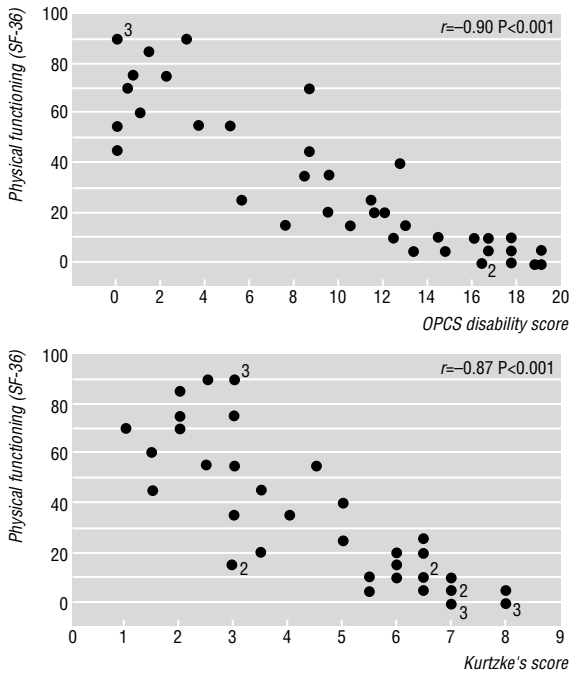

Fig 2 Correlation between score for physical function in SF-36 assessed by patients and (*bottom*) score on the Kurtzke's scale assessed by neurologist and (*top*) OPCS disability score assessed by non-medically qualified assistant. The number beside some dots shows the number of points overlying each other

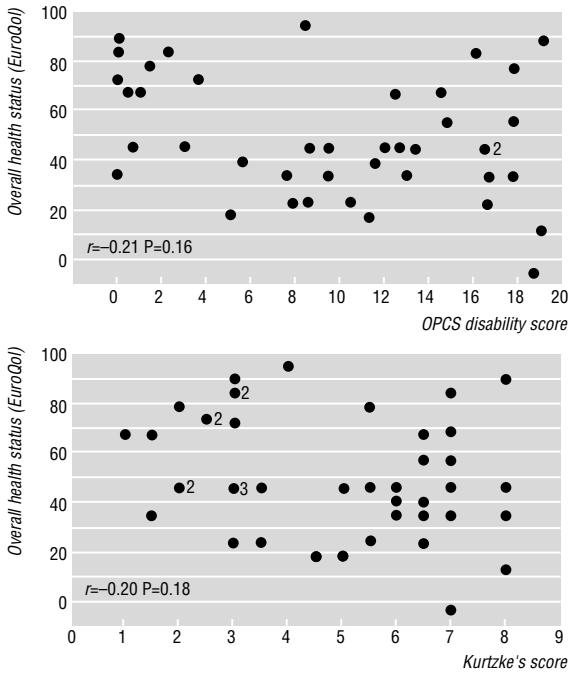

Fig 3 Correlation between patients' assessments of their overall health related quality of life derived from EuroQol questionnaire and (*bottom*) the score on Kurtzke's scale assessed by neurologist and (*top*) OPCS disability score assessed by non-medically qualified assistant. The number beside some dots shows the number of points overlying each other

correlated with the score on Kurtzke's scale, and only the score for vitality was correlated with the OPCS disability score (table 2). Neither the score on Kurtzke's scale nor the OPCS score was correlated with overall health related quality of life estimated by the patients using the visual analogue scale in the EuroQol questionnaire (fig 3). Overall health related quality of life was, however, correlated significantly with vitality, general health, mental health, and physical role limitation in the SF-36 (table 2).

Discussion

Concerns of patients

Doctors are not good at estimating the overall quality of life of their patients.[14 15] O'Boyle and colleagues argued that a valid measure of quality of life should quantify the level of functioning of each individual in those areas of life that he or she believes to be most important.[16] We have shown, at least in multiple sclerosis, that doctors' assessments of the relative importance of the domains of health related quality of life differ from those of patients. The pattern of domains reported to be important by the patients and that reported by the clinicians both differed significantly from the distribution expected by chance alone. In other words, there was a degree of agreement within the patient group and within the medical group as to which domains were most important. However, there was significant disagreement between the two groups. Generally the clinicians were more concerned than the patients about the physical manifestations of disease; the patients were more concerned with less tangible quantities such as mental health and vitality.

Although we did not study the reliability of these assessments, we have produced some evidence of their importance. Firstly, the patients with multiple sclerosis had significantly lower scores than the general population controls for three of the four domains of the SF-36 (mental health, vitality, and general health) that they rated higher than the clinicians did. Secondly, these domains were highly correlated with their estimates of overall health related quality of life. It does not necessarily follow, however, that the patient's viewpoint is more important than that of the doctor. Doctors will usually have a better understanding of the natural course and possible clinical manifestations of a particular disease, and their opinions may be based on experience of treating many patients. Nevertheless, doctors should at least bear in mind that their concerns may not coincide with those of their patients when considering whether to prescribe treatment, and, as has been suggested recently, trialists should consider taking patients' views into account when designing trials.[17 18]

Assessment of physical disability

Irrespective of which domains of health related quality of life are important to patients with multiple sclerosis, physical disability should clearly still be measured in trials of treatments that potentially modify disease. Although Kurtzke's scale is the most commonly used measure of disability in multiple sclerosis, it is expensive and time consuming to perform as it requires a full clinical examination by a neurologist. Questionnaire based disability scales, such as the OPCS scale, have the advantage that they can be administered by non-clinical assistants, although this will still be relatively costly in a large multicentre trial. Moreover, both options require blinding of assessors to treatment allocation. Non-blinded assessment can

significantly bias the results of multiple sclerosis trials in favour of treatment,[1] and blinding is difficult to maintain in practice without going to considerable lengths. Self assessment by patients has the advantage of eliminating the potential for bias by an external assessor, although it does not avoid bias resulting from placebo effects experienced by patients. Postal or telephone follow up with the SF-36, or a similar instrument, would be easily standardised and relatively inexpensive.

Assessment of physical disability by a non-clinically qualified assistant using the OPCS disability scale was correlated with that obtained by a neurologist using Kurtzke's scale. The functional limitations profile has also been shown to be highly correlated with Kurtzke's scale.[19] We have shown that self assessment of disability by patients, using the physical functioning domain of the SF-36, can provide closely similar information to that obtained with Kurtzke's scale or the OPCS disability scale. Patients could provide their self assessment by post or telephone, and, at least on a cross sectional basis, this would provide about 80% of the information obtained with Kurtzke's scale or the OPCS scale ($r^2 = 0.76$ and $r^2 = 0.81$ respectively). However, none of the measurements of physical disability was correlated with the patients' assessments of their overall health related quality of life based on the EuroQol questionnaire. Overall health related quality of life was correlated most significantly with vitality, general health, and mental health in the SF-36. Given that these domains were all thought to be important by patients and that the scores obtained from patients for each of the domains were lower than those expected on the basis of control data—that is, the scores had been affected by the disease process—the assessment of overall health related quality of life seems to have some validity. Other studies have shown that measurement of health related quality of life in multiple sclerosis can be reproducible.[20]

Side effects of treatment

The finding that patients rate variables such as vitality, general health, and mental health as important determinants of their overall health related quality of life is important when considering how best to incorporate the side effects of treatments into the results of clinical trials. Side effects are rarely incorporated into the overall trial result and are usually simply listed separately. Whether the benefits of treatment justify the side effects is therefore left for doctors to decide for themselves. However, as the concerns of doctors and patients may not coincide this may be inappropriate. Treatments given to patients with multiple sclerosis frequently cause side effects that are sufficiently severe to influence patients' quality of life.[21] For example, interferon beta may cause reactions at the injection site, flu-like symptoms, nausea, myalgia, fever, depression, and malaise,[2-5 22] each of which is likely to have an adverse effect on the very domains of health related quality of life that patients consider more important than doctors. Measuring outcome with an overall measure of health related quality of life would at least record the patients' perspective on whether the treatment was worse than the disease itself.

Key messages

- Patients can accurately assess their own physical disability
- Physical disability may not always be the main determinant of overall health related quality of life
- Patients and clinicians differ in their assessments of the relative importance of different elements of health related quality of life
- The opinions of patients should be taken into account in the selection of outcome measures for clinical trials

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Influence of cholesterol on survival after stroke: retrospective study

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Abstract

Objective: To investigate the association between serum cholesterol concentration and cerebrovascular disease.

Design: Retrospective study.

Setting: Acute stroke unit of inner city general hospital.

Subjects: 977 patients with acute stroke.

Main outcome measures: Serum total cholesterol concentration, type of stroke investigated by computed tomography or magnetic resonance imaging, three month outcome (good (alive at home) or bad (dead or in care)), long term mortality.

Results: After adjustment for known prognostic factors, higher serum cholesterol concentrations were associated with reduced long term mortality after stroke (relative hazard 0.91 (95% confidence interval 0.84 to 0.98) per mmol/l increase in cholesterol) independently of stroke type, vascular territory and extent, age, and hyperglycaemia. Three month outcome was also influenced independently by serum cholesterol ($P = 0.024$).

Conclusions: Our data suggest an association between poor stroke outcome and lower serum cholesterol concentration. Until a prospective controlled study has confirmed the benefits of lowering cholesterol concentration in elderly subjects, the application of cholesterol lowering guidelines cannot be justified as secondary prevention of acute stroke.

Introduction

The association between total serum cholesterol concentration and coronary artery disease is well established, but the relation between stroke and cholesterol remains elusive. Epidemiological studies in Japanese[1 2] and Japanese Americans[3] failed to associate cerebral infarction with raised cholesterol but found an inverse relation with the incidence of intracerebral haemorrhage. A study of middle aged men in the United States noted a positive association between raised cholesterol concentration and ischaemic stroke and a negative association with haemorrhagic events.[4]

A meta-analysis of the prospective follow up of 450 000 patients within 45 separate cohorts over an average period of 16 years detected no relation between cholesterol concentration and the overall incidence of stroke. In most cohorts there was no differentiation between stroke type; thus, cerebral infarction or intracerebral haemorrhage and competing associations with infarction and a negative association with haemorrhage could not be excluded. Postmortem examination of Japanese stroke patients suggested that low serum cholesterol concentration was associated with cerebral haemorrhage while high cholesterol was linked with large carotid vessel atherothrombotic infarction. There was no association between subcortical lacunar events and raised cholesterol.[5] It is therefore likely that cholesterol influences certain types of stroke, and any effect is likely to be diluted if strokes are not divided into the appropriate diagnostic grouping.

Meta-analyses of published trials of lipid lowering treatment and incidence of stroke are fraught with potential bias and pitfalls, but few trials support the hypothesis that lowering cholesterol concentration effectively reduces the incidence of stroke. In the west of Scotland coronary prevention study treatment of a middle aged population with pravastatin reduced cholesterol concentration and the primary incidence of coronary events but not stroke.[6] A further secondary prevention study in patients who had survived a myocardial infarction did, however, suggest a significant reduction in stroke events (3.8% in placebo group *v* 2.6% in the group given pravastatin, $P = 0.003$).[7] In the Scandinavian simvastatin survival study patients given simvastatin (as secondary prevention of coronary heart disease) also had a significantly reduced number of cerebrovascular events.[8]

There are several reasons why a reduction in the incidence of stroke has not been consistently reported in studies of lipid lowering treatment. These large trials were designed to detect a reduction in coronary deaths in middle aged patients with and without coronary artery disease. Coronary events are 10 times more common than stroke in this age group, and as a result relatively few stroke end points were reported in each study. The few studies that have been conducted in elderly patients do, however, suggest a reduction in the incidence of stroke with lipid lowering treatment.

As yet there are no published data regarding the effect of cholesterol concentration on survival after stroke. We therefore investigated the effect of cholesterol in patients presenting with acute stroke.

Patients and methods

The admission criteria and protocol of the acute stroke unit of the Western Infirmary, Glasgow, have been described in detail elsewhere.[9] Briefly, all patients within a well defined geographical region suffering a new focal or global neurological deficit are admitted, regardless of age or severity of neurological deficit. Computed tomography or magnetic resonance imaging is performed routinely within 72 hours of admission. Serum cholesterol is measured in blood samples taken from fasting patients on the morning after admission. Details of each patient's risk factors, presenting complaints, neurological examination, results of investigations, and final diagnosis are prospectively recorded and transferred to a computerised database.

The patients included in this study represent a series of consecutive admissions to this unit. Patients whose symptoms were found to be caused by a con-

dition other than stroke were excluded from the analysis.

Outcome measures

Serum total cholesterol concentration was measured by a standard cholesterol oxidase method. Types of stroke (primary intracerebral haemorrhage or infarction) were diagnosed from the early computed tomograms or magnetic resonance images. Stroke was classified according to the system used by the Oxford Community Stroke Project.[10] This describes patients as having total anterior circulation infarction, partial anterior circulation infarction, posterior circulation infarction, or lacunar infarction.

Follow up was by record linkage[11] to death records from the registrar general of Scotland and to hospital discharge records to obtain information on medical events after stroke. This technique has been validated in an epidemiological study of hypertension[12] and has been used for monitoring end points in a large clinical trial.[13] The method of record linkage is reliable, but, admissions to private hospitals or institutions outside Scotland are not detected. Outcome is categorised as alive at home, alive in care, or dead at two, three, six, or 12 months after stroke. We used only the three month data in our study, with good outcome defined as alive at home and bad outcome as alive in care or dead. This subdivision at three months is a marker for three month functional outcome, an end point commonly used in trials of therapeutic agents in stroke.

Statistical analysis

We assessed the effect of serum cholesterol on survival using a Cox proportional hazard model, in which we controlled for known confounding factors affecting outcome—stroke type (haemorrhage *v* infarction); duration of symptoms (transient ischaemic attack or reversible neurological deficit *v* sustained stroke); Oxford classification of stroke; plasma glucose concentration; age; packed cell volume; presence of ischaemic heart disease; and smoking history. Since stroke type may not satisfy the proportionality assumption of the Cox proportional hazard model, we stratified the analysis into five groups for type of stroke and Oxford category (primary intracerebral haemorrhage, total anterior circulation infarction, partial anterior circulation infarction, posterior circulation infarction, and lacunar infarction). Age and serum cholesterol were entered as continuous independent variables. Plasma glucose did not satisfy the proportionality assumption and was entered as a dichotomous variable ($\leq$8 mmol/l *v* >8 mmol/l).

Estimates of survival rates at 1000 days in example cases were provided from the Cox proportional hazard model.[14] The model predictions were checked by comparison with Kaplan-Meier survivorship functions for selected subgroups of patients. Variables which predict dichotomous outcome at three months were assessed by logistic regression, using the same independent variables as above (that is, age, Oxford classification, serum cholesterol, and serum glucose). We performed the statistical analysis with Statistica for Windows Version 5.0 (StatSoft, Tulsa, OK, USA). Statistical significance was declared at $P < 0.05$.

Results

Of 1392 patients in the database, 26 had inadequate data for analysis because of immediate death, etc, 140 had a final diagnosis other than cerebrovascular disease, 80 could not be classified according to the Oxford categories or died before computed tomography, 97 had transient ischaemic attacks (symptoms lasting <24 hours), 43 had reversible neurological deficits (symptoms lasting 1-3 days), and in nine the duration of symptoms was not noted. Thus, complete data were available for 997 patients with acute stroke. Their mean (SD) age was 69.9 (12.5) years (range 23-95), their mean serum total cholesterol concentration was 5.93 (1.41) mmol/l, and their plasma glucose concentration was 7.3 (2.7) mmol/l.

The mean follow up of survivors was 895 days (range 105-2032), with total mortality during follow up of 39.4%. Median survival time could not be estimated, but the 25th centile was 254 days. The 1000 day survival of the 109 patients with primary intracerebral haemorrhage was 38%, of the 198 with total anterior circulation infarction it was 38%, of the 326 with partial anterior circulation infarction 63%, of the 91 with posterior circulation infarction 71%, and of the 287 with lacunar infarction 64%. Plasma glucose concentration also significantly influenced mortality: 1000 day survival was 60% in normoglycaemic subjects (concentration $\leq$8 mmol/l) compared with 43% in hyperglycaemic subjects (concentration >8 mmol/l). Age significantly influenced outcome, with 1000 day survival being 71% in patients aged $\leq$69 years and 46% for older patients. Serum cholesterol concentration did not correlate with plasma glucose ($r^2 = 0.002$) or with age ($r^2 = -0.004$).

Figure 1 shows the Kaplan-Meier cumulative survival curves for patients grouped according to their cholesterol concentrations. In the Cox proportional hazard model (after adjustment for stroke type and Oxford group) higher plasma glucose, lower serum cholesterol, and greater age were independent predictors of mortality after stroke (table 1). The relative hazard was 9% lower for each 1 mmol/l rise in serum cholesterol concentration. The effect of cholesterol

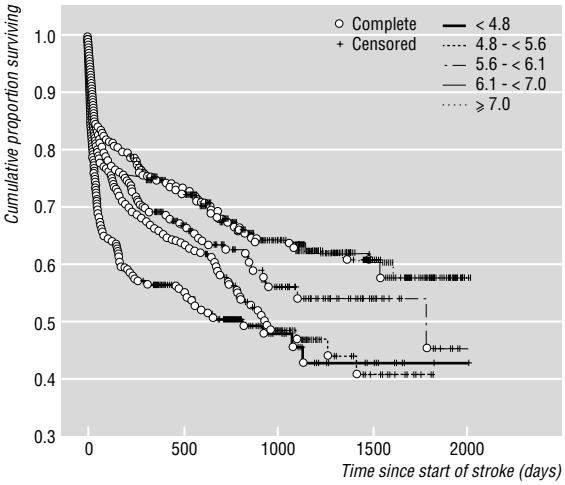

Fig 1 Kaplan-Meier cumulative survival curves for patients with stroke grouped according to their serum total cholesterol concentrations (demarcation of groups by quartiles of cholesterol concentration)

Table 1 Cox proportional hazard model for mortality after stroke in 997 patients, adjusted for stroke type and Oxford classification* (χ^2=124.2, df=7, P<0.00001)

Risk factor†	Relative hazard (95% CI)	P value
Plasma glucose (>8 mmol/l *v* ⩽8 mmol/l)	1.79 (1.40 to 2.27)	<0.00001
Serum cholesterol (per 1 mmol/l increase)	0.91 (0.84 to 0.98)	0.012
Age (per 1 year increase)	1.04 (1.03 to 1.05)	<0.00001

*Primary intracerebral haemorrhage, total anterior circulation infarction, partial anterior circulation infarction, posterior circulation infarction, or lacunar infarction.
†Other risk factors did not have significant predictive effects: packed cell volume (P=0.83), smoking (P=0.9), alcohol intake (P=0.052), presence of ischaemic heart disease (P=0.81).

Table 2 Relative hazard for mortality after stroke in 997 patients associated with serum cholesterol concentration within each stroke type or Oxford category

Group	Relative hazard (95% CI)
Primary intracerebral haemorrhage (n=109)	1.00 (0.81 to 1.25)
Total anterior circulation infarction (n=193)	0.90 (0.79 to 1.03)
Partial anterior circulation infarction (n=322)	0.85 (0.73 to 0.99)
Posterior circulation infarction (n=90)	1.11 (0.82 to 1.50)
Lacunar infarction (n=283)	0.88 (0.75 to 1.04)

Table 3 Cox proportional hazard model's prediction of survival after stroke for 997 patients by type of stroke and Oxford classification, age, and serum total cholesterol concentration

Stroke type	1000 Day survival (%)
Total anterior circulation infarction	
Patients aged 50 years:	
Cholesterol concentration 5.2 mmol/l	54
Cholesterol concentration 7.5 mmol/l	62
Patients aged 75 years:	
Cholesterol concentration 5.2 mmol/l	31
Cholesterol concentration 7.5 mmol/l	40
Lacunar infarction	
Patients aged 50 years:	
Cholesterol concentration 5.2 mmol/l	88
Cholesterol concentration 7.5 mmol/l	91
Patients aged 75 years:	
Cholesterol concentration 5.2 mmol/l	61
Cholesterol concentration 7.5 mmol/l	69

seemed similar for each type of stroke, though it reached significance only in the largest subgroup of patients, those with partial anterior circulation infarcts (table 2). Table 3 shows examples of the predicted 1000 day survival for patients with different stroke types, serum cholesterol concentrations, and ages.

Logistic regression showed that, when outcome at three months was coded as good (alive at home) or bad (alive in care or dead), increasing levels of cholesterol were still associated with better outcome after adjustment for age, plasma glucose concentration, stroke type, and Oxford classification.

Discussion

Our data suggest that the concentration of cholesterol independently influences survival of patients with acute stroke. Higher cholesterol concentrations were associated with improved survival. The effect seemed to be greatest in elderly patients—for example, 1000 day survival would be 51% for a 75 year old patient with a serum cholesterol concentration of 5.2 mmol/l and 59% if the cholesterol concentration was 7.5 mmol/l, an absolute risk reduction of 8% and a relative risk reduction of 16%. Cholesterol concentration did not correlate with either blood sugar or age. The prognostic effect was robust after adjustment for known prognostic indicators such as stroke type, Oxford group, blood sugar concentration, and age. Further adjustment for factors that were not significant within our model—such as smoking, alcohol intake, packed cell volume, and ischaemic heart disease—did not alter the results.

Possible bias

This counter-intuitive effect of cholesterol cannot be taken at face value without considering possible sources of bias in our study. This was a retrospective study based on prospectively collected data. We examined a hospital population and, while referrals were admitted unselectively, even from nursing homes, some patients with minor symptoms would not have been referred as inpatients. A community study would be equally likely to miss patients with severe stroke who die within 24 hours of the onset of stroke.

Serum cholesterol concentrations may reflect the severity of stroke, rather than the premorbid concentration. Our results are consistent across a range of stroke severity, however. In addition, serum cholesterol concentration did not correlate with stroke severity, as measured by the National Institutes of Health stroke scale, in a subgroup of our population (Spearman correlation coefficient -0.081, $n=323$, $P=0.15$).

Our blood samples were collected on admission or within 24 hours. We have data on serial samples collected for up to three months in a subgroup of patients, and these show no downward trend as a result of stress or poor nutrition. A recent formal study has shown that serum cholesterol measurements within the first 48 hours are identical to those after three months, although between these times a fall in concentration does occur.[15] Only a community based survey that measured premorbid cholesterol concentrations could conclusively establish the effect of cholesterol on stroke outcome, but we consider that obvious sources of bias have been excluded.

Explanation of results

There is no established biological mechanism that explains these results, but cholesterol is known to have effects on the vasculature and is essential for normal membrane fluidity. High blood cholesterol concentrations modify the action of platelets such that exposure to low density lipoprotein cholesterol enhances platelet aggregation by its action on platelet activating factor.[16] Rabbits fed a high cholesterol diet have larger experimentally induced infarcts associated with an increase in platelet deposition in the thrombus at the infarct.[17] Exposure to high levels of cholesterol also reduces the responsiveness of large blood vessels, though not small vessels, to vasodilatory stimuli.[18] All of these effects suggest that a higher serum cholesterol concentration would predispose to a poorer outcome from a stroke event.

High cholesterol concentrations may exhibit a neuroprotective effect by modulating the action of the enzyme γ-glutamyltransferase and acetylcholinesterase: a high cholesterol diet results in increased γ-glutamyltransferase activity but reduced acetylcholinesterase activity.[19] γ-Glutamyltransferase has a role in amino acid uptake and transport; thus, its increase in patients with higher serum cholesterol could reduce the neurotoxic effects of excitotoxic amino acids. Early survival does not, however, seem to be affected by cholesterol: rather it seems that the difference in the survival curves of stroke patients with high and low cholesterol concentrations gradually increases over time. This suggests that the protective mechanism may have a more prolonged effect.

Cholesterol therefore seems to be a marker for long term rather than short term survival. Why this should be so is unclear as all conventional rationales suggest that patients with higher cholesterol concentrations would have an increased risk of coincidental cardiac disease, of subsequent sudden cardiac death, and of larger cerebral infarctions. A low cholesterol concentration is, however, known to be associated with underlying serious illness, and it might be expected that such patients would have a poorer outcome than those with higher cholesterol concentrations. A retrospective analysis of our data dividing patients into five groups according to cholesterol concentration confirmed that both early and long term mortality were greater in the groups with lowest cholesterol concentrations. It is possible that lower cholesterol in these relatively elderly patients (mean age 69.9 (SD 12.4)) may simply have reflected poor nutritional status, which could predispose to a poor outcome after stroke.

Conclusion

Trials of lipid lowering drugs have concentrated on middle aged patients with a low risk of stroke, and as a result it is unknown whether lipid lowering treatment is beneficial in older patients with elevated cholesterol concentration and cerebrovascular disease. Epidemiological data suffer from failure to subclassify stroke into different types, which masks any positive relation between infarction and cholesterol and a negative relation with cerebral haemorrhage. Postmortem examination of stroke patients also suggests that high cholesterol concentrations may increase the risk of only certain types of infarct, such as large vessel atherothrombosis, but not lacunar events.

A prospective study of the incidence and outcome of stroke in relation to blood cholesterol in elderly patients (with appropriate computed tomography and clinical diagnosis) is required to define this relation accurately. A randomised controlled trial of lipid lowering drugs in elderly patients would evaluate the safety and cost effectiveness of lowering cholesterol and could reveal effectiveness in terms of reducing the incidence of both stroke and coronary events. Current practice recommends reducing blood cholesterol in patients aged over 55 with a high risk of vascular disease despite the lack of evidence for benefit in patients over 70 years old. As all trials of lipid lowering drugs have studied middle aged patients their results cannot be extrapolated to elderly people.

Key messages

- Although the link between cholesterol and stroke is controversial, the balance of evidence suggests higher cholesterol is associated with an increased risk of atherothrombotic stroke but a reduced risk of intracerebral haemorrhage
- Trials of lipid lowering drugs have concentrated on middle aged patients with a relatively low incidence of stroke events
- We investigated the association between serum cholesterol and cerebrovascular disease in 977 patients hospitalised with acute stroke and found that higher serum total cholesterol concentrations were associated with a reduction in long term mortality after stroke
- This relation was independent of the type or extent of the stroke, vascular territory, age, and hyperglycaemia—all factors known to influence survival independently after stroke
- Until conclusive benefit is shown in elderly patients with cerebrovascular disease, the routine application of lipid lowering treatment after stroke cannot be justified

Our data suggest that lower serum cholesterol concentrations may have an independent adverse effect on survival after stroke. Further studies to investigate and confirm this relation are required. The efficacy of cholesterol lowering drugs as primary or secondary prevention of coronary and cerebrovascular events in elderly patients remains unproved.

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Review by a local medical research ethics committee of the conduct of approved research projects, by examination of patients' case notes, consent forms, and research records and by interview

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Abstract

Objective: To monitor the conduct of medical research projects that have already been approved by the local medical research ethics committee.
Design: Follow up study of ethically approved studies (randomly selected from all the studies approved in the previous year) by examination of patients' case notes, consent forms, and research records and by interview of the researchers at their workplace.
Setting: Tayside, Scotland (mixed rural and urban population).
Subjects: 30 research projects approved by Tayside local medical research ethics committee.
Main outcome measures: Adherence to the agreed protocol, particularly for recruitment (obtaining and recording informed consent) and for specific requirements of the ethics committee, including notification of changes to the protocol and of adverse events.
Results: In one project only oral consent had been obtained, and in a quarter of the studies one or more consent forms were incorrectly completed. Inadequate filing of case notes in five studies and of consent forms in six made them unavailable for scrutiny. Adverse events were reported, but there was a general failure to report the abandoning or non-starting of projects; in two studies the investigators failed to notify a change in the responsible researcher.
Conclusions: Monitoring of medical research by local medical research ethics committees promotes and preserves ethical standards, protects subjects and researchers, discourages fraud, and has the support of investigators. We recommend that 10% of projects should undergo on-site review, with all others monitored by questionnaire. This would require about six person hours of time and a salary bill of £120 per study monitored.

Introduction

Medical research on patients or healthy volunteers in Britain is subject to appraisal by local medical research ethics committees, which may impose conditions on or changes to the proposed protocols. Follow up is not routine, so we do not know whether an approved protocol is the protocol that is subsequently used. We therefore conducted an on-site audit of how a random sample of approved research projects had been or were being conducted.

Subjects and methods

The Tayside local medical research ethics committee was formed in 1990 as a joint committee of the Tayside Health Board and the University of Dundee and consists of 12 members—six medically qualified members, a nurse, a lay chairman, and four lay members—supported by a secretariat. The committee meets monthly to discuss all submissions for Tayside—some 310 a year. The submissions are on detailed standard proposal forms, which contain guidance to researchers on the committee's requirements, including the need, after approval, to notify subsequent protocol amendments and adverse events.

We selected at random a stratified sample of 39 of the 311 projects approved one year previously, and in each case we asked the investigators for a review meeting at their workplace and to complete a questionnaire. At the meeting the assessors—one lay member and one medically qualified member—(*a*) discussed responses to the questionnaire; (*b*) completed a reviewers' questionnaire for recording further details of the visit; (*c*) inspected consent forms; and (*d*) inspected case records.

The assessors recorded details in a standard format, from which data were subsequently entered into the committee's database (Microsoft Access) to facilitate analysis of the results. They also wrote to the researchers summarising the main points raised at the meeting and advising them of any changes in

procedures needed to meet the committee's requirements. Substantial problems or issues of interest were reported at the next committee meeting.

Results

Had the study begun?

Of the 39 studies initially selected for review, nine had not begun because they had not been funded; eight of these had been abandoned without the committee being notified. Of the 30 projects that had begun, 20 were independent projects initiated from universities, the government, or the NHS; eight were hospital based (five) or general practice based (three) studies sponsored by pharmaceutical companies; and two were from a contract research drug development unit.

Non-recruitment, refusal, and withdrawal

A common reason given for difficulties in recruitment was that doctors did not wish to recruit patients who were stabilised on current treatment, with the result that potential patient numbers were restricted. In six studies some recruited patients had withdrawn, either unilaterally or in discussion with the researcher. These were isolated events, affecting only 1% of recruited patients, and they did not seem to raise ethical concerns. In addition, doctors terminated other patients' participation through failure of symptoms to improve, excess pain from the underlying condition, and symptoms of Alzheimer's disease.

Complying with protocol

One department failed to inform the committee of a change in principal researcher and another that a group of patients had been placed in the care of a previously unidentified researcher. In three studies important protocol amendments had been made without being reported to the committee; two studies had been terminated prematurely without notification, one because of poor recruitment. In five other studies minor protocol amendments, not directly affecting patients, had not been notified.

Adverse events

Patients had experienced adverse events in nine studies. Although unpleasant, most of these events were not serious. Serious adverse events had been notified to the committee.

Availability of a study report

Researchers in the 20 independent projects were committed to publish, with three full papers and two abstracts published in professional journals, two local reports, and four papers in preparation.

Informed consent

In 29 studies patients gave informed consent. In the remaining study, which involved patients with Alzheimer's disease, consent was obtained from relatives. In one, non-invasive study, the researcher approached patients for oral consent while they were in labour.

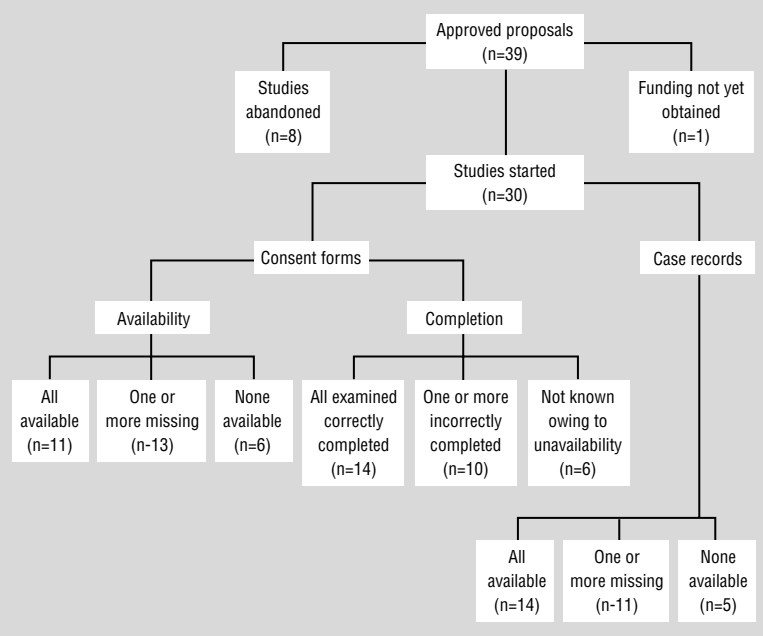

Fig 1 Availability and accuracy of completion of consent forms and case records (values are numbers of studies)

Availability and completeness of consent forms and case records

Figure 1 shows the availability and completeness of consent forms and case records. In six studies consent forms, although completed, were not available for scrutiny when needed. In 13 other studies one or more consent forms were initially missing. In one study doctors had thought that consent was not necessary for non-invasive research, in another a collaborating researcher thought that oral consent was enough, and in a third the researcher's supervisor had been a healthy volunteer without following the recruitment requirements.

In one third of studies one or more consent forms had been completed incorrectly. Although patients had signed all of the forms, they had either not answered individual questions or had ticked a box indicating that they had not received enough information, not had an opportunity to ask questions, etc. In five studies the doctor had not countersigned all of the forms.

In nearly half of the studies a proportion of research case records were not available for inspection because they were filed with the patient's hospital case notes in use elsewhere (fig 1). Some hospital case notes were bulky, and researchers and reviewers had considerable difficulty in finding relevant consent forms and records. On an additional five occasions no case records were available. In two studies sponsored by drug companies the legibility of copies of records retained by the researcher was poor. The errors in completion detected in several studies were all minor and did not affect their validity.

Discussion

Concern for research subjects

We were reassured that, in recruiting participants, investigators were placing the interests of patients

before those of their projects and were willing to withdraw patients from studies when it was against their interests to continue. Most projects attracted sufficient participants and yielded potentially valuable information. Investigators were strongly committed to publishing their results.

Notifying changes and adverse events

Investigators did not sufficiently recognise the need for protocol amendments to be approved either by the full local medical research ethics committee meeting if important or by the chairman's action if minor. (Investigators have approval to implement only what a local medical research ethics committee originally endorses.) There was no evidence of failure to report serious adverse events: in such cases investigators and sponsors are anxious to share responsibility for allowing the study to continue.

Availability of case records and valid consent forms

Examination of case records provides a means of assessing the thoroughness with which data relevant to the scientific validity of the study and the patients' welfare are being recorded. There was a clear need for improving access to research case records.

Care needs to be given to the timing of the consent process. Researchers and coworkers must always obtain informed consent from patients, even in non-invasive studies and even when the subject is a research collaborator, such as a supervisor. Researchers must scrutinise consent forms to ensure that participants have completed them correctly and are satisfied with the information provided before they countersign them and start the study. We found no instances of researchers failing to obtain written consent from patients in studies involving identifiable risk or discomfort.

Educational value of ethical review

Researchers have recently strongly criticised local medical research ethics committees on various grounds,[1-4] summarised in a recent editorial[5] to which one of us replied with points in their favour.[6] Uncertainty has been expressed by the professional bodies about the need for review after ethical approval has been given.[7-9] The 30 principal researchers whom we approached were, however, supportive of the work of the committee and of the need for such review.

Constructive feedback to the specific researchers was an essential part of the exercise. The discussions were of educational value through the reviewers to the committee, contributing then to advice given to researchers with the proposal forms and in the annual reports. Awareness of the possibility of review after ethical approval provides an added incentive not to cut corners and to maintain standards, thereby helping to protect research participants from potential harm and investigators from criticism, as well as providing a disincentive to potential fraud.

The Tayside local medical research ethics committee intends to seek the views of patients and healthy volunteers on their experiences during and after participation in research projects. Several researchers expressed the need for this.

Key messages

- Positive monitoring or audit of research projects by local medical research ethics committees is supported by medical researchers
- Ten per cent of projects should undergo on-site review
- All other projects should be monitored by questionnaire
- Patients should also complete a questionnaire

Cost of ethical review

A review visit involves about six person hours of work for the assessors and was estimated as costing some £120, based on the time of a medical consultant and a lay scientific adviser. In addition, an hour or more of the researcher's time for the visit and preparation time must be taken into account.

Conclusion

Public confidence in medical research and the willingness to cooperate will continue only if the research is seen to be conducted to the highest ethical standards. The existence of an effective system for the ethical review of research shows that efforts are being made to maintain these high standards. That there is room for improvement in Tayside, which has high standards of patient care and staff training, leads us to recommend that all local medical research ethics committees should randomly select a minimum of 10% of approved projects for subsequent on-site review by members of the committee; that all remaining projects should be reviewed by questionnaire; and that patients should also be asked to complete a questionnaire.

We gratefully acknowledge the willing help given by the researchers who participated in this study. We also thank Mr H D Sheldon (chairman), Mr M Finlayson (secretary), and other members of the Tayside committee on medical research ethics for their support and encouragement; and Tayside Health Board, the University of Dundee, and Dundee Teaching Hospitals NHS Trust for providing the facilities that made the study possible.

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Conflict of interest: None.

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Intentions of newly qualified doctors to practise in the United Kingdom

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In a series of cohort studies of newly qualified British medical graduates, respondents were asked about intentions to practise in the United Kingdom.[1 2] We compared the intentions of those who qualified in 1993 with the intentions and subsequent employment of earlier cohorts.

Methods and results

The studies were undertaken by mailed questionnaires. Members of each cohort was asked in their preregistration year, "Apart from temporary visits abroad, do you intend to practise in the United Kingdom for the foreseeable future?" There were five possible responses: "yes—definitely," "yes—probably," "undecided," "no—probably not," and "no—definitely not." For this report, these five categories were reduced to three by combining the "yes" and the "no" categories.

Of those qualifying in 1993, 75.7% (1969/2600) definitely or probably intended to practise in the United Kingdom (75.1% (967/1287) of men, 76.3% (1002/1313) of women) compared with 89.2% (2812/3154) of those qualifying in 1983 (table1), a decrease of 13.5% (95% confidence interval 11.5% to 15.4%). This 1993 figure is lower than that for any cohort previously surveyed. The large percentage in the "undecided" category in 1993, compared with earlier cohorts, is notable. The increase since 1983 in those who probably or definitely do not intend to practise in the United Kingdom is more modest and is no higher than in 1974. The percentage of those of non-British nationality who definitely or probably intended to practise in the United Kingdom was roughly constant across the cohorts. The decrease in intention to practise in the United Kingdom occurred among those with British nationality and residence.

The response rate for the 1993 cohort was lower than for earlier cohorts. Even if every non-respondent in each cohort intended to practise in the United Kingdom, the percentage of the whole 1993 cohort intending to practise in the United Kingdom was still historically low, at 82.7% (3031/3662) compared with 85.7% (2014/2350) for the 1974 cohort, 86.9% (2724/3136) for 1977, 92.1% (3167/3437) for 1980, and 91.1% (3503/3845) for 1983.

We analysed the association between expressed early intentions and later career destinations for the three cohorts that we followed for at least nine years (1977) or 11 years (1974 and 1983). In the combined cohorts, 91.2% (4994/5473) of doctors who said in their preregistration year that they probably or definitely intended to practise medicine in the United Kingdom eventually did so. This compares with 77.9% (300/385) of those who were undecided and 62.5% (250/400) of those who said that they would definitely or probably not practise in the United Kingdom. The table also shows, for each of the four earlier cohorts, the number and percentage of respondents employed in medicine in the United Kingdom five and nine years after graduation. These comparisons of intentions in the preregistration year with later employment show that in previous cohorts early intention to practise in the United Kingdom has been a reasonable indicator of subsequent employment.

Comment

The wording of the question that was asked combined the idea of working in medicine with that of working in the United Kingdom. It was evident from the replies in full, however, that most of those who were not committed to practising in the United Kingdom were committed to practising medicine. Although early intentions do not invariably correspond with future careers, the doctors' responses show an increased lack of commitment to a medical career in the United

See editorial by Richards et al

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Table 1 Intentions of medical students to practise medicine in the United Kingdom and their employment five and nine years after qualification. Values are numbers (percentages)

Intended and subsequent employment in medicine in Britain	Year of graduation				
	1974	1977	1980	1983	1993
Employment					
Intentions in preregistration year:					
Definitely or probably in medicine in Britain	1596 (82.6)	2217 (84.3)	2579 (90.5)	2812 (89.2)	1969 (75.7)
Undecided	146 (7.6)	230 (8.7)	98 (3.4)	132 (4.2)	380 (14.6)
Definitely not or probably not in medicine in Britain	190 (9.8)	182 (6.9)	172 (6.0)	210 (6.7)	251 (9.7)
5 Years after graduation:					
In medicine in Britian	1538 (86.4)	2371 (87.7)	2304 (89.6)	2284 (90.3)	
Not in medicine in Britain	242 (13.6)	334 (12.3)	266 (10.4)	244 (9.7)	
9 Years after graduation:					
In medicine in Britain	1681 (85.2)	2168 (87.7)		2323 (88.8)	
Not in medicine in Britain	291 (14.8)	304 (12.3)		292 (11.2)	
Response rates					
Total in cohort	2350	3136	3437	3845	3662
Preregistration year	1932 (82.2)	2629 (83.8)	2849 (82.9)	3154 (82.0)	2600 (71.0)
5 Years after graduation	1780 (75.7)	2705 (86.3)	2570 (74.8)	2528 (65.7)	
9 Years after graduation	1972 (83.9)	2472 (78.8)		2615 (68.0)	

Kingdom, which may be an "early warning" of either higher loss or higher disenchantment in the future. The large group of "undecided" doctors may be open to persuasion, through good subsequent experience of work, to remain in Britain to practise.

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Role of medical factors in 1000 fatal aviation accidents: case note study

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Sudden illness in flight is said to cause 1.5% of fatal general aviation accidents.[1] This study reviews the experience from the United Kingdom.

Methods and results

We reviewed the findings of 1000 consecutive accidents between 1956 and 1995 for which a consultant from our department either performed or attended the necropsies. Medical or toxicological factors caused or were a contributory cause in 47 accidents (table 1). Cardiac disease in the pilot was the most common factor. In one case the collapse of a front seat passenger in a light aircraft was thought to have distracted the pilot and caused the accident. The other medical causes in private pilots included nine cases of alcohol intoxication and three definite suicides. Central nervous system disorders contributed to seven accidents; three pilots were thought to have had epileptic fits, one had encephalitis, one a pituitary tumour, one haemorrhage from a cerebellar arteriovenous malformation, and one with a history of migraine radioed a report of visual disturbances and numbness before his crash.

Comment

Finding disease in the crew does not mean that it is the cause of the accident. Usually it is a coincidental finding. The main problems of interpretation are that the signs of trauma are superimposed on the disease process and that the victims often have such serious injuries that meaningful examination of their organs is impossible. For example, we know of a case where a young helicopter pilot collapsed on his way to his aircraft. He was admitted to hospital where he died of a haemorrhage into a cerebral metastasis from a minute testicular teratoma. Had he died while flying his brain would probably have been severely traumatised. Even if the tumour had been found it would have been difficult to determine if the haemorrhage caused the accident or was caused by it.

The history of the flight and accident is essential for accurate interpretation of pathological findings in aviation accidents. In this series most of the pilots had cardiac disease. Often there are no signs of acute changes and pathologists rely entirely on history. Haemorrhage into an atheromatous plaque has been seen occasionally, and in these cases staining for iron showed the presence of haemosiderin laden macrophages suggesting that there had been previous bleeds into the plaque. This contrasts with the bleeding caused by direct trauma to the heart which is adventitial rather than within the plaque and has no demonstrable haemosiderin.

The commonest cause of incapacitation in flights not resulting in accidents is neurological disorders.[2 3] However, neurological disorders were under represented in our series, probably because of the difficulty in postmortem diagnosis and the severe cerebral damage that often occurs in aviation accidents.

The 2.4% rate of alcohol intoxication in private pilots is comparable with that reported elsewhere[4] but is much less than the third of private pilots quoted in the British Medical Association booklet *Alcohol and accidents*.[5] Interestingly, five pilots were clearly drinking while flying as the remains of spirit bottles were found in the wreckage. One of these cases may have been suicide and in three others in which the pilot was not intoxicated we are certain that the pilot took his own life.

Table 1 Role of medical factors in fatal aviation accidents

Category	Total accidents	Medical factors		
		Cardiac	Other	Total (%)
Glider	67	6	2	8 (12)
Private	375	9	17	26 (7)
Commercial	114	4	1	5 (4)
Military	407	3	5	8 (2)
Parachutists, hang gliders	37	0	0	0
Total	1000	22	25	47 (4.7)

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Drug points

Interaction of thyroxine sodium with antimalarial drugs

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Every year over 25 million non-immune people visit areas of the world where the prevalence of malaria is high. Although *Plasmodium falciparum* is resistant to the combination of chloroquine and proguanil, this type of prophylaxis is still common. To date, few adverse side effects and hardly any drug interactions have been described with these drugs. We report a case of interaction between thyroxine sodium and chloroquine and proguanil.

A 52 year old white woman, who had no history of medical illness except for hypothyroidism, which was stabilised by treatment with thyroxine sodium 125 µg daily, went to Mali for two weeks. As prophylaxis against malaria she was given chloroquine 100 mg daily and proguanil 200 mg daily for two months, starting on the day of intended travel. Four weeks after starting prophylaxis her thyroid stimulating hormone concentration was 44.8 mU/l (normal range 0.35-6.0 mUI/l) at a routine visit. The regimen for thyroxine sodium was not changed. Chloroquine and proguanil were stopped as intended two months after the day of intended travel, and a week later her concentration of thyroid stimulating hormone was back to normal.

After 16 months—during which she had received the same regimen of thyroxine sodium—she spent two weeks in Indonesia. The same prophylaxis was prescribed. Because of what had happened the previous year her concentration of thyroid stimulating hormone was checked before her departure (3.2 mU/ml), when she came back (26 mU/l), and eight weeks after starting the prophylaxis (54.7 mU/l). When she came back free tri-iodothyronine concentration was 2.7 pmol/l (normal range 2.6-5.9), free thyroxine concentration 11 pmol/l (6-18), and thyroglobulin concentration 1.1 pg/l (< 25). The dose of thyroxine sodium remained unchanged. Four weeks after the end of prophylaxis her concentrations of thyroid stimulating hormone and free tri-iodothyronine had returned to normal (0.7 mU/l and 14.6 pmol/l, respectively).

One of the antimalarial drugs might have had a central effect on the hypothalamus, but a drug interaction between thyroxine sodium and chloroquine and proguanil seems more likely to have enhanced the induction of liver enzymes. In this case chloroquine probably increased the catabolism of thyroid hormones by enzymatic induction. Chloroquine reduces the sedation produced by diazepam,[1] but it enhances the effect of cyclosporin.[2]

Intrinsically, such an interaction[3] is likely because it recurred when the drug was reintroduced and because the concentration of thyroid stimulating hormone was normal before antimalarial treatment. To our knowledge, this is the first reported case of an interaction between chloroquine and thyroxine sodium, although interactions with chloroquine have been reported in association with ciprofloxacin and methotrexate.[1 2 4 5]

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Acute renal failure due to rhabdomyolysis in presence of concurrent ciprofibrate and ibuprofen treatment

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We report a case of acute renal failure due to rhabdomyolysis which occurred while the patient was taking ciprofibrate and ibuprofen.

A 29 year old Asian man with type M hyperlipidaemia attended the lipid clinic with characteristic xanthomata. Initial cholesterol and triglyceride concentrations were 14.8 mmol/l and 6.86 mmol/l, respectively. In addition to a lipid lowering diet, he received ciprofibrate 100 mg daily, which resulted in his cholesterol concentration dropping to 8.2 mmol/l and his triglyceride concentration to 3.3 mmol/l. Further improvement followed when the dose was increased to 200 mg daily, cholesterol and triglyceride concentrations falling to 6.6 mmol/l and 1.9 mmol/l, respectively.

After six months of treatment and three weeks after tests showing normal liver function and creatine kinase activity he developed a painful heel. He obtained ibuprofen 200 mg over the counter, and the dose was increased to 400 mg by his general practitioner. The pain became generalised, his urine turned "muddy," and he presented as an emergency complaining of a "stiff body." Urea concentration was 6.9 mmol/l. Intravenous urography was performed because renal colic was suspected. Two days later he developed renal failure, which required transfer to a renal unit (urea concentration 23 mmol/l, creatinine concentration 647 µmol/l, potassium concentration 6.2 mmol/l). Creatine kinase activity was 13 740 U/l. Subsequently, he recovered fully.

In the month that this patient developed renal failure (April 1995) the data sheet for Modalim (ciprofibrate) was amended, reducing the maximum recommended dose to 100 mg daily because of the high incidence of rhabdomyolysis reported in France with 200 mg.

Ibuprofen and ciprofibrate are heavily bound by protein (Sanofi Winthrop, technical brochure for ciprofibrate, 1992)[1 2] and contain propionic acid groups.[2] We postulate that ibuprofen displaced ciprofibrate, making what had been a safe dose for our patient become toxic, causing rhabdomyolysis and renal failure. This situation was probably exacerbated by radiological contrast medium. This case is important because ibuprofen can be bought without a prescription and can affect the pharmacokinetics of concurrent drug treatment.

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