HSE

Health and Safety Executive

Feasibility of using urinary biomarkers to identify occupational musculoskeletal disorders of the lower limbs

Prepared by the **Health and Safety Laboratory**
for the Health and Safety Executive 2010

RR773
Research Report

HSE

Health and Safety Executive

Feasibility of using urinary biomarkers to identify occupational musculoskeletal disorders of the lower limbs

Howard Mason
Health and Safety Laboratory
Harpur Hill
Buxton
Derbyshire
SK17 9JN

Interest has developed in biomarkers in body fluids, which are indicative of degradation of bone, cartilage and synovial tissue. These are currently being investigated in the clinical context in terms of diagnosis, prognosis and efficacy of treatment for rheumatoid and osteo-arthritis. There is also interest in the use of such biomarkers in sports medicine and now occupational medicine. The cause of occupational lower limb musculoskeletal disorders may involve complex multifactorial issues, such as psychological, and organisational factors as well as abnormal biological processes caused by over-use or abnormal loading of the knees, hips and ankles during various work activities. There is an interest in whether non-invasive biomarkers of bone, cartilage and synovium metabolism may add to other tools by objectively identifying the involvement and extent of specific abnormal biological processes in those who present with lower limb problems. Such biomarkers could be cost-effective tools in studying the efficacy of various intervention strategies in controlling the risk and incidence of occupationally related lower limb musculoskeletal disorders.

This report and the work it describes were funded by the Health and Safety Executive (HSE). Its contents, including any opinions and/or conclusions expressed, are those of the author alone and do not necessarily reflect HSE policy.

HSE Books

First published 2010

CONTENTS

EXECUTIVE SUMMARY

Objectives

This project was initiated as a pilot study to investigate whether non-invasive urinary biomarkers may have value as objective measures of early, occupational musculoskeletal disorders of the lower limbs (LLMSDs).

Background

Interest has developed in biomarkers in body fluids, which are indicative of degradation of bone, cartilage and synovial tissue. These are currently being investigated in the clinical context in terms of diagnosis, prognosis and efficacy of treatment for rheumatoid and osteo-arthritis. There is also interest in the use of such biomarkers in sports medicine and now occupational medicine. The cause of occupational LLMSDs may involve complex multifactorial issues, such as psychological, and organisational factors as well as abnormal biological processes caused by over-use or abnormal loading of the knees, hips and ankles during various work activities. There is an interest in whether non-invasive biomarkers of bone, cartilage and synovium metabolism may add to other tools by objectively identifying the involvement and extent of specific abnormal biological processes in those who present with lower limb problems. Such biomarkers could be cost-effective tools in studying the efficacy of various intervention strategies in controlling the risk and incidence of occupationally related LLMSDs.

Study design and population

A cross-sectional cohort study was undertaken involving three working cohorts and two case cohorts. The power calculation for the study had defined overall case and working cohort sizes of fifty plus as adequate. These cohort sizes were achieved, even though the tracing of relevant, occupational cases proved difficult. The two case cohorts were individuals, aged between 18-65 years, and were derived from either GP referrals to hospital specialists with significant problems in their lower limbs (n=38) or those who had been occupationally redeployed due to such problems (n=24). Two working cohorts were from the major construction sector (n=60) and other trades at high risk of LLMSDs (n=59); the third working cohort (n=27) consisted of staff at the Health and Safety Laboratory. Informed consent was gained from all volunteers. Working and case cohorts were investigated using urine measurements of the c-telopeptides of collagen I and collagen II and applying two validated quantitative questionnaires. The questionnaires used were the well-established, generic SF12 quality of life questionnaire with summary physical and mental components and the clinically validated Rheumatoid and Arthritis Outcome Score (RAOS) questionnaire specific for symptoms and functional problems related to joints of the lower limbs. Both questionnaires give quantitative outcome scores across a number of health-related dimensions.

Main Findings

This cross-sectional study suggests that at least one urinary biomarker may have potential value as an objective measure of occupational-associated LLMSD. Urinary c-telopeptide of collagen II, as a marker of cartilage breakdown, had largely the same discriminate power in distinguishing the working cohorts from case cohorts as did several item scores in the RAOS questionnaire. Other finding related to c-telopetide collagen II, perceived pain and discrimination between case and working cohorts are noted in points 3 and 4 below. In contrast, the equivalent c-telopeptide collagen I marker showed no discriminant power between cases and

working cohorts, suggesting that increased bone turnover was not a significant feature in the LLMSD cases.

Other findings include;

1. The SF12 scores showed that perceived general physical health in the working cohorts was good in comparison to published, general population norms. However, the RAOS scores suggested that some lower limb problems were to be found within the working cohorts. The 'case cohorts' showed significantly poorer perception of both their general physical and mental status in comparison to general population norms. Those of the occupational cases in employment at the time of the study tended to have better RAOS and SF12 scores that those not in employment or the clinical cases. This was statistically significant for the physical summary score of the SF12 quality of life questionnaire and the pain and sport items of the RAOS questionnaire.

2. The distributions across the cohorts of item scores from the clinically validated RAOS questionnaire suggested that this questionnaire may be an appropriate tool for use in occupational studies of lower limb problems, giving quantitative indices of perceived extent of symptoms and deficit in functionality. Further analysis (see 4 below) may indicate that questions related to the pain item score may be particularly relevant.

3. Increased perceived pain (RAOS item) in the lower limb joints was statistically correlated to higher levels of urinary c-telopeptide of collagen II in all subjects, and also within the combined working cohorts. However the levels of correlation (Spearman Rank correlation coefficients of 0.58 and 0.34 respectively) suggested that neither was a good surrogate of the other.

4. Both perceived pain (RAOS item) and urinary c-telopeptide of collagen II were significant variables in distinguishing subjects in case cohorts from those in working cohorts. High levels of current or past involvement in sports involving the lower limbs were not significant explanatory variables.

5. Our estimate of the total intra-individual coefficient of variation (biological and analytical) of around 50% for c-telopeptide of collagen II for single, spot urine samples suggests that care would need to be exercised about interpretation for an individual based on a single sample. Higher values of this biomarker may also be encountered in pregnancy and those in their adolescent growth phase.

Recommendations

The use of biomarkers of cartilage degradation, such as c-telopeptide collagen II, should be considered in occupational health cohort studies of LLMSDs.

Consideration should be given to the use of the quantitative RAOS and SF12 self-reporting questionnaires in studies of LLMSDs in order to capture quantitative data on perceived poor health outcomes

Due to cost/time constraints together with technical issues, we were not able to measure the biomarker related to synovium tissue degradation that had also been identified as a possible useful candidate marker. Any further investigation of these urine biomarkers should consider its incorporation.

1 INTRODUCTION

This is a pilot study investigating whether two non-invasive, urinary biomarkers of collagen metabolism may have potential value as an objective measure of early, occupational musculoskeletal disorders in the lower limbs (LLMSD).

Considerable effort in recent years has been expended in developing diagnostic tests of 'later life diseases', which include those related to joints and bones. While radiological and imaging methods have been considered as gold-standard diagnostic tests, several assay systems based on body fluid measurements have been shown to have some value in the diagnosis and monitoring of osteoarthritis and rheumatoid arthritis[1-6]. Some of these assays have been commercialised diagnostic assays (Chondrex DCR/YKL40; Cartilaps, Cross-laps Nordic Biosciences). HSE has identified that assays measuring cartilage breakdown products as indices of osteoarthritic conditions may have potential use in the occupational context [4]. Some MSDs, particularly those related to the lower limbs, are due to occupational over-use and abnormal loading of joints, and may reflect the pathological (and age-related) process of increased destruction of normal cartilage and structural components in bone-joints. Thus they may reflect an osteoarthritic state. However, it is recognised that psychological and psychosocial factors may also be important in the presentation of LLMSDs [7-9].

Many of these body fluid assays related to abnormal biological processes are non-invasive measurements and are attractive as being readily applicable in the occupational setting. They are also being investigated in sports medicine research [10]. However, the clinical value of these biomarkers is not fully undisputed [11], but they potentially offer objective evidence of a physical problem and its extent, which would otherwise have to rely on subjective, self-reporting of largely non-specific symptoms. The issue of reported pain reflecting psychosocial and organisational pressures, rather than a physical problem, is well accepted [7, 8], but not necessarily easily distinguished from that purely related to a physical problem. Thus a validated biomarker sensitive to the early stages of clinically relevant joint disorders, and based on urine samples, could be a cost-effective tool to study the efficacy of various intervention strategies in controlling the risk and incidence of physically-driven occupational LLMSDs.

Markers of collagen type I metabolism have been associated with bone turnover, while marker of collagen type II have been associated with cartilage turnover [12]. The urinary excretion of c-telopeptide of collagen II has been shown to increased in both osteoarthritis and rheumatoid arthritis [13]. Urinary levels of glucosyl-galactosyl pyridinoline have been suggested as useful measures of degraded synovial tissue in joints [5].

Clinical studies have also recognised that outcomes such as pain and functional problems are not necessarily closely related to X-ray measurements of joint destruction, which is the 'gold standard' of diagnosing osteoarthritic joints. However, there are differences between the clinical and occupational context that makes further investigation of these suggested biomarkers warranted. The clinical use of biomarkers is as a cost-effective and early means of helping to diagnose a specific disease entity in an individual who presents with significant symptoms, or in monitoring the efficacy of treatment in individual. More invasive tests, such as blood tests are also more acceptable in the clinical environment than in the occupational health setting. In the occupational context, the health outcomes which impinge on employment status and working days lost are likely to include severe joint pain and functional loss, and the value of biomarkers would be as prognostic indicators of real functional loss and/or pain symptoms reflecting physical damage. Biomarkers may still have value even if their 'diagnostic power' in the individual is limited. In this case they may still allow on a cohort basis the identification of specific work activities associated with likely poor health outcomes that have not been

previously identified, or confirm evidence of biologically-driven poor health outcome in trades which has previously relied on anecdotal information from self-reporting or staff turn-over data. Also in the occupational setting, biomarkers based on urine measurements are invariably sought, because they are non-invasive, cost-effective to collect and readily obtainable in workplace studies.

While the establishment of a biomarker of LLMSDs would be useful in the occupational setting, currently we have to rely on self-reporting questionnaires for health outcomes. Concerns about the reliance on questionnaires as health outcome measures centre around their subjective nature, their potential for bias and that invariably they have been validated as cohort tools rather than as risk indicators for an individual. Such questionnaires cannot identify that problems being suffered by an individual, or within a cohort, reflect the extent of a real abnormal biological process as opposed to other psychological or organisational factors.

Several self-reporting questionnaires have been validated and formalised for use in the clinical investigation of LLMSDs and the study of the efficacy of interventions/treatments [14-16]. Many of these questionnaires include quantitative scores across separate dimensions of pain, other symptoms, Activities of Daily Living and Quality-of-Life. They have also been investigated in terms of their validity, reliability, consistency, and responsiveness to various specific conditions and interventions. Likewise generic Quality-of-Life questionnaires, such as the SF36 or SF12, have been very widely utilised and shown themselves useful in quantitatively comparing cohorts against general population scores [17]. Our experience in Hand-Arm Vibration Syndrome (HAVS has convinced us that employing established, quantitatively scored questionnaires, where both validation and comparative data are available, are more useful as health outcome measures than using (a) descriptive questionnaires, (b) ad-hoc alteration of existing questionnaires or (c) formulating new questionnaires. There is also benefit from using either generic health outcome questionnaires, or quality-of-life questionnaires, as well as those specific for the lower limbs. They may allow comparison with population normal values and other occupational health problems, and allow some identification of the level of 'psychological distress' involved. Such an approach has to be balanced by the possibility of redundancy in questionnaires and volunteer fatigue in completing a number of questionnaires. The established questionnaires we have used are relatively quick to complete (e.g. 10 minutes to complete) and have been well trialled in terms of volunteer understanding and ease of answering.

This study employs a combination of urine biomarkers associated with cartilage turnover and joint/bone damage and a set of validated questionnaires covering specific lower limb symptoms and functionality (Rheumatoid and Arthritis Outcome Score questionnaire (RAOS)), generic quality of life (SF12 questionnaire) and demographic/work history information. The ROAS questionnaire was employed as it is self-administered, has high reliability, its construct validity determined with the SF36 and has been relatively widely used [15]. It also includes the questions which form the well-established Western Ontario MacMaster (WOMAC) questionnaire which has been validated in subjects with osteoarthritic hips and knees [18, 19]. The SF12 quality of life questionnaire was included because of its widespread use in the public health arena and that it produces physical and mental summary scores compatible with the longer SF36 questionnaire.

This study is of a 'pilot' nature, attempting to see if the urine biomarkers discriminate between normal subjects and those who would be classified as suffering from LLMSD and the relationship between urine biomarkers and quantitative scores derived from self-administered symptom/functionality questionnaires in workers.

2 POPULATION AND METHODS

2.1 POPULATIONS AND STUDY DESIGN

The first part of the study investigated the variability of the urine markers across the working day. This is an important factor to be defined as any interpretation of abnormality, either from a single spot urine sample biomarker or serial samples over time, depends on the likely extent of normal variation in its excretion. The two urine biomarkers investigated are recommended to be creatinine corrected (www. Nordicbioscience.com). Creatinine correction is relatively standard practise in the UK for the majority of samples taken for biological monitoring or biological effect monitoring purposes, where interpretation is often routinely based on the results from a single spot urine sample.

Volunteers for study of intra-individual variability were recruited from Health and Safety Laboratory (HSL) and deemed as non-manual working referents. There were no exclusion criteria applied and volunteers were anonymous to the research team. The questionnaires and two urine collection bottles were supplied to volunteers, requesting on a single day the completion of the questionnaire and collection of 'start of working day' and 'end of working day' spot urine samples. These were returned to the sample reception office at HSL. Urine samples were stored at –40C until analysis. Analysis was carried out with samples randomised in batches.

The second part of the study applied the same questionnaire and collected a single, random urine sample in a range of volunteers recruited from;

(a) GP referrals to specialist clinical units because of functional and/or symptoms in their lower limbs, these were termed 'clinical cases'. None of these subjects were working at the time of the study.
(b) Workers who were currently not working or had been re-deployed to another job due to a LLMSD condition- these subjects were identified through workplace management and occupational health professionals and are termed 'occupational cases'. In the main, those in employment at the time of the study were sedentary workers.
(c) A range of occupations where significant LLMSD have been reported (e.g. floor-layers/carpet fitters, construction trades such as carpenters and roofers, freight logistics)
(d) Workers within the construction industry as defined by SIC codes undertaking major construction activities.

Significant attempts were made to trace and include subjects from workplaces who had, or had had, an LLMSD recognised by occupational health professional and in some cases were no longer undertaking their original trade. However, the study did not attempt to be truly cross-sectional of any work-activity or industry sector. It was an attempt to collect a significant number of individuals with LLMSD problems that were likely to be occupationally associated, as well unselected working subjects.

Inclusion criteria for clinical 'cases' were to be of working age (18-65 years), reporting significant problems in their hips, knees or ankles that were not related to a recent acute trauma. In many cases a formal diagnosis had not been made, but the majority were thought to have an osteoarthritic condition in their knees and/or hips.

Inclusion criteria for 'occupational cases' were workers who were aged 18-65 years and had reported significant problems in their hips, knees or ankles in the last six months, which had

been reflected by a change in job or currently being off-sick, but unrelated to any recent, acute trauma. These cases had been either notified through an occupational health provider or workplace management. Such cases may be in some form of work at the time of study.

Inclusion criteria for volunteers in the working populations were to be of working age (18-65 years) and currently in employment, with no changes in job or work activities within the last six months because of health issues. These were approached through their workplace management. While the large majority of these subjects were current manual workers, a number of them were previously manual workers, now undertaking managerial functions and a few were sedentary workers.

2.2 URINE BIOMARKERS

Two biomarker measurements were undertaken. The measurements in urine of c-telopeptide of collagen type II have been shown to reflect cartilage degradation and turnover in animals and humans [4, 20]. The c-telopeptide of collagen type I reflects bone turnover. Some initial work was carried out on the synovial biomarker glucosyl-galactosyl pyridinoline [5], but technical difficulties precluded its use in the main study.

Other biomarkers of bone or cartilage metabolism that have been measured and validated in blood serum were not included, as such samples would always be impractical to collect except in a research occupational setting. This excluded assays such YKL-40 [6, 21], cartilage oligomeric matrix protein (COMP), N-propeptide of collagen I, N-propeptide of collagen II, hyaluronic acid [22, 23].

This study used commercially available enzyme immunoassay kits- 'Cartilaps' for collagen type II and 'Cross-laps' for collagen type I (Nordic Bioscience Diagnostics A/S Herlev, Denmark). In our hands the within-day analytical imprecision of both these assays is 7-8%; the between-day imprecision is 12-14%. The assays were carried out according to the kit instructions, using a robotic system to automate and standardise all elements of the assay.

Urinary levels of c-telopeptide collagen II have been shown to be increased in patients with hip osteoarthritis, correlated significantly with minimum joint space assessed by hip radiography and significantly differentiated on a mean basis between rapidly destructive from slowly progressive osteoarthritis [1]. Urinary levels of c-telopeptide collagen II have also been shown to be strongly associated with radiographic knee osteoarthritic disease severity in a well defined general population study of males with a mean age of 65 years [24], although in a clinical study of obese women, these markers levels were not able tot distinguish subjects with progressive radiographic or symptomatic knee osteoarthritis from those with stable disease [11]. Two other studies have shown C-telopeptide of both type I & II collagen to be predictive of radiographic damage over 1 and 5 years independent of rheumatoid arthritis disease activity and radiographic damage at baseline [25, 26]. In the ECHODIAH study (Evaluation of the CHOndromodulating effect of DIAcerein in osteoarthritis of the Hip -patients with hip osteoarthritis over a 3 year follow-up) c-telopeptide of collagen II was correlated with pain, functional impairment, joint space narrowing and subchodral sclerosis [22]. A recent sports medicine study showed that urinary C-telopeptide of collagen II was increased in training endurance athletes, with runners showing highest increases (mean +83%) over controls and was assumed to reflect stressed joints in the lower limbs in terms of cartilage remodelling or degradation [10].

There is conflicting evidence about the association between collagen I biomarkers of bone turnover and osteoarthritis, showing either increases [27], decreases [2] or no association [1]. This difference may be related to the definition, stage and site of osteoarthritis or the biomarkers used as markers of bone turnover, the body fluid used and other cohort factors. Interestingly a

recent occupational study of construction workers showed an increase in both serum carboxyterminal propeptide of collagen I, reflecting bone synthesis, and serum carboxyterminal telopeptide of collagen I reflecting bone breakdown [28]. These results may reflect adaptive bone remodelling in those undertaking heavy manual work rather than sedentary occupations. Interestingly the previously identified study of endurance athletes [10], who may reflect the extreme end of heavy manual occupational activity, also showed an increase in bone breakdown, but using a urine measurement of N-terminal rather C-terminal telopeptide of type I collagen [10]. Again the data may suggest that physical loading of the skeleton may influence bone remodelling, as increases in biomarkers of bone degradation are usually tightly coupled to rates of bone synthesis.

2.3 DEMOGRAPHICS AND QUESTIONNAIRES

The questionnaire included three elements, covering joint problems in the lower limbs, general quality of life and a limited amount of personal, occupational and medical data.

All subjects in the study completed two validated, and widely used, health-related questionnaires. These were the Rheumatoid and Arthritis Outcome Score for the lower extremity (RAOS questionnaire) and the SF-12v2 quality-of-life questionnaire.

The RAOS has been validated against the Health Assessment Questionnaire, SF-36 and Arthritis Impact Score [15, 29]. It covers 5 subscales, namely pain, symptoms, activities of daily living, sport/recreation and quality of life, derived from 42 items scored on a 5 choice Likert scale. Scores are normalised to 100 points for each subscale, with the accepted format of using higher scores reflecting better outcomes. The questions and scoring system replicate the original system [15]. We have also averaged across the subscales to give a simple average score.

The SF-12v2 questionnaire has increasingly been used in public health studies or clinical studies, specifically looking for general health outcomes after specific medical interventions [29]. The larger SF36 has been used in a general health study related to knee pain and disability [30]. The SF12v2 has been calibrated against the more widely used SF36 questionnaire, both producing summary scores for the physical and mental components of quality-of-life. The SF12 is a smaller questionnaire, only 12 questions compared to 36 questions in the SF36, allowing its completion in a shorter time. However the two summary outcome scores for the physical (PCS) and mental components (MCS) have been shown to be almost identical between the SF12 and SF36 [31]. There is a considerable published data including UK general population studies. However, standard practice has been to normalise SF36 and SF12 data to US general population data giving a mean of 50 with a standard deviation of 10. Recent data has suggested that normalisation of UK data to the US general population does not introduce significant basis in comparison to using a smaller UK general population database [32]. The scoring convention is that normalised SF12 summary scores above 50 are associated with better quality-of-life (mental or physical) than the general population mean, while lower scores are associated with poorer quality of life.

Basic personal data on sex, age, weight, height, and their past and present exposure to vigorous sport/exercise involving the lower limbs were collected. Body mass index (BMI) was estimated using the standard formulation (weight kg/height m^2) on self-reporting of height and weight. Volunteers' current trade was also collected. Medical history included questions on visits to their GP in the last 12 months for back, arm and lower limb problems, whether they have family

history of rheumatic disease and any current medication. A body map (front and back) was also included to allow subjects to note areas of current or recent pain.

3 RESULTS

Twenty-seven HSL-based referents completed the questionnaires and collected both urine samples. Thirty-eight case subjects derived from the clinical route, twenty-four case subjects derived from the occupational route, sixty working subjects involved in major construction activity and fifty-nine working subjects from high risk trades completed the questionnaires and collected a single urine sample. Those involved in major construction activity were derived from two companies. Occupational cases and those working in high-risk trades (mixed occupational cohort) were derived from a number of sources. Key work activities and trades included some specific construction trades [28, 33-35], floor layers [36-38]

3.1 NORMAL VARIATION IN URINE BIOMARKERS

The HSL volunteers had collected a urine sample at the start of the working day and a further sample at the end of the working day. Results for the two biomarkers were corrected for urine creatinine concentration. The geometric means (medians) of the morning and afternoon samples for c-telopeptide of collagen I were 265 (285) and 167 (158) μg/mmol creatinine respectively. These differences with the morning sample giving apparently higher c-telopeptide collagen I results were statistically significant (Wilcoxon test, $p<0.01$). Bland-Altman plot analysis suggested that on average the difference between the morning and afternoon samples in each individual was +43% of the average value of the two results with +/-two standards deviation of 103% around this mean value. This suggests that while statistically the end of working day sample tended to be higher than the morning sample, many samples also showed a reverse pattern. No urine sample for the HSL volunteers were outside the quoted normal range for the c-telopeptide of collagen I assay of 54-539 μg/mmol creatinine.

A similar pattern was seen for c-telopeptide of collagen II measured in start-of-working day and end-of-working day samples. The geometric means (medians) of the morning and afternoon samples for c-telopeptide of collagen I were 0.33 (0.22) and 0.173 (0.159) μg/mmol creatinine respectively. However, the differences between results in the morning and afternoon were not statistically significant ($p>0.05$). Bland-Altman plot analysis suggested that on average the difference between the morning and afternoon samples in each individual was +25% of the average value of the two results and with a distribution of +/-two standard deviations of 113% around this mean. Two subjects gave c-telopeptide collagen II results greater than the quoted reference range; one marginally increased and one significantly increased. Both were females and reported a family history of arthritis; the highest value was associated with a pregnant volunteer in the third trimester of pregnancy; the second highest was also from a pregnant volunteer at the end of the first trimester of pregnancy. We could find no published evidence that pregnancy has been associated with an increase in collagen II degradation products.

Thus this data suggest a total intra-individual (biological +analytical) coefficient of variation of approximately 50%. Given the level of our within-run imprecision (approx. 8%) is compatible with that quoted by the assay developers, this suggests a significant uncertainty in a single c-telopeptide collagen I or II measurement. Whether this level of variation ascribed to a spot urine sample reflects that the c-telopeptide is not produced at a constant process or other factors is unknown.

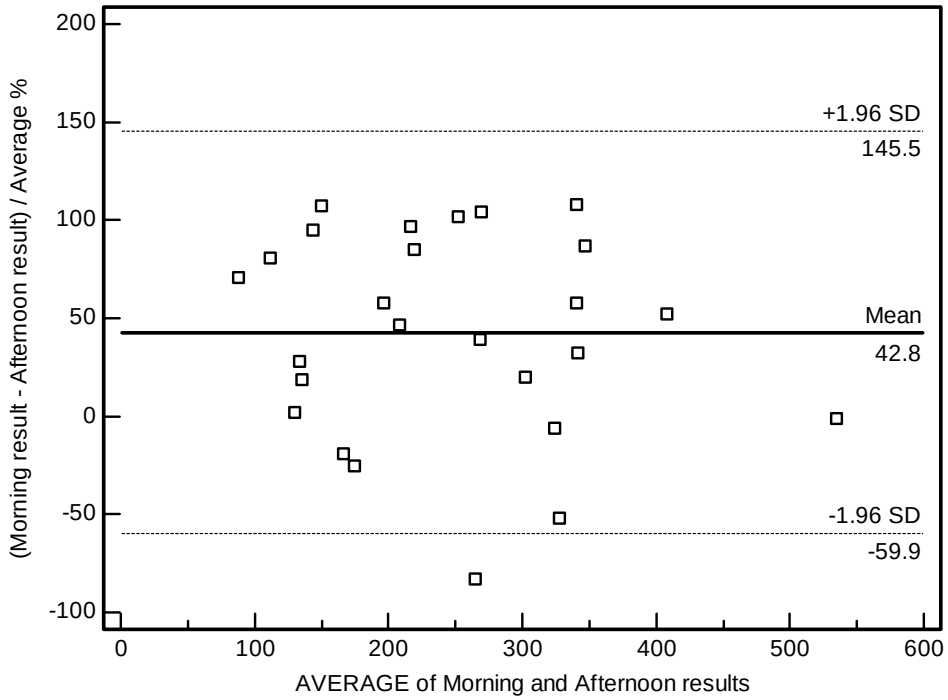

Figure 1. Bland-Altman plot of results of morning and afternoon urine samples for c-telopeptide collagen I

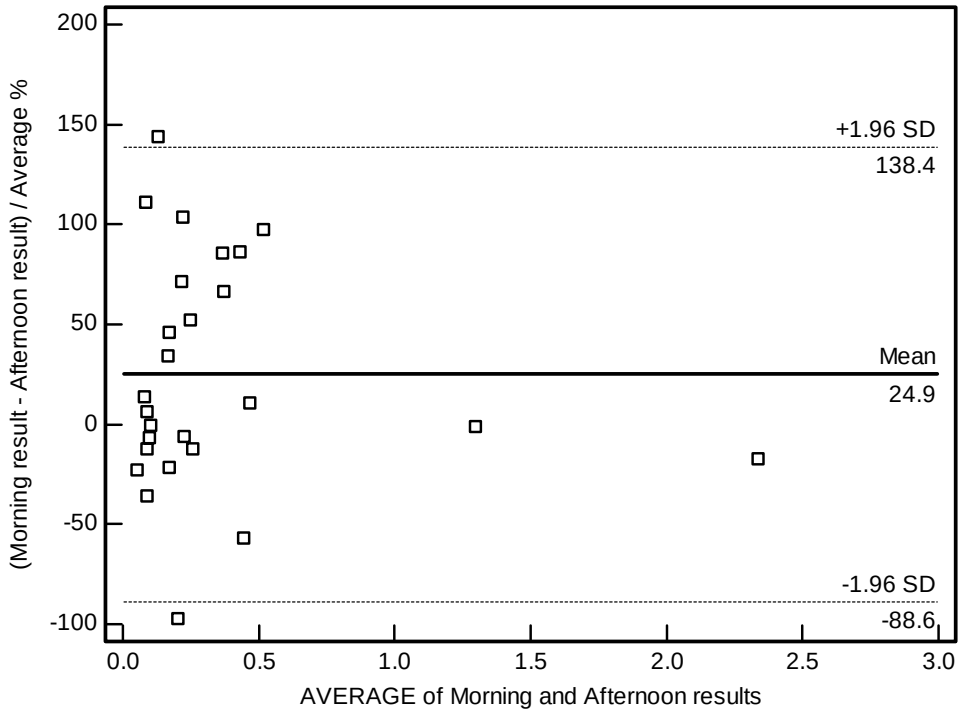

Figure 2. Bland-Altman plot of results of morning and afternoon urine samples for c-telopeptide collagen II

3.2 DEMOGRAPHICS OF COHORTS

The demographic information for the five cohorts is detailed in table 1. Comparisons between cohorts for continuous variables were undertaken using the non-parametric Kruskal-Wallis test with Dunn's post-test comparison where significance was indicated. Categorical data in table 1 was analysed using Chi-squared tests.

Cohorts	**HSL**	**Clinical cases**	**Occup. cases**	**Construction**	**Occupational**
Age	43 27.4-52.4	51.5 47.5-56.3	52.4 38-60.4	39.5 18-57	41 23-56
Number	27	38	24	60	59
Sex (F/M)	12/15	14/24	2/22	3/57	4/55
BMI	26.1 21.9-32.9	27.8 22.9-31.8	27.5 23.9-33.1	25.5 22.8-30.6	25.7 22.3-31.1
Currently sport activity involving LL	66%	3%	0%	63%	56%
Past moderate + sport activity involving LL	74%	58%	75%	56%	61%
GP visit in last 12 months for leg problem	20%	100%	100%	5%	15%
GP visit in last 12 months for lower back	19%	24%	29%	25%	34%
GP visit in last 12 months-neck/shoulders	11%	5%	4%	13%	15%
Visited GP in last 12 months for arms/hands	15%	3%	4%	6%	20%
NSAID use in last week	4%	69%	74%	5%	19%

Table 1. Demographic characteristics of the five cohorts, median and 10^{th} & 90^{th} percentiles shown for continuous variables. Proportions of cohorts presented for categorical data.

Both 'case' cohorts were significantly ($p<0.01$) older than the three working cohorts, but there were neither significant difference in age between the cases derived from the 'clinical route' and those via the 'occupational route', nor between the three working cohorts. As expected, both 'case cohorts' had a statistically lower prevalence of current sports activity involving the lower limbs than the current working cohorts. However, there were no differences between any of the cohorts in their historical involvement in sports using the lower limbs to a moderate to significant extent. Excluding the two case cohorts, the proportion of workers having visited their

GP for any problem in their legs was lower for the construction cohort compared to the other two working cohorts. There was no significant difference ($p>0.05$) in the proportions within the five cohorts who had visited their GP in the last 12 months for a problem with their lower back or neck/shoulders. Interestingly there were significant differences between the working cohorts in the proportion of subjects who had visited their GP in the last 12 months for a problem in their hands or arms. The proportion in the mixed occupation group was significantly higher than the construction group ($p<0.05$). Interestingly, this mixed occupational group also had significantly higher use ($p=0.02$) of non-steroidal anti-inflammatory agents in the week prior to the survey compared to the other two working cohorts (approximately four-fold increase in use). All three working cohorts were by definition in work, all the clinical cases were not working at the time of the study and 55% of the occupational cases were in some form of full or part-time employment at the time of the study.

3.3 OUTCOME MEASURES IN COHORTS

Two quantitative outcome measures are derived from the SF12 Quality of Life questionnaire. One relates to a physical summary component (PCS) and one a mental health summary component (MCS). Both the MCS and PCS scores are continuous variables between 0-100, with 50 representing the average score in the general population [31, 32]. The RAOS questionnaire gives five item scores; - symptoms, pain, functions related to activity of daily living, sports/recreation and quality of life. These five items are scaled between 0-100 with lower scores reflecting poorer outcomes. The two urinary biomarkers are expressed creatinine-corrected.

Comparisons between cohorts for continuous variables were undertaken using the non-parametric Kruskal-Wallis test with Dunn's post-test comparison where significance was indicated. Categorical data in table 2 was analysed using Chi-square test.

A similar pattern of results to those for age (table 1) was found in table 2 for the median physical summary score (PCS) from the SF12 questionnaire. Even though the median PCS in the clinical cases was lower than the occupational cases, it failed to reach statistical significance ($p>0.05$). The PCS scores in both case cohorts were lower ($p<0.01$) than the working cohorts, but PCS scores were not different ($p>0.05$) between the two case cohorts or between the three working cohorts. For the mental summary scores (MCS) from the SF12 questionnaire, both case cohorts were lower ($p<0.01$) than the working cohorts, but there was no difference between the two case cohorts. The HSL cohort had lower MCS outcomes than the construction cohorts, suggesting poorer self-perception of mental well-being in the HSL cohort, but there was no difference between the two other working cohorts. Thus generally perceived quality of life for both physical and mental components were significantly lower in those subjects where lower limb problems had significantly affected their normal work activities. 71% and 21% of the clinical or occupational case cohorts respectively had SF12 physical summary scores (PCS) lower or equivalent with the worst 2.5% of the general population. In contrast all subjects within the three working cohorts were within the mean plus or minus one standard deviation of the estimated PCS in the general population, with the median PCS in each of the working cohorts being slightly higher than the general population mean.

A similar pattern of statistical results was obtained comparing cohorts for the ROAS questionnaire outcomes related to symptoms, pain, ADL, and sports. The two case cohorts reported worse symptom, pain, ADL and sports/recreation scores ($p<0.001$) than the three working cohorts, but with no statistical difference between the case groups even though the medians for clinical cases were lower for the four RAOS outcomes. Interestingly the mixed

occupational cohort, where high risk trades were represented, showed a worse pain and symptom score ($p<0.05$) than the construction cohort, whereas there were no statistical differences between the three working cohorts for perceived problems in sports/recreation or ADL. The perceived Quality of Life (QoL) outcome scores suggested a slightly different pattern to the other outcomes. The lower values found in the clinical cases compared to the occupational cases were statistically significant ($p<0.05$), and consistent with pattern shown for the PCS and MCS data from the more general SF12 questionnaire. The three working cohorts had statistically better QoL scores than both 'case' cohorts ($p<0.001$). However, both the HSL and mixed occupation cohorts had significantly worse QoL scores ($p<0.05$) than the construction group.

So clearly using both a lower limb specific as well as a more generalised questionnaire tool, the working cohorts perceived their quality-of-life as very significantly better than the case cohorts and with less symptoms and pain.

Figures 3 and 4 represent the urinary biomarkers concentrations (creatinine corrected) for all subjects in the five cohorts. For the c-telopeptide biomarker of collagen I (bone degradation), the HSL cohort was significantly lower than the two other working cohorts. No other statistical differences between the five cohorts were found.

The cartilage degradation biomarker (c-telopeptide of collagen II) showed no significant difference in medians between the three working cohorts or between the two case cohorts. The biomarker in both 'case' cohorts was significantly ($p<0.001$) higher than the three working cohorts. There was overlap in distribution between c-telopeptide of collagen II in the working cohorts and the case cohorts. However, the two highest results in the HSL cohort were from pregnant females; the highest value from a volunteer in the third trimester of pregnancy. The highest value in the construction cohort was from the youngest volunteer of 18 years old. When the values from the two pregnant women and the 18 year old were removed from the working cohorts there was evidence that c-telopeptide of collagen II was higher than in the mixed occupation cohort than the sedentary HSL cohort ($p=0.03$).

There was a very significantly increased prevalence of non-steroidal anti-inflammatory medication in the case cohorts, where the long-term use has been reported as being associated with decrease in the biomarker, c-telopeptide of collagen II [39]. These factors may operate to reduce the separation in the biomarker values between working and case cohorts.

Cohorts	HSL	Clinical cases	Occup. cases	Construction	Occupational
SF12 questionnaire outcome measures					
SF12 PCS	53.4 42.2-60.8	28.3 18.3-33.2	39 27.6-41.2	54.8 46.7-58.8	51.8 40.7-59.7
SF12 MCS	48.9 38.4-57.2	31 21.6-34	35 26.8-43.2	56.6 49.8-62.9	50.3 39.7-60
RAOS questionnaire outcome measures					
RAOS Symptoms (0-100)	89 95.4-100	37 26.3-45.8	44.7 34.5-59	93 72.5-100	84 61.8-96
RAOS Pain (0-100)	92 75-100	34.5 18-41	46 33.2-62.1	100 74-100	92 62.4-100
RAOS ADL (0-100)	100 89-100	40.2 22.6-49	43.3 23.8-56.4	100 79-100	95 70.8-100
RAOS Sport (0-100)	90 61-100	25.5 14.9-37.9	30.5 17-46.7	100 75.5-100	90 62.5-100
RAOS QoL (0-100)	75 40.4-100	28 9.9-37.5	36.2 9-56.3	100 75-100	75 52.4-100
Urine biomarker outcome measures					
c-telopeptide collagenI (bone biomarker) µg/mmol creatinine	299 187-512	373 205-589	350 262-503	397 221-604	466 287-608
c-telopeptide collagenII (cartilage biomarker) µg/mmol creatinine	0.217 0.081-0.735	0.884 0.444-1.49	0.931 0.493-1.724	0.27 0.095-0.464	0.288 0.097-0.600

Table 2. Outcome measures from questionnaires and urine samples for the five cohorts. Medians and 10-90th percentiles are shown.

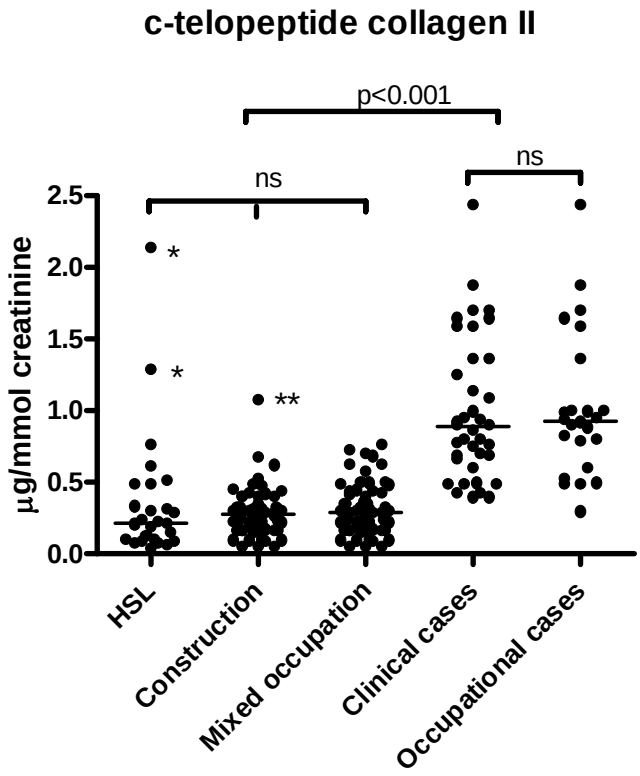

Figure 3. Graph of the cartilage breakdown biomarker (c-telopeptide of collagen II) in five cohorts.* indicates pregnant subjects. ** indicates youngest(18 year old) male subject. Medians marked as horizontal lines.

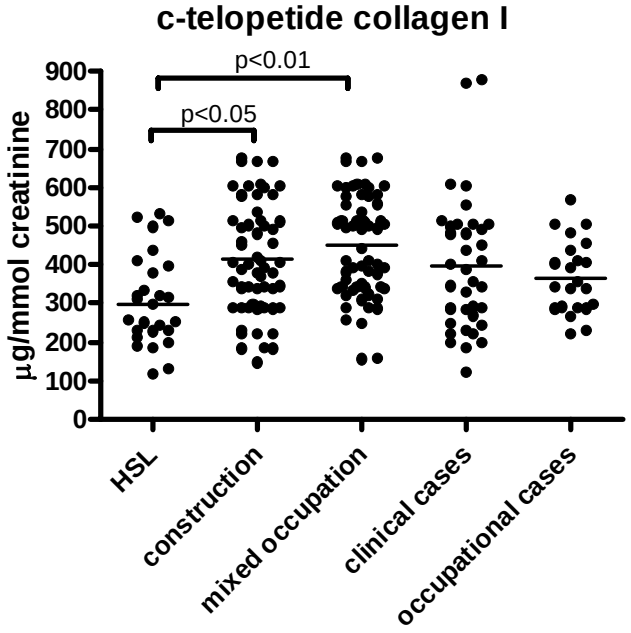

Figure 4. Graph of the bone breakdown biomarker (c-telopeptide of collagen I) in five cohorts. Medians marked as horizontal lines.

Given the apparent discrimination shown by c-telopeptide collagen II between case cohorts and working cohorts (figure 3), a formal ROC analysis was carried out comparing the three working cohorts combined with the combined two case cohorts. An upper limit for 'normality' was derived from the combined working populations by excluding those who had reported visiting their GP for a lower limb problem in the last twelve months or reported significant pain or problems in their lower limbs. The area under the ROC curve was 0.93 (95% CI =0.90-0.97) using a cut-off value of 0.485 μg/mmol creatinine. The sensitivity and specificity were 91.4% and 84.4% suggesting good diagnostic discriminatory power. However, this analysis needs to be treated with some caution as the case definition is not based on a defined pathology, but rather subjects had reported lower limb problems that were significant enough for them to be absent from work, have changed their trade or be seeking expert clinical advice for their lower limb problems.

Figures 5 and 6 show comparisons for items in the RAOS questionnaire and the SF12 questionnaire for case cohorts combined and working cohorts combined.

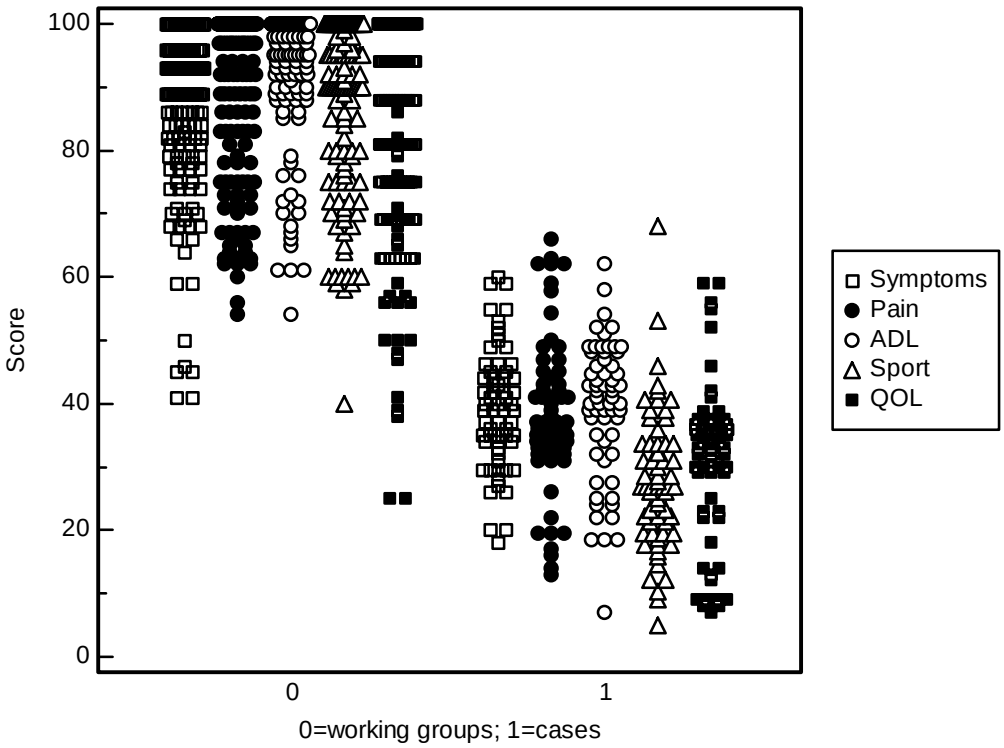

Figure 5. Comparison of 'working population' versus 'cases' for items scores in the RAOS questionnaire

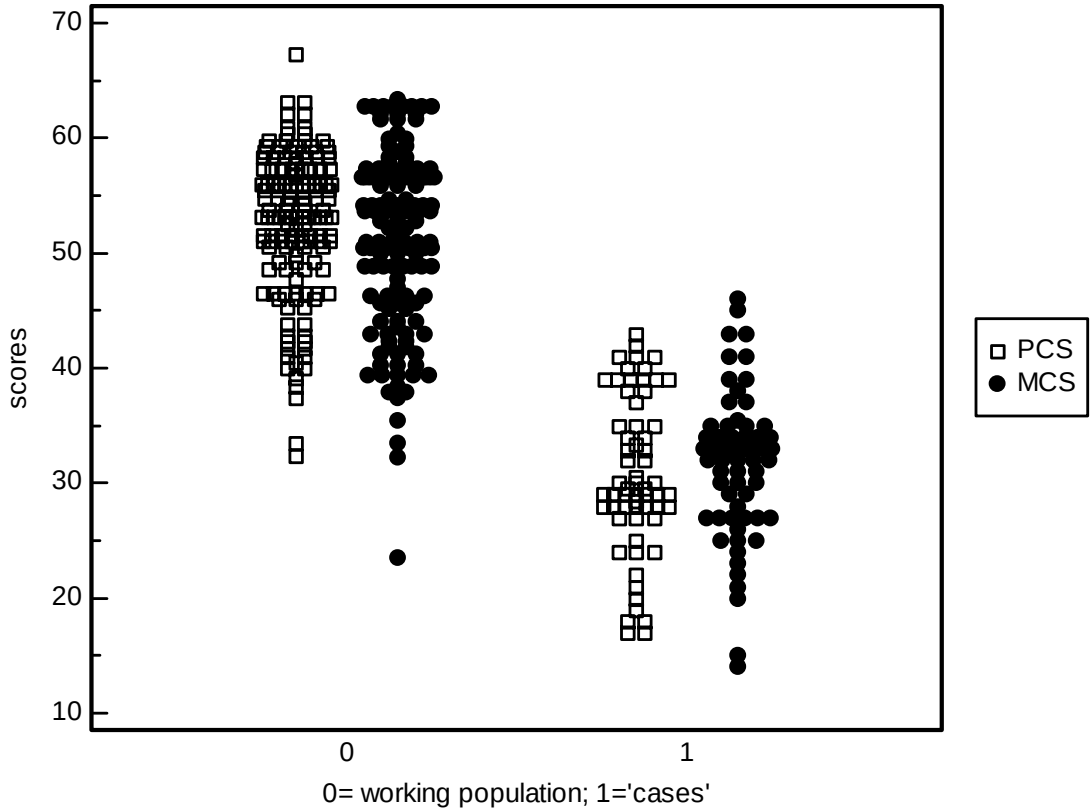

Figure 6. Comparison of 'working population' versus 'cases' for the physical summary score (PCS) and mental summary score derived from the SF12 questionnaire

3.4 RELATIONSHIPS BETWEEN OUTCOME MEASURES

Cross-tabulations between outcome measures and key variables such as age and BMI were carried out for the whole study population (table 3). There were high internal correlations between items within in the RAOS questionnaire (Spearman Rank coefficients between 0.82-0.91) and between the two summary scores in SF12 questionnaire. The correlation between RAOS item scores and the SF12 summary scores were less but still highly significant (Rank Spearman coefficients between 0.54-0.77). Correlations with RAOS items tended to be higher for the physical component score (PCS) rather than the mental summary score (MCS).

No significant association was found between the collagen I and collagen II biomarkers reflecting that they relate to degradation pathways in two distinct tissues (bone and cartilage respectively) and that these two processes do not seem coupled in the overall population where there is a significant proportion of subjects with lower limb problems. There was a significant negative correlation between age and c-telopeptide collagen I but not the collagen II peptide. The latter biomarker was significantly negatively correlated with BMI.

		Crosslaps Collagen I	Cartilaps Collagen II	BMI	ADL score	Pain score	QOL score	Sport score	Symptoms score	PCS	MCS
Age	R P N	-0.31 <0.001	-0.17 ns	0.38 <0.001	-0.48 <0.001	-0.50 <0.001	-0.49 <0.001	-0.53 <0.001	-0.50 <0.001	-0.51 <0.001	-0.41 <0.001
Crosslaps Collagen I	R P		0.19 ns	-0.07 ns	0.02 ns	-0.03 ns	0.10 ns	0.21 ns	-0.17 ns	-0.13 ns	0.14 ns
Cartilaps Collagen II	R P			0.24 <0.01	-0.33 <0.001	-0.58 <0.001	-0.33 <0.01	-0.29 <0.01	-0.52 <0.001	-0.38 <0.001	-0.21 <0.05
BMI	R P				-0.19 ns	-0.25 <0.01	-0.31 <0.001	-0.33 <0.001	-0.26 <0.01	-0.17 ns	-0.19 ns
ADL	R P					0.84 <0.001	0.81 <0.001	0.84 <0.001	0.82 <0.001	0.65 <0.001	0.55 <0.001
Pain	R P						0.91 <0.001	0.90 <0.001	0.85 <0.001	0.77 <0.001	0.59 <0.001
QOL	R P							0.88 <0.001	0.84 <0.001	0.72 <0.001	0.73 <0.001
Sport	R P								0.84 <0.001	0.77 <0.001	0.54 <0.001
Symptoms	R P									0.75 <0.001	0.63 <0.001
PCS	R P										0.73 <0.001

Table 3. Cross tabulation of items in the RAOS and SF12 questionnaires and urine biomarkers with Spearman Rank correlation coefficients for all the working and case cohorts combined.

		Crosslap Collagen I	Cartlaps CollagenII	BMI	ADL score	Pain score	QOL score	Sport score	Sympt oms score	PCS	MCS
Age	R P	-0.31 <0.05	-0.43 <0.01	0.44 <0.001	-0.03 ns	-0.19 ns	-0.21 ns	-0.23 ns	-0.01 ns	-0.107 ns	0.06 ns
Crosslaps Collagen I	R P		0.615 <0.001	-0.07 ns	0.02 ns	-0.03 ns	0.01 ns	0.229 0.05	-0.17 ns	-0.13 ns	0.34 <0.01
Cartilaps Collagen II	R P			-0.05 ns	-0.03 ns	-0.335 <0.05	-0.17 ns	0.04 ns	-0.24 ns	-0.135 ns	0.189 ns
BMI	R P				-0.02 ns	-0.32 <0.05	-0.29 0.05	-0.24 ns	-0.11 ns	-0.09 ns	0.14 ns
ADL	R P					0.60 <0.001	0.43 <0.001	0.62 <0.001	0.43 <0.001	0.46 <0.001	0.266 <0.05
Pain	R P						0.77 <0.001	0.74 <0.001	0.58 <0.001	0.36 <0.01	0.27 ns
QOL	R P							0.66 <0.001	0.61 <0.001	0.30 <0.05	0.48 <0.001
Sport	R P								0.57 <0.001	0.24 ns	0.52 <0.001
Symptoms	R P									0.34 <0.05	0.29 0.05
PCS	R P										-0.22 ns

Table 4. Cross tabulation of items in the RAOS and SF12 questionnaires with Spearman Rank correlation coefficients for the three working cohorts combined.

In contrast to analysis within the whole study population, there was no significant relationship between the physical and mental summary scores (PCS & MCS) from the general quality of life questionnaire within the combined three working cohorts (table 4). There were good correlation coefficients (0.43- 0.77) internally between the items of the RAOS lower limb questionnaire. The lower correlation between items of the RAOS and the summary scores from the SF12 likely reflect the more general nature of the latter questionnaire i.e. not focussed on the lower limbs.

Urine c-telopeptide of collagen I appeared to be significantly negatively correlated with age, but not with BMI (table 4). This urine biomarker also appeared correlated with MCS and to a weaker extent with the sports item from the RAOS questionnaire.

Urine c-telopeptide of collagen II also appeared significantly negatively correlated with age, but not with BMI (table 4). The cartilage degradation biomarker was strongly correlated with the collagen I biomarker and weakly negatively correlated with pain score of the RAOS questionnaire suggesting that an increase in the urine biomarker was associated with more perceived pain in the lower limbs.

A model based on a backwards multiple regression analysis were carried out to investigate the relationships between the two urine biomarkers and the pain and symptom score of the RAOS questionnaire, age, body mass index, the current and past level of moderate or greater sports activity involving the legs and GP visit in the last 12 months for a lower limb or other site specific problems (Given the co-linearity between scored items derived from the RAOS questionnaire, only the two most correlated items were included in the analysis). This was carried out for the whole population (cases and working cohorts combined) and the three working cohorts combined.

For the bone biomarker (c-telopeptide collagen I) only age was included in both final model (Coefficient –3.94; $p<0.04$). No other variables were included in the model.

For the cartilage biomarker (c-telopeptide collagen II), there were differences between the final models. For the whole population, BMI (coefficient +0.55; $p= 0.03$) and pain score (coefficient –0.045; $p<0.001$) were included in the final model. For the working cohorts combined the final model included age (coefficient-0.0346; $p<0.03$) and pain score (coefficient =-0.011; $p=<0.05$). Thus the whole population model suggested that increased body mass index (BMI) and more perceived pain were associated with increased c-telopeptide collagen II; while the analysis restricted to the working cohorts suggested the biomarker was negatively influenced by increasing age but was also positively related to perceived pain as derived from the RAOS questionnaire.

A logistic model was carried out using case or non-case as the dependent variable and explanatory variables of age, BMI, urine biomarkers, symptom and pain items from the RAOS questionnaire, sports activity and the MCS from the SF12 as a measure of psychological distress. Urinary c-telopeptide of collagen II ($p<0.01$) and pain score ($p<0.01$) remained as influencing measures. The increased psychological distress as defined by the MCS just failed to reach significance ($p=0.052$) as an additional influence. Further logistic regression failed to identify the level of current or past significant sports activity involving the lower limbs as significant influences on the risk of being defined as ‘case’.

4 DISCUSSION

This study has been an initial investigation at the potential value of two objective biomarkers reflecting potential occupational stress on the lower limbs and poor occupational outcomes. It appears to be one of the first to have done so. Originally established biomarkers of bone, cartilage and synovium degradation were to be investigated, based on urinary measurements of c-telopeptide of collagen I, c-telopeptide of collagen II and glucosyl-galactosylpyridinoline respectively [5, 24, 25, 40]. Technical difficulties with the development of a high-pressure liquid chromatography (HPLC) and its standardisation for measurement of glucosyl-galactosylpyridinoline within the time and cost constraints of the project made us concentrate on c-telopeptides of collagen I and II as measured by enzyme immunoassay. There is a growing body of publications that suggest that particularly the c-telopeptide of collagen II may respond to biological process involved in both rheumatoid and osteoarthritis [2, 4, 13, 24, 41]. It has been suggested that osteoarthritis reflects the biological process leading to the common occupationally-associated problems found in the lower limb joints of some workers in particular trades [38].

Cohorts were defined as either cases or working groups. There was no attempt to ensure appropriate cross-sectional representation of specific working populations. Two cohorts of 'cases' were obtained. One was a group of subjects of working age who by GP referral were attending a specialised centre for assessment or treatment of problems in their lower limbs. The other group of 'cases' were those who had either changed their job in agreement with their management or left their job because of significant problems with their lower limbs. All had been seen by an occupational health professional. Recruitment of cases, especially the latter cohort, proved challenging. Although lower limb MSDs are thought to be relatively common in certain trades, identifying individuals as potential study volunteers appeared difficult. Discussions with a number of GPs highlighted that non-trauma related lower limb problems in subjects of working age are very small compared to those related to those of pensionable age. Often they involve only a single visit to the GP and period of sickness absence from work. Discussion with occupational health professionals and company managers suggested an ill-defined picture of any referral pattern or job modification, while there were good examples of both medical referral and job modification to address the issue. Many workers may be simply leaving jobs, which cause them problems, for other less-stressing employment on the lower limbs, possibly going on long-term sick, or 'soldering-on' in the job accepting reduced functionality and a certain level of symptoms. It was not considered necessary for individuals in the 'clinical case' cohort to have a formal diagnosis (e.g.osteoarthritis) for inclusion, as it is pain or functional problems that impinge on a worker's ability to remain or function effectively in employment, rather than the medical diagnosis per se. However, lower limb pain such as knee pain is relatively common in the general population and certain occupations [9, 30, 35, 38, 42-44] and may have psychological, organisational drivers as well as a biological basis [7-9, 45] The clinical cases in this study had been referred by their GP to a specialised clinic for either assessment or treatment of problems in the joints of their lower limbs not related to recent acute trauma. At the time of study, all the clinical cases and most of the cases obtained via the occupational route were not working.

The working populations had no exclusion criteria associated with them and include one cohort derived from HSL staff that may be assumed to be a largely sedentary workforce compared to the other two. Another working cohort was derived from volunteers recruited in trades that have been associated with risk of LLMSDs and osteoarthritis [35, 36, 38]. They included roofers, carpenters, floor layers, and the logistic/transport sector. The third cohort was derived from volunteers from two major construction companies [33].

We applied two quantitative and established questionnaires. The SF12 quality of life questionnaire and the lower limb specific RAOS. The former questionnaire, or its SF36 version, has been used widely in public health issues, including the occupational health context and one study involving knee pain [30]. To our knowledge this is the first time that the clinically validated RAOS questionnaire has been used in occupational health. Experience in hand-arm vibration syndrome has suggested that quantitatively scored questionnaires are useful tools in diagnosis and to explore the relationship between health outcomes and workplace stressors. While it must be borne in mind that such questionnaires are self-reporting and therefore by definition at best reflect the individual's perception of their health or problems, it is the individual's perception of the level of pain or loss of functionality they suffer that may influence their remaining in that specific workplace. The SF12 questionnaire is general in nature but includes a mental health/psychological status score (MCS). However, while psychosocial factors and levels of psychological distress may influence the perception of symptoms like pain or functional capability [7, 8] it is difficult in a cross-sectional study to prove whether poor MCS scores indicates causation or reflects the effects of pain or poor functionality. Historically the Nordic questionnaire has been used in MSDs. This questionnaire is geared to locating sites of problems and also the duration of symptoms in some specific anatomical sites [46], rather than attempting to quantitate the level of symptoms or functional problems. However, we would argue that a combination such as SF12/RAOS can give more useful information, where complex physiological and psychological factors may be involved.

As such any self-reporting questionnaire is open to bias in real-life use and this is partly the driver for looking for more objective biomarkers. However, in this research study where there may be less reason for individuals to bias their responses, the items of the RAOS questionnaire appeared highly related and showed good discrimination between cases and working populations. As expected the SF12 outcome were not as discriminatory, being based on general health, and therefore susceptible to other the effects of MSDs or health problems elsewhere in the body. However, it still significantly discriminated between cases and working populations. We feel that the spread of scores from the RAOS questionnaire and its discrimination between working cohorts and cases substantiates the further use of this clinically derived questionnaire in occupational health studies as a useful tool in defining both symptom and functional issues in the lower limbs. The SF12 has already been substantiated in public health work. All three working groups had median physical summary scores above the general population mean and all values within +/- standard deviation of the general population mean reflecting the general healthy condition of those subjects in workers cohorts. However this is not to exclude the possibility of some lower limb problems to some extent within the working cohorts, as possibly reflected within the RAOS questionnaire and urinary biomarkers.

The results largely discount the potential value of the bone marker, c-telopeptide of collagen I, to discriminate between working subjects, manual and sedentary, and those who have a significant problem in their lower limbs. This may reflect that any adverse biological process does not involve bone metabolism or that skeletal effects outside the lower limbs masks any effect in the lower limbs. It is interesting to note that the sedentary working cohort appeared to have lower excretion of c-telopeptide of collagen I than the other two non-sedentary working groups. It has been suggested that increased bone degradation is found in manual workers due to the increased physical loading of the skeleton, but may only reflect a non-pathological response in bone turnover given the usual tight coupling of rates of bone formation and degradation [28].

The urinary, c-telopeptide of collagen II showed more promise in distinguishing 'cases' from working populations as may be expected from the published literature investigating this biomarker in terms of arthritic diseases. Simple comparisons of areas-under-the curve in ROC analysis suggested that there was no significant difference in the discriminant power between

working population and cases between this biomarker and several quantitative items scores (e.g. pain, symptoms) derived from the RAOS questionnaire.

The inverse relationship found in the working cohorts between c-telopeptide collagen II and age is probably reflecting the reported similar finding in the healthy general population. Interestingly, we found high levels of this urinary biomarker in comparison to their respective cohort distributions for two pregnant females as well as an 18 year old male.

It is interesting that increased severity of reported pain (lower RAOS pain score) was significantly associated with increasing levels of c-telopeptide collagen II as a marker of cartilage degradation in both the whole study population and the combined working groups. Also in terms of discriminating 'cases' from workers by logistic regression both pain score and c-telopeptide collagen II were significant. This suggests that while the urine biomarker is linked to a key health outcome (i.e. perceived lower limb joint pain), and possibly the pathological processes causing the pain, the perception of pain and the objective measure are stronger in combination as measures of risk of becoming a 'case'.

However the data on intra-individual variability c-telopeptide collagen II, which in our hands appears wider than that reported by the commercial developer of the assay, suggests that care would have to made in not over-interpreting a single measurement in an individual in comparison to its use in cohort studies. Measurements from pregnant females and young males may also appear to give values that could be misinterpreted by use of a simple adult normal range. These findings should reinforce the knowledge that such a urinary biomarker is not specific to an anatomical part of the body but rather to a tissue (in this case cartilage), which is associated with articular joints through the body and areas such as ribs, bronchial tubes and spinal discs.

It is impossible in a study of this nature (cross-sectional) to prove indisputably the value of a biomarker 'as an objective measure of early musculoskeletal disorders of the lower limbs'. Prospective studies may be necessary but are expensive. It is unfortunate that we have not managed to analyse the same samples for glucosyl-galactosyl-pyridinoline as this marker is present in synovium tissue but relatively absent in bone, cartilage and its excretion has been reported to related to synovial degradation [2, 5]. Thus the measurement of both urinary c-telopeptide of collagen II and glucosyl-galactosyl-pyridinoline may have given a fuller picture of the biological processes involved in joints and reflect more the symptoms and functional deficit that impinge on the ability to remain in trade or continue to work effectively.

The data from this study of biomarkers of cartilage degradation, such as c-telopeptide collagen II, suggest that they should be considered in further occupational health cohort studies of LLMSDs. Consideration should be given to the use of the RAOS and SF12 self-reporting questionnaires in studies of LLMSDs in order to capture quantitative data on perceived poor health outcomes.

5 REFERENCES

1. Garnero P, Conrazier T, Christgau S et al. *Urinary type II Collagen C-telopeptide levels are increased in patients with rapidly destructive hip osteoarthritis.* Ann Rheum Dis, 2003. **62**: p. 939-942.

2. Garnero P, Piperno M, Gineyts E et al. *Cross-sectional evaluation of biochemical markers of bone, cartilage, and synovial tissue metabolism in patients with knee osteoarthritis: relations with disease activity and joint damage.* Ann Rheum Dis, 2001. **60**: p. 619-626.

3. Landewe R, Geusens P, Boers M. et al. *Markers of type II collagen breakdown predict the effect of disease-modifying treatment on longterm radiographic progression with patients with rheumatoid arthritis.* Arthritis and Rheumatism, 2004. **50**(5): p. 1390-1399.

4. Christgau S, Garnero P, Fledelius E et al. *Collagen type II C-telopeptide fragments as an index of cartilage degradation.* Bone, 2001. **29**(3): p. 209-215.

5. Gineyts E, Garnero P, and P Delmas. *Urinary excretion of glucosyl-galactosyl pyridinoline: a specific biochemical marker of synovium degradation.* Rheumatology, 2001. **40**: p. 315-323.

6. Johansen J, Jenson H, and P Price. *A new biochemical marker for joint injury. Analysis of YKL-40 in serum and synovial fluid.* British Journal of Rheumatology, 1993. **32**: p. 949-955.

7. Nahit E, Pritchard C, Cherry N et al. *The influence of work related psychological factors and psychological distress on regional musculoskeletal pain: a study of newly employed workers.* Journal of Rheumataology, 2001. **28**(6): p. 1378-1384.

8. Nahit E, Hunt I, Lunt M et al. *Effects of psychosocial and individual psychological factors on the onset of musculoskeletal pain: common and site-specific effects.* Ann Rheum Dis, 2003. **62**: p. 755-760.

9. Jones G, Harkness E, Nahit E et al. *Predicting the onset of knee pain: results from a 2-year prospective study of new workers.* Ann Rheum Dis, 2007. **66**: p. 400-406.

10. O'Kane J, Hutchinson E, Astley L et al. *Sport-related differences in biomarkers of bone resorption and cartilage degradarion in endurance athletes.* Osteoarthritis and Cartilage, 2006. **14**: p. 71-76.

11. Mazzuca S, Brandt K, Eyre D et al. *Urinary levels of type II collagen c-telopeptide crosslinks are unrelated to joint space narrowing in patients with knee osteoarthritis.* Ann Rheum Dis, 2006. **65**: p. 1055-1059.

12. Billinghurst R, et al. *Enhanced cleavage of type II collagen by collagenases in osteoarthritic articular cartilage.* J Clin Invest 1997. **99**: p. 1534-1545.

13. Charni N, Juillet F and P. Garnero. *Urinary type II collagen helical peptide (HELIX-II) as a new biochemical marker of cartilage degradation in patients with osteoarthritis and rhematoid arthritis.* Arthritis and Rheumatism, 2005. **52**(4): p. 1081-1090.

14. Roos E, Klassbo M, and L. Lohmander, *WOMAC Osteoarthritis Index: relaibility, validity, amd responsiveness in patients with arthroscopically assessed osteoarthritis.* Scandanavian Journal of Rheumatology, 1999. **28**: p. 210-215.

15. Bremander A, Peterssen I, and E Roos. *Validation of the Rheumatoid and Arthritis Outcome Score (ROAS) for the lower extremity.* Healthy and Quality of Life Outcomes, 2003. **1**: p. 55-66.

16. Jaglal S, Lakhani Z, and J Schatzker. *Reliability, validity and responsiveness of the lower extremity measure for patients with a hip fracture.* Journal of Bone and Joint Surgery, 2000. **82A**(7): p. 955-962.

17. Poole K. *Review of the phenomenon of temporary threshold shifts in sensory perception following acute exposure to vibration, and its relationship to the long-term effects of vibration exposure*. 2001, Health & Safety Laboratory: Sheffield.

18. Bellamy N. *Pain assessment in osteoarthritis:Experience with the WOMAC osteoarthritis index.* Semin. Arthritis Rheum., 1989. **18**: p. 14-17.

19. Bellamy G. et al. *Validation study of WOMAC. A health status instrument for measuring clinically importamt patient relevant health outcomes to antirheumatic drug therapy in patients with osteo-arthritis of the hip or knee.* Journal of Rheumatology, 1988. **15**: p. 1833-1840.

20. Ceunick F, et al. *Urinary collagen type II telopetide fragments are sensitive markers of matrix metallo-proteinase dependent cartilage degradation in rat adjuvant arthritis.* Journal of Rheumatology, 2003. **30**: p. 1561-1564.

21. Johansen J, Hvolris J, Hansen M, et al. *Serum YKL-40 levels in healthy children and adults. Comparison with serum and synovial fluid levels of YKL-40 in patients with osteoarthritis or trauma of the knee joint.* British Journal of Rheumatology, 1996. **35**: p. 553-559.

22. Mazieres B. *Marquers biologiques de l'arthrose: les donnees de la cohorte ECHODIAH.* Presse Med, 2004. **33**: p. 1513-1515.

23. Heinegard D and T Saxne. *Molecular markers of processes in cartilage in joint disease.* British Journal of Rheumatology, 1991. **30(Suppl 1)**: p. 21-24.

24. Jordan K, Syddall, H, Garnero P, et al. *Urinary CTX-II and glucosyl-galactosyl-pyridinoline are associated with the presence and severity of radiographic knee osteoarthritis in men.* Ann Rheum Dis, 2006. **65**: p. 871-877.

25. Garnero P, et al. *Association of baseline levels of urinary glucosyl-galactosyl-pyridinoline and type II collagen c-telopetide with progress of joint destruction in patients with early rheumatoid arthritis.* Arthritis and Rheumatism, 2002. **46**: p. 21-30.

26. Garnero P, et al. *Association of baseline levels of markers of bone and cartilage degradation with longterm progression of joint damage in patients with early*

rheumatoid arthritis: the COBRA study. Arthritis and Rheumatism, 2002. **46**: p. 2847-2856.

27. Garnero P. et al. *Cross-sectional association of 10 molecular markers of bone, cartilage, and synovium with disease activity amd radiological joint damage in patients with hip osetoarthritis: the ECHODIAH study.* J Rheumatol, 2005. **32**: p. 697-703.

28. Kuiper J, et al. *Serum markers of collagen metabolism: construction workers compared to sedentary workers.* Occupational Environmental Medicine, 2005. **62**: p. 363-367.

29. Suk, M, et al. *AO Handbook Musculoskeletal Outcomes Measures and Instruments*. AO publishing. 2004, New York: Thieme.

30. O'Reilly S, Muir K, and M Doherty. *Knee pain and disability in the Nottingham community: association with poor health status and psychological distress.* British Journal of Rheumatology, 1998. **37**: p. 870-873.

31. Jenkinson C. *The SF-36 physical and mental health summary measures: an example of how to interpret scores.* J Health Serv. Res. Policy, 1998. **3**(2): p. 92-96.

32. Jenkinson C. *Comparison of UK and US methods for weighting and scoring the SF-36 summary measures.* Journal of Public Health Medicine, 1999. **21**(4): p. 372-376.

33. Merlino L, Rosecrance J, Anton D et al. *Symptoms of musculoskeletal disorders among apprentice construction workers.* Applied Occupational and Environmental Hygiene, 2003. **18**(1): p. 57-64.

34. Hunting K, Welch L, Cuccherini B, et al. *Musculoskeletal symptoms among electricians.* American Journal of Industrial Medicine, 1994. **25**: p. 149-163.

35. O'Reilly S, Muir K, and M. Doherty, *Occupation and knee pain: a community study*. Osteoarthritis and Cartilage, 2000. **8**: p. 78-81.

36. Jensen L, and L Kofoed. *Musculoskeletal disorders among floor layers: Is prevention possible?* Applied Occupational and Environmental Hygiene, 2002. **17**(11): p. 797-806.

37. Jensen L, Mikkleson S, Loft I, et al. *Work-related knee disorders in floor layers and carpenters.* Journal of Occupational and Environmental Medicine, 2000. **42**(8): p. 835-842.

38. Jensen L, et al. *Radiographic knee osteoarthritis in floorlayers and carpenters.* Scandanavian Journal of Work and Environmental Health, 2000. **26**(3): p. 257-262.

39. Gineyts E, Mo J, Ko A, et al. *Effects of ibuprofen on molecular markers of cartilage and synovium turnover in patients with knee osteoarthritis.* Ann Rheum Dis, 2004. **63**: p. 857-861.

40. Mouritzen U, et al. *Cartilage turnover assessed with a newly developed assay measuring collagen II degradation products: influence of age, sex, menopause, hormone therapy amd body mass index.* Ann Rheum Dis, 2003. **62**: p. 332-336.

41. Young-Min S, Cawston T, Marshall N, et al. *Biomarkers predict radiographic progression in early rheumatoid arthritis and perform well compared with traditional markers.* Arthritis and Rheumatism, 2007. **56**(10): p. 3236-3247.

42. Bergenudd H, Nilsson B and F Lindgarde. *Knee pain in middle age and its relationship to occupational work load and psychosocial factors.* Clinical Orthopedics, 1989. **245**: p. 210-215.

43. Chen J.-C, Linnan L, Callahan L et al. *Workplace policies and prevalence of knee osteoarthritis: the Johnston County Osteoarthritis Project.* Occupational Environmental Medicine, 2007. **64**: p. 798-805.

44. Miranda H, Viikari-Juntura E, Martikainen R, et al. *A prospective study of knee pain and its risk factors.* Osteoarthritis and Cartilage, 2002. **10**: p. 623-630.

45. Nahit E, Pritchard C, Cherry N, et al. *Short-term influence of mechanical factors on regional musculoskeletal pain: a study of new workers from 12 occupational groups.* Occupational Environmental Medicine, 2001. **58**: p. 374-381.

46. Kuorinka I, Johnsson B, Kilbom A, et al. *Standardised Nordic questionnaires for the analysis of musculoskeletal symptoms.* Applied Ergonomics, 1987. **18**(3): p. 233-237.

Published by the Health and Safety Executive 02/10

HSE

Health and Safety Executive

Feasibility of using urinary biomarkers to identify occupational musculoskeletal disorders of the lower limbs

Interest has developed in biomarkers in body fluids, which are indicative of degradation of bone, cartilage and synovial tissue. These are currently being investigated in the clinical context in terms of diagnosis, prognosis and efficacy of treatment for rheumatoid and osteo-arthritis. There is also interest in the use of such biomarkers in sports medicine and now occupational medicine. The cause of occupational lower limb musculoskeletal disorders may involve complex multifactorial issues, such as psychological, and organisational factors as well as abnormal biological processes caused by over-use or abnormal loading of the knees, hips and ankles during various work activities. There is an interest in whether non-invasive biomarkers of bone, cartilage and synovium metabolism may add to other tools by objectively identifying the involvement and extent of specific abnormal biological processes in those who present with lower limb problems. Such biomarkers could be cost-effective tools in studying the efficacy of various intervention strategies in controlling the risk and incidence of occupationally related lower limb musculoskeletal disorders.

This report and the work it describes were funded by the Health and Safety Executive (HSE). Its contents, including any opinions and/or conclusions expressed, are those of the author alone and do not necessarily reflect HSE policy.

RR773

www.hse.gov.uk