could not be stated with any conviction for the patients in the retrospective study, many of whom died while still hypothermic and before a definite diagnosis was made.

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Paget's disease of bone: the Lancashire focus

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Summary and conclusions

The radiological prevalence of Paget's disease of bone has been studied in 31 towns in Britain. A remarkably localised area of high prevalence has been shown in Lancashire. Although environmental influences seem dominant in the aetiology of the disease, no hypothesis about the environmental cause of the Lancashire focus can be advanced.

Introduction

In a recent survey of the radiological prevalence of Paget's disease of bone in 14 towns in England and Wales the highest prevalences recorded were in three towns in Lancashire.[1] To investigate further this apparent focus of high prevalence in Lancashire, and to define its boundaries, we extended the survey to include an additional 17 towns.

Methods

The 17 towns were Aberdeen, Birkenhead, Blackpool, Burnley, Carlisle, Chester, Glasgow, Lancaster, Macclesfield, Middlesbrough, Newcastle upon Tyne, Oldham, Portsmouth, Rochdale, Warrington, Whitehaven, and Wigan. They were selected to include areas of Britain not studied in the initial survey, and to provide additional data on Lancashire and the towns bordering it. The survey method was identical with that used previously.[1] In each town a sample of abdominal radiographs of people aged 55 and over was taken from the stored films within the radiological department of a general hospital. The selected films showed the entire pelvis and sacrum, the femoral heads, and all lumbar vertebrae—sites that are affected in 95% of patients with Paget's disease.

Wherever possible samples of about 1000 radiographs were drawn for each town, with similar numbers for men and women. In some towns the records systems were such that retrieval of radiographs, of the kind required and for patients of known age, was excessively time-consuming. In these circumstances smaller size samples were used. The films were initially classified by a trained observer (ATC) into three groups: positive (unequivocal signs of Paget's disease), doubtful, and negative. A second observer (PBG, a radiologist) then examined all the positive and doubtful films and a one-in-ten sample of the negative ones. Standardised criteria were used for the diagnosis of Paget's disease. In the previous survey the radiologist's observations were shown to have a high level of repeatability. After completion of the present survey a sample of 109 films from six towns was re-examined to determine whether this level of repeatability had been maintained.

Results

The table shows the prevalence of Paget's disease in the 17 towns, together with those in the 14 towns in the previous survey. The prevalence rates for each sex were directly standardised to allow comparisons corrected for the differing age distributions of the subjects in the various towns. The standardisation procedure used five-year age distributions from 55 to 90 and over. The combined population of the 14 towns was again used as the standard so that results of the two surveys are directly comparable. In the table the towns are listed in descending order of the overall age- and sex-standardised prevalences (final column), which varied from 8·3% in

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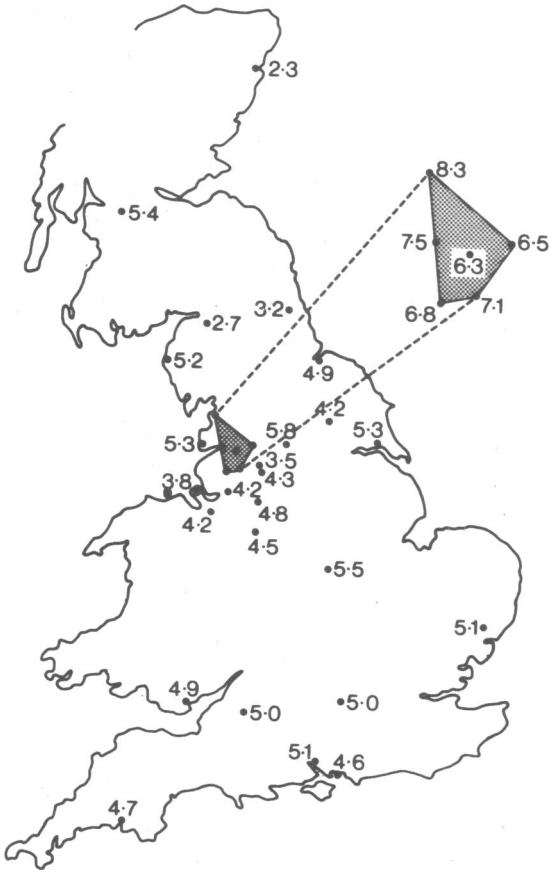
Lancaster to 2·3% in Aberdeen. A map displaying these prevalences (figure) shows that rates above 6·0% occur only in a cluster of six Lancashire towns—Lancaster (8·3%), Preston (7·5%), Bolton (7·1%), Wigan (6·8%), Burnley (6·5%), and Blackburn (6·3%). Outside this small area of high prevalence the rates fall sharply, with the bordering towns having prevalences of around the average for all towns. Of special interest is the sharp drop that is seen between towns lying within and just outside the area. For example, Wigan and Warrington are only 10 miles apart but the prevalences are 6·8% and 4·2% respectively. There is no evidence of any other focus of high prevalence in the country.

Prevalence of Paget's disease among hospital patients aged 55 years and over in 31 towns

Town	No of patients	No with disease	Prevalence (%) of Paget's disease		
			Men* (n = 14 444)	Women* (n = 14 610)	Both sexes†
Lancaster	626	58	6·5	10·0	8·3
Preston	1000	82	8·6	6·3	7·5
Bolton	602	42	7·7	6·4	7·1
Wigan	600	42	8·1	5·4	6·8
Burnley	979	74	8·2	4·9	6·5
Blackburn	595	39	8·8	3·8	6·3
Bradford	1000	59	7·9	3·6	5·8
Glasgow	938	50	6·3	4·6	5·4
Leicester	1021	57	7·8	3·1	5·5
Hull	1000	53	7·6	3·1	5·3
Blackpool	949	63	6·5	4·1	5·3
Whitehaven	1002	58	7·1	3·4	5·2
Ipswich	997	50	6·5	3·8	5·1
Southampton	1000	53	6·6	3·6	5·1
Reading	989	56	7·3	2·7	5·0
Bath	998	52	5·3	4·7	5·0
Middlesbrough	734	39	5·9	3·9	4·9
Cardiff	999	41	6·6	3·3	4·9
Macclesfield	890	47	5·3	4·4	4·8
Plymouth	959	48	6·8	2·7	4·7
Portsmouth	999	55	5·4	3·9	4·6
Stoke	1000	40	4·7	4·2	4·5
Oldham	917	45	5·4	3·2	4·3
York	1000	41	5·8	2·5	4·2
Chester	970	43	5·6	2·9	4·2
Warrington	809	37	4·5	3·9	4·2
Birkenhead	994	39	4·4	3·2	3·8
Rochdale	1104	54	4·0	3·1	3·5
Newcastle	1002	32	3·9	2·6	3·2
Carlisle	1482	44	3·9	1·5	2·7
Aberdeen	899	23	2·0	2·6	2·3
All towns	29054	1516	6·2	3·9	5·0

*Age-standardised rates. †Age- and sex-standardised rates.



Age- and sex-standardised prevalences of Paget's disease among hospital patients aged 55 years and over in 31 British towns.

In the previous survey it was shown that the radiological prevalence of Paget's disease is higher in radiographs taken specifically to show the skeleton than in other radiographs of the pelvis—namely, those taken during intravenous pyelography, barium studies, and plain abdominal examinations. In the combined results for the 31 towns the age-standardised prevalence was 6·3% in skeletal radiographs (which comprise about 20% of all the radiographs) compared with 4·6% in the remainder. The prevalence recorded in a town will therefore be influenced by the proportion of the radiographs which were skeletal. Standardisation to allow for the differing proportion of skeletal radiographs, however, has little effect on the ranking of towns according to prevalence as shown in the table. Indeed, the difference between the six Lancashire towns with a high prevalence and the remainder is somewhat enhanced by this standardisation.

The extent of between- and within-observer variation in the interpretation of the radiographs was assessed. In each town the radiologist examined a 10% sample of films initially classified as negative. For only two radiographs out of about 1500 was reclassification necessary. In the two towns where this occurred the radiologist examined an additional 20% sample of negative films to ensure that no systematic bias, perhaps due to quality of radiographs, had occurred. No further reclassifications were necessary.

At the end of the survey the radiologist re-examined a sample of 109 radiographs drawn from six towns. Of this sample 45 (41%) had been initially classified as doubtful, a proportion far greater than that of 2·2% in the main survey. The radiologist's re-examination was carried out in such a way that he was unaware of his earlier report on the films. The agreement between the first and second examination was 88%, with only 13 radiographs reclassified. These 13 films, all initially classified as doubtful, were drawn from four towns, and there was no evidence of a systematic change in the radiologist's diagnostic criteria during the course of the survey. When the results of this observer variability study were put in the context of the mix of radiographs classified in the town survey they indicated an overall within-observer agreement level greater than 99%.

Discussion

This survey of Paget's disease in 31 towns in Britain has shown a remarkably localised area of high prevalence within Lancashire. Although the prevalence of the disease among patients attending for routine radiological investigations is a biased estimate of the true prevalence of the disease in the community, analyses carried out in the initial survey suggest that comparisons between towns will not be greatly affected by these biases. The higher prevalences in the six Lancashire towns did not depend on a higher level of clinical suspicion of Paget's disease among doctors referring patients for skeletal radiographs. A study of radiology request forms in Blackburn and Bolton showed that in patients with radiological evidence of Paget's disease this diagnosis was rarely stated on the form. The higher prevalences in the six towns did not depend only on the rates recorded in skeletal radiographs. Among other radiographs of the pelvis—namely, those taken during intravenous pyelography, barium studies, and plain abdominal examinations—the average age-standardised prevalence was 6·3% in the six towns compared with 4·3% in the remaining 25. Although the prevalence was higher among patients referred for skeletal radiographs, most of whom would presumably have had skeletal symptoms such as backache, standardisation to allow for the differing proportions of skeletal radiographs did not alter the rankings of the six towns according to prevalence.

The results of epidemiological studies in Australia and America[2 3] indicate that environmental influences are dominant in the aetiology of the disease. We can advance no hypothesis about the environmental cause of the Lancashire focus. There are no geological or climatic characteristics exclusive to the area. Although the drinking water in the area is soft, and is supplied by what was formerly the Lancashire River Authority, it has

no known unique quality. Historically a major commitment to the cotton industry has been a feature of the high prevalence area, yet other towns also associated with the cotton industry—for example, Rochdale—have low prevalences.

We are most grateful to the radiologists, radiographers, and x-ray records staff in the towns surveyed. Their kindness and help made the survey possible. The study was funded by a grant from the Department of Health and Social Security.

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SHORT REPORTS

Prenatal chromosomal analysis of fetal blood obtained at fetoscopy

Cultivation of amniotic fluid cells for prenatal diagnosis may take several weeks. But the fetal karyotype in a mother who presents in an advanced stage of pregnancy may need to be determined within a few days. We describe here a method of doing a fetal chromosomal analysis within 72 hours. Fetoscopy samples of mixtures of fetal blood and amniotic fluid[1 2] are cultured by a modified technique of microculturing whole blood. We also report our first clinical application of the method.

Patients, methods, and results

The fetuses of 14 women in the 16th to 23rd week of gestation were studied. Thirteen of the women had been admitted for therapeutic abortion. In one of them fetal trisomy 18 had been diagnosed by amniocentesis. The remaining woman was a case for diagnosis. She had had a child with Down's syndrome. In the 22nd week of her gestation it became obvious that a second attempt to culture amniotic fluid cells had failed. Since she refused to accept the risk of having another affected child she agreed to prenatal diagnosis by analysis of fetal blood obtained at fetoscopy.

The site of the placenta and the position of the fetus were determined by ultrasound immediately before fetoscopy. Under direct visual control (Dyonics Needlescope) a placental vessel was punctured without any attempt at cannulation. A sample (2 ml) of blood mixed with amniotic fluid was aspirated and immediately transferred to a glass tube containing 3·5 IU heparin. After centrifugation at 125 *g* for 10 minutes the supernatant was discarded and the cell button was deposited in a 6-ml Falcon plastic tube containing 1 ml McCoy's 5a medium (Flow Laboratories) with 20% fetal bovine serum and 0·026 ml phytohaemagglutinin supplemented with streptomycin and benzyl penicillin. After incubation for 65-70 hours at 37°C cell growth was stopped by adding colchicine in a final concentration of 0·125 μg/ml. After hypotonic treatment with potassium chloride 0·075 mmol/l for 10 minutes at room temperature the cells were fixed in three parts methanol and one part acetic acid. Slides were prepared with the standard air-drying technique. The chromosomes were stained with the trypsin-Giemsa banding technique according to Seabright[3] as slightly modified by Özkinay and Mitelman.[4]

Analysable metaphase plates were obtained in 12 out of the 14 cases (table). The fetus of the woman who had borne a child with Down's syndrome had a normal karyotype (46,XY) and at birth was normal, as predicted. The diagnosis in the case of fetal trisomy 18, established by amniocentesis, was verified in the fetoscopy sample. Examination of the fetuses after the abortion showed that sex prediction was correct in all of them.

*Chromosomal analysis in fetoscopy samples**

Fetoscopy case No	Gestational age (weeks)	Amount of blood (μl)	No of analysable metaphases	Karyotype
1	18	130	2	46,XY
2	16	70	3	46,XY
3	19	<10	0	—
4	19	400	58	46,XX
5	22	280	>100	46,XX
6	23	90	73	46,XX
7	19	20	1	47,XY,+18
8	19	<10	0	—
9	17	30	7	46,XY
10	18	40	4	46,XY
11	22	50	>100	46,XX
12	18	280	>100	46,XX
13	22	130	24	46,XX
14†	22	520	>100	46,XY

*Mixtures (2 ml) of fetal blood and amniotic fluid. The percentage of fetal red blood cells (Kleihauer technique) was 80-90% in samples in cases 1 and 4; the remaining samples contained >95% fetal red blood cells.
†Diagnostic fetoscopy in a woman who had borne a child with Down's syndrome.

Comment

The essential step in the procedure seems to be centrifuging the mixed sample before culture. Our first attempts to culture the cells without previous centrifugation usually failed. Pure fetal blood uncontaminated with amniotic fluid[5] would certainly be more suitable for culture than a mixed sample. But sampling pure fetal blood at fetoscopy requires cannulation of the placental vessel with a risk of penetrating the chorionic plate and consequent admixture of maternal blood in the sample. The procedure proved diagnostically reliable in the clinical case. Within three days we could tell the woman that the results of our chromosomal studies were normal. This was later confirmed by the birth of a normal infant.

Although amniocentesis remains the method of choice for prenatal diagnosis of chromosomal abnormalities the procedure described may prove a useful alternative when it is impracticable to wait about three weeks for the results of amniotic fluid cell culture.

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"Catabolic" loss of body protein after human liver transplantation

Little is known about the metabolic response to orthotopic liver transplantation. The trauma and length of the operation would be expected to evoke a major "catabolic" response in normal patients. Furthermore, the routine postoperative high doses of prednisolone to prevent graft rejection would be expected to increase catabolism further. On the other hand, such a response, with increased urea nitrogen excretion in the urine, would be suppressed if the transplanted liver failed to function normally since protein is oxidised and urea synthesised by the liver. Moreover, patients depleted of protein,