Elevated calcium concentration – is it dangerous?

Long-term follow-up in primary care

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ABSTRACT

Background and aims: Patients with hypercalcaemia are relatively common in primary care; the most frequent causes are primary hyperparathyroidism (pHPT) and cancer. Many patients with pHPT have such discrete symptoms that they are difficult to detect without a calcium analysis. To increase the detection of pHPT, more calcium analyses are recommended by Swedish authorities. The aim of this thesis was to study the care of patients with elevated calcium concentrations and to investigate factors contributing to the variation in calcium analyses between physicians and health care centres (HCC) in primary care.

Material and Methods: First, we investigated all patients with elevated calcium concentrations (n=142) at Tibro HCC between the years 1995−2000. In the following studies, HCC patients with normal calcium concentrations were used as controls. Both groups were offered an examination after 10 years with new blood analyses and questions concerning diseases, medication and quality of life.

In the last study, the variation in the ordering of calcium analyses between 457 physicians and 24 HCCs was investigated through a multilevel analysis.

Results: In the first study we tried to survey the underlying causes in patients with elevated calcium concentrations; however, no cause was found in 70 %

of the patients. pHPT and cancer were among the most common diagnoses. At follow-up, 88 % of the patients with elevated calcium concentrations turned out to have an underlying disease. Many women had pHPT, while men showed an increased mortality from cancer. Patients with elevated calcium concentrations had poorer quality of life and increased health care utilisation than patients with normal calcium concentrations.

There were large differences in the number of calcium analyses ordered, both between physicians and HCCs. A patient's likelihood of an analysis could increase 2.5 times if both the physician and the HCC were changed. Physicians in education ordered more and locums fewer calcium analyses than the average general practitioner.

Keywords: Hypercalcaemia, primary care, primary hyperparathyroidism, cancer, gender, mortality, longitudinal studies, quality of life, health care costs, Physician's Practice Patterns

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SAMMANFATTNING PÅ SVENSKA

Trötthet och nedsatt välbefinnande är symtom som leder till att patienter söker sin vårdcentral. Det kan vara svårt att avgöra om dessa patienter har någon sjukdom. Många läkare beställer då blodanalyser av bland annat kalcium.

Höga kalciumvärden i blodet är inte ovanligt i primärvården, en vanlig orsak är cancersjukdom. Det kan också handla om en godartad knuta i bisköldkörtlarna, primär hyperparatyreoidism (pHPT). pHPT drabbar framförallt kvinnor som passerat klimakteriet, och sjukdomen kan botas kirurgiskt. Många patienter med pHPT har så diskreta symtom att de är svåra att hitta utan analys av kalcium.

Vid Tibro vårdcentral hade knappt 150 patienter höga kalciumvärden i blodet mellan 1995–2000. Vi kartlade i studie I de bakomliggande orsakerna till höga kalciumvärden genom journalstudier. De vanligaste diagnoserna vi fann var pHPT hos 15 % och cancer hos 3 % av patienterna. Hos hela 70 % av patienterna kunde vi inte hitta någon bakomliggande diagnos i journalen.

Patienterna med höga kalciumvärden erbjöds en ny undersökning efter 10 år. I studie II och III har dessa jämförts med kontrollpersoner med normalt kalciumvärde. Alla har tagit nya blodprover, besvarat frågor om sina sjukdomar och läkemedel samt fyllt i ett självskattningsformulär om livskvalitet.

Vid återundersökningen var det få av de med höga kalciumvärden som inte hade fått någon diagnos. Många kvinnor hade pHPT, medan männen hade en ökad dödlighet i cancer. Patienterna med höga kalciumvärden hade också sänkt livskvalitet samt ökad sjukvårdskonsumtion jämfört med patienterna med normala kalciumvärden i blodet.

Syftet med studie IV var att undersöka faktorer som bidrar till skillnader i antalet kalciumanalyser i Primärvården i Skaraborg med 24 vårdcentraler, 457 läkare och 154 629 patienter. Det var stor skillnad i antalet kalciumanalyser som beställdes både mellan olika vårdcentraler och mellan olika läkare. Läkare under utbildning beställde fler och stafettläkare färre kalciumanalyser än genomsnittet. Om en patient byter både vårdcentral och läkare kunde sannolikheten för kalciumanalys i medeltal öka 2,5 gånger.

Högt kalciumvärde i blodet hos män ger en ökad dödlighet. Både män och kvinnor med högt kalciumvärde i blodet har en sänkt livskvalitet samt en ökad sjukvårdskonsumtion.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. **Dalemo S**, Hjerpe P, Bostrom Bengtsson K. Diagnosis of patients with raised serum calcium level in primary care, Sweden. Scand J Prim Health Care. 2006; 24:160-165.

II. **Dalemo S**, Eggertsen R, Hjerpe P, Jansson S, Almqvist E, Bengtsson Boström K. Long-term follow-up of patients with elevated serum calcium concentration in Swedish primary care. Scand J Prim Health Care. 2013; 31:248-254.

III. **Dalemo S**, Eggertsen R, Hjerpe P, Jansson S, Almqvist E, Bengtsson Boström K. Quality of life and health care consumption in patients with elevated serum calcium concentrations in Swedish primary care. Submitted.

IV. **Dalemo S**, Hjerpe P, Ohlsson H, Eggertsen R, Merlo J, Bostrom KB. Variation in plasma calcium analysis in primary care in Sweden − a multilevel analysis. BMC Fam Pract. 2010; 11:43.

CONTENT

ABBREVIATIONS

ALP	Alkaline phosphatase
Ca	Calcium
CI	Confidence interval
DIC	Deviance Information Criterion
ESR	Erythrocyte sedimentation rat
Equalis	External quality assessment for clinical laboratory investigations
GP	General practitioner
HCC	Health care centre
ICD	International statistical classification of diseases and related health problems
MCMC	Markov chain Monte Carlo
MCV	Mean corpuscular volume
MEN	Multiple endocrine neoplasia
MLRA	Multilevel logistic regression analysis
MOR	Median odds ratio
N	Number
NORIP	Nordic reference interval project
NS	Not significant
OR	Odds ratio
P-Ca	Plasma calcium

PHCC	Primary health care centre
pHPT	Primary hyperparathyroidism
PTH	Parathyroid hormone
R&D	Resource and Development
S-Ca	Serum calcium
SF-36	Medical outcome study 36 item short form health survey
SD	Standard deviation
SMR	Standardised mortality ratio
SPCD	Skaraborg Primary Care Database
SWEDAC	Swedish Board for Accreditation and Conformity Assessment
QoL	Quality of life

1 INTRODUCTION

Fatigue and general malaise are some of the most common symptoms leading to a visit to the doctor in Swedish primary care. In these cases, it can be difficult to determine whether the patient has an underlying disease or not. Usually a broad battery of blood tests is used to identify or rule out disease. An analysis of the calcium concentration is often included. Which laboratory tests to perform in these cases is not always a matter of course.

A process was carried out in the 1990s in Sweden, to optimise the clinical chemical work at health care centres (HCCs) for instance, Professor Nils Tryding provided further education for general practitioners, under the theme of ”Correct and optimal use of clinical chemistry in primary care” [1]. The idea was that some analyses provided no further information to the general practitioners, and the number of analyses should therefore be reduced. On the other hand, other analyses should increase in number as they could help identify people with certain diseases. An evaluation of the education showed that a doubling of certain analyses could be seen among the course participants [2]. One of the analyses recommended for an increase was calcium concentration.

1.1 Calcium

Calcium is essential to many cell functions, such as the contraction of muscles, nerve impulses and the release of important substances from different cells. The calcium concentration in the blood is normally very carefully regulated. About 50 % is protein-bound in the blood, mainly to albumin, 45 % is free, physiologically active calcium, known as ionised calcium, and about 5 % is bound as calcium citrate or calcium phosphate. The body’s calcium content is about 1 kg, 99 % of which is bound to the bones. The daily intake of calcium for an adult should be at least 800 mg. The most common sources of calcium are dairy products, which account for more than 60 % of the daily intake. Other calcium sources include cereals, seeds and fresh vegetables. The uptake of calcium occurs primarily in the proximal part of the small intestine.

1.1.1 Calcium regulation

The blood calcium level is primarily regulated by the parathyroid hormone (PTH). PTH increases the calcium concentration in the blood by reducing the excretion of calcium though the kidneys and increasing the influx of calcium from the skeleton. Intact PTH has a half-life in the circulation of about four minutes [3]. PTH stimulates the formation of active vitamin D in the kidney, which, in turn, increases the absorption of calcium from the small intestine. Normally, at falling calcium concentrations in the blood, more PTH is released, which causes an increase in the calcium level. Thus, adequate kidney function is essential for a normal concentration of serum calcium.

1.1.2 The parathyroid glands and hormone

PTH is produced in the parathyroid glands located behind the thyroid at the front of the neck. Usually, the parathyroid glands are four in number, two on either side. Each gland is about the size of a grain of rice, consequently, they cannot be palpated. The release of PTH is regulated by a receptor in the parathyroid cell.

The parathyroid glands were first discovered in 1877 by medical student Ivar Sandström in Uppsala [4]. They are therefore sometimes referred to as Glandulae Sandström. Dr Sandström published his discovery in Swedish in 1880, in a local journal [5]. This may have led to his discovery not being fully appreciated. In 1891, Friedrich von Recklinghausen reported on one of the most severe findings in advanced parathyroid disease, a typical disorder of the skeleton in a patient who had experienced recurrent fractures of several bones with negligible trauma [6]. Jacob Erdheim described the relationship between bone disease and abnormalities of the parathyroid glands in 1906 [7]. Felix Mandl in Vienna performed the first parathyroidectomy under local anaesthesia, in 1925 [8]. That same year, James Collip discovered the parathyroid hormone, PTH [9]. Rosalyn Yalow and Solomon Berson developed the immunoassay that made it possible to measure parathyroid hormone levels in 1963 [10]. Analysis of intact PTH was introduced in the clinical chemistry laboratories in the early 1990s [11].

1.2 Hypercalcaemia

Disturbances of the calcium metabolism lead to high or low calcium concentrations in the blood. Hypercalcaemia occurs when the flow of calcium from the skeleton and gastrointestinal tract into the blood exceeds the kidneys' excretion ability. Hypercalcaemia is seen in 1−4 % of outpatients and in 0.2−3 % of hospital patients.

Primary hyperparathyroidism (pHPT) is the most common cause of hypercalcaemia in outpatient care, whereas malignancies constitute 30–50 % of the hypercalcaemic cases among hospital patients. There are several other, less common causes of hypercalcaemia. Hypercalcaemia can be divided into two groups according to the concomitant PTH concentration, Table 1.

Table 1. Common causes of hypercalcaemia.

Conditions with increased PTH	**Conditions with decreased PTH**
1. Primary hyperparathyroidism	1. Malignancy
2. Renal Impairment - GFR < 60–80 ml/min	2. Calcium and or vitamin D medication
3. Benign familial hypocalciuric hypercalcaemia	3. Sarcoidosis
4. Intake of lithium and thiazides	4. Immobilisation, thyrotoxicosis
	5. Milk alkali syndrome

1.2.1 Primary hyperparathyroidism

Primary hyperparathyroidism (pHPT) is the third most common endocrine disorder after diabetes mellitus and thyroid disease. In pHPT, the release of parathyroid hormone is increased due to a pathologic process in one or more of the parathyroid glands. In most cases, 80–85 %, just one gland is affected with an adenoma, releasing excessive amounts of parathyroid hormone. Hyperplasia, a general growth of all cells in all parathyroid glands, causes about 15 % of all cases of pHPT. pHPT most often arises without any known underlying cause. Rarely, pHPT occurs a part of MEN (Multiple endocrine neoplasia) 1 or 2, a hereditary disorder of several hormonal organs, or as an isolated instance of familial pHPT.

pHPT mainly affects postmenopausal women. The disease has been found in 3.4 % of postmenopausal women in a population-based screening study in Sweden [12]. pHPT is detected relatively frequently in routine blood sampling. The clinical presentation of pHPT nowadays is dominated by subtle symptoms such as depressed mood, confusion, fatigue, sleep disorders, neuromuscular symptoms or other less obvious symptoms [13-15]. For this reason, the first contact with the health service is usually in general practice. The severity of the psychiatric symptoms is not linearly related to the degree of hypercalcaemia [16]. Formerly, in the 1960s and 1970s, pHPT patients came to the hospital due to kidney stones, massive bone decalcification with many fractures and severe muscle weakness. This picture is still seen in developing countries. pHPT patients have an increased risk of cancer [17]

and increased mortality from cardiovascular disease [18, 19]. The disease can be cured surgically with few complications. In Sweden, patients with obvious symptoms or elevated calcium concentrations over 2.70 to 2.80 mmol/l are offered surgery [20]. The patient's symptoms normally disappear postoperatively and the calcium concentration is normalised. pHPT patients who do not undergo surgery should be examined regularly, including checks of calcium, creatinine and bone density [21].

1.2.2 Malignancy

Hypercalcaemia is a serious complication of malignancy, affecting 5–10 % of patients with cancer during the course of the disease. Hypercalcaemia due to malignancy may arise from local osteolysis releasing calcium bound in the bones. This is often caused by metastases, for instance, from breast, bladder, lung and kidney cancer, as well as myeloma, leukaemia, and lymphoma. Another mechanism of hypercalcaemia in malignancy is when cancer cells produce a circulating PTH-like hormone, the parathyroid hormone-related peptide, which stimulates the PTH receptors in the bone and kidney and causes generalised bone resorption. Both conditions result in the release of calcium from the skeleton.

1.2.3 Other causes of hypercalcaemia

Hypercalcaemia may also be caused by various medications, such as lithium and thiazides. The hereditary benign familial hypocalciuric hypercalcaemia is an important differential diagnosis to pHPT that has to be ruled out during the preoperative investigation. In sarcoidosis, the hypercalcaemia is caused by the production of active vitamin D, which increases calcium absorption in the intestine.

Secondary hyperparathyroidism is a physiological response to low levels of calcium in the blood, for example, due to chronic renal failure or severe vitamin D deficiency. These patients have low calcium and high PTH concentrations. Tertiary hyperparathyroidism is a result of prolonged secondary hyperparathyroidism. The parathyroid hyperplasia is then so severe that the autonomous overproduction of PTH continues even if the patient is kidney-transplanted or treated with vitamin D. Tertiary hyperparathyroidism is usually seen in patients with chronic renal failure. These patients have high concentrations of both calcium and PTH.

1.2.4 Hypercalcaemia in the population

The prevalence of hypercalcaemia in the population is about 1–2 % [22-25], Table 2. In older studies, the calcium concentration defining hypercalcaemia

was higher [22]. The prevalence of pHPT in population surveys has been shown to vary between 0.2–0.36 % [22-25], Table 2. Studies limited to elderly and women showed a higher prevalence of hypercalcaemia and pHPT [12, 26].

Several population-based screening studies also examined the mortality associated with hypercalcaemia. In a study from Gävle, the mortality was significantly higher in the hypercalcaemic group than in the control group, mainly due to disorders of the circulatory organs [23]. A study from Malmö also found increased mortality from cardiovascular disease, most pronounced in a group of men younger than 50 years of age [25].

Table 2. Hypercalcaemia in population survey in Scandinavia

Author, year	Selection	No. of subjects	Age years	Design	Ca test	Ca limit Mmol/L	Hyper-calcaemia prevalence	pHPT prevalence
Christensson, 1976	Employees of Stockholm City and Country Council	15 903	20–63	Health survey	Ca	>2.78	1.1 %	0.36 %
Palmer, 1987	Inhabitants in a district in Gävle	16 401	>25	Population survey	Ca albumin corrected	>2.60	0.7 %	0.21 %
Lindstedt, 1992	Inhabitants in Mölnlycke	368	75–95	Health survey	Ionised Ca	>1.3	5%	2.2 %
Leifsson, 1996	Inhabitants in Malmö	33 346	>20	Health survey	Ca	2.60	2.1 %	unknown
Lundgren, 1997	Mammography screening (women), Uppsala	5 202	55–75	Health survey	Ca	>2.55	3.5 %	2.1 %
Jorde, 2000	Inhabitants in Tromsö	25 733	25–75	Population survey	Ca	>2.55	1.4 %	0.25 %

A draw-back of screening studies is that the participating individuals often differ from the non-participants. For instance, in the study from Malmö, it was found that non-participants in the screening programme had higher mortality, morbidity and alcohol consumption than the participants [27, 28]. These studies are thus likely to include healthier individuals and do not give the true prevalence of the studied conditions.

1.2.5 Hypercalcaemia studies in clinical practice in primary care

There are many studies of hypercalcaemia and pHPT in in-patient care [29, 30]. In contrast, there are very few studies of the prevalence of hypercalcaemia in primary care. A study from Mölnlycke [26] showed that screening for pHPT in an elderly population is not clinically useful as many of these patients would not be considered for surgical intervention. Furthermore, this was a population screening study that only included elderly individuals. This calls for more studies of the prevalence, underlying causes and management of hypercalcaemia in primary care.

1.2.6 Quality of Life

Patients with pHPT and disseminated cancer, often associated with hypercalcaemia, generally have impaired quality of life, QoL [31, 32]. According to the WHO, QoL is the individual's perception of their situation in relation to the prevailing culture and norms and to their own objectives, expectations, values and interests. By definition, it is a personal experience that is affected by changes in the life situation and that varies with time. QoL is a common concept in many types of research and the use of validated survey forms is an accepted way of measuring QoL. The Short Form (36) Health Survey, the SF-36 [33], is a widespread health status measurement, which has gained great acceptance because of its brevity and breadth [34]. The SF-36 is applicable regardless of the patient's condition [35].

As mentioned earlier, hypercalcaemia is not an unusual finding in patient's with fatigue and general malaise. The patient's QoL has become an increasingly important component in the treatment of disseminated cancer disease in recent years [36]. Researchers disagree on whether parathyroidectomy improves QoL in patients with pHPT [32, 37].

A Swedish screening study shows that women with formerly unknown, mild pHPT visit physicians more often and have more sick leave days during the years prior to the diagnosis [38, 39]. However, there is still a lack of studies of QoL in patients with hypercalcaemia per se. It is not known whether there

is an association between QoL and health care consumption in patients with hypercalcaemia in primary care.

1.3 Hypocalcaemia

Hypocalcaemia may have several causes; with iatrogenic hyperparathyroidism after thyroid surgery being the most common cause. Hypocalcaemia may also occur due to calcium or vitamin D deficiency caused by malnutrition and lack of sunlight. Other causes of low calcium concentrations might be kidney and intestinal disease or malignancies lead to low albumin concentrations. Several studies from intensive care units have shown that low calcium concentrations are associated with increased mortality without any specific underlying cause [40-42].

1.4 Analytical methods

At the HCC, calcium is usually analysed in serum or plasma. Analysis of whole blood is equivalent to the analysis of serum. The standard method during the 1990s was analyses in serum, but in the 2000s, plasma analysis was introduced. Both analyses use the same reference values [43, 44], which implies that analysed calcium in serum and plasma are equivalent. As calcium is highly bound to albumin, the calcium concentration in serum or plasma should be corrected for the concomitant albumin level. There are many different formulae for this calculation, most of them not validated. Analysis of ionised calcium, however is the most reliable method, with the highest specificity and sensitivity. This analysis is unusual in primary care, probably because it is more expensive than the plasma calcium analysis.

In the 1990s, some HCC laboratories received point-of-care equipment, enabling them to perform more than just simple analyses like glucose and blood count on the spot. Some of these point-of-care analyses were calcium and albumin. In many HCCs, calcium is a common analysis, as it is included in the diagnostic procedure in dementia, fatigue and general malaise. Several HCCs also have standardised group analyses including calcium analysis; for instance, analyses of electrolytes.

1.5 The role of primary care and point-of-care analyses

Primary care in Sweden was developed in the 1970s and 1980s. Each municipality was served by at least one HCC. Skaraborg is a rural area in the

southwest of Sweden with 15 municipalities and 256 000 inhabitants. In the former county of Skaraborg, each HCC worked independently and had the medical responsibility for its geographical area. Until 2009, primary care in Skaraborg was delivered by one private and 24 public HCCs, as well as by a few independent, private GPs. At the time of the study the public primary care in Skaraborg covered 97 % of the population. In-patient care is offered by four public hospitals.

During the 1990s, many HCCs in Skaraborg were given the opportunity to perform extended point-of-care tests, for example, calcium analyses. The rationale behind this was that the patient should receive the result of the blood analysis already in connection with the visit to the HCC, which would improve communication with the patient and speed up the diagnostic process.

At the same time, medical records in primary care were computerised. Initially, several different computer systems were introduced in the municipalities. In 1999, the entire public primary care service in Skaraborg changed their system to ProfDoc Journal III (PDIII, Prof-Doc AB, Uppsala). All previous medical records were converted to this new system in connection with the changeover. The fact that all 24 public HCCs use the same computerised medical record system makes it easier to use patient record data for research purposes.

1.5.1 Tibro Health Care Centre

Tibro HCC was one of the 24 public HCCs in Skaraborg. The municipality is a typical Swedish rural community, 25 km from the nearest hospital in Skövde, with approximately 11 000 inhabitants and low migration rates. There is only one HCC in Tibro municipality, used by the vast majority of patients. Until the early 2000s, there was also a private practitioner working in Tibro municipality. In this sense, Tibro HCC is typical and representative of Swedish primary health care in rural areas.

Medical records at the HCC were computerised already in the autumn of 1991 when a fully computerised medical record system, Swedestar (Tieto, Helsinki), was introduced. In the 1990s, a Resource and Development (R&D) unit specialised at using computerised patient record data for research and development purposes, was located to Tibro HCC. The early computerisation and the presence of the R&D unit made it possible to retrieve data from Tibro HCC's record already in the 1990s. Extended point-of-care analyses were introduced at Tibro HCC in 1993. Between 1993 and 2006, calcium was analysed in whole blood at the laboratory by Vision (Abbott, North Chicago).

After 2006, calcium was analysed in plasma centrally at the primary care laboratory by Integra 400 (Roche).

In the 1990s, Tibro HCC was well staffed, with all six medical posts filled. Several interns and resident physicians also performed their primary care practice in Tibro. However, during the 2000s it became increasingly difficult to recruit physicians and about half of the medical posts at Tibro HCC became vacant. The number of physicians in education was also reduced. The staffing situation was resolved by hiring locums who worked for just one or a few weeks at a time.

As shown here, there are few studies of elevated calcium concentrations diagnosed in primary care. Accordingly, there is a lack of knowledge of how to handle elevated calcium concentrations in general practice. The four studies in this thesis were conducted in order to bridge this knowledge gap.

2 AIMS

2.1 General aims

The overall aim of this thesis was to examine the underlying cause, mortality, quality of life and health care consumption among patients with elevated calcium concentrations in primary care in a defined municipality. A further aim was to explore the variation in calcium analyses in Skaraborg, with respect to patient characteristics, physicians and HCCs.

2.2 Specific aims

To achieve the overall aims, four specific studies were conducted:

Study I. The number of calcium analyses and diagnoses in patients with elevated calcium concentrations

This study was designed to investigate whether the number of calcium analyses and detected patients with elevated calcium concentrations increased at Tibro HCC during the period 1992 to 2000, according to the National Recommendations [1]. We also wanted to examine the diagnoses of the patients with elevated calcium concentrations from 1995 to 2000.

Study II. Examination of diagnoses and long-term mortality

This study was designed to investigate the underlying diagnoses after ten years, especially in patients who had no diagnosis at the first investigation. Furthermore, we wanted to analyse long-term mortality in patients with elevated calcium concentrations compared with patients with normal calcium concentrations.

Study III. Quality of life and health care consumption

This study was designed to investigate QoL in patients with elevated calcium concentrations compared with patients with normal calcium concentrations and the Swedish norm population. The secondary aim was to study whether elevated calcium concentrations affect sick leave, drug prescriptions and the number of visits and admissions to HCCs and hospitals.

Study IV. Variations in calcium test ordering

This study was designed to investigate determinants of and variations in calcium test ordering. Data from computerised medical records were used to clarify the relative importance of different levels in the health care organisation for the ordering of calcium analyses.

3 PATIENTS AND METHODS

3.1 Design of the individual studies

Studies I and IV are both based on data retrieved from computerised medical records. In Study I, data from Tibro HCC were used, while all 24 public HCCs in Skaraborg were included in study IV. Studies II and III are based on examinations of the patients in study I with elevated calcium concentration at Tibro HCC in the 1990s, Figure 1, compared with patients with normal calcium concentrations. The designs of the individual four studies are shown in Table 3. The table defines different study periods, inclusion criteria, number of participants and outcome variables.

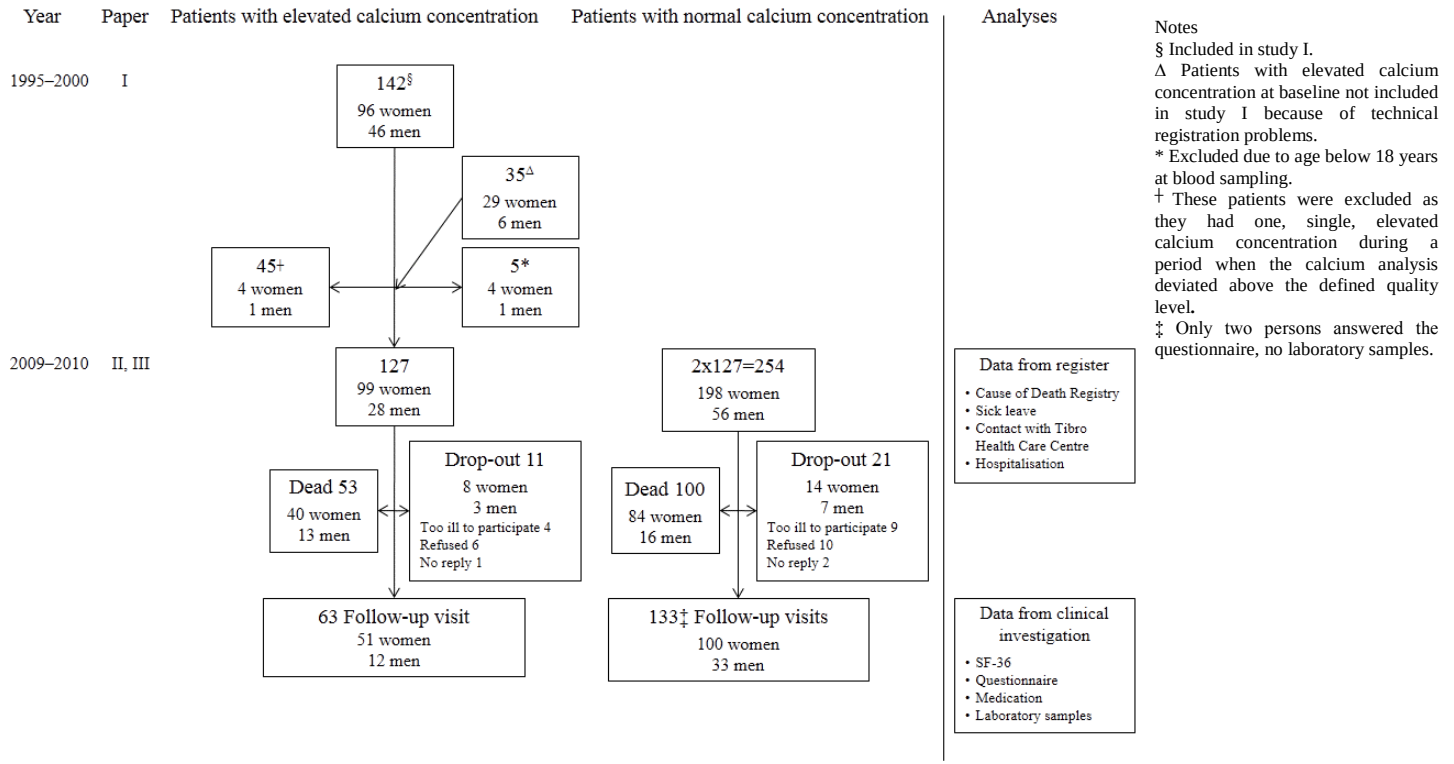

Figure 1. Flow chart of patients with elevated calcium concentrations at Tibro Health Care Centre, Sweden, 1995–2000, and examination of the patients with elevated and normal calcium concentrations during 2009–2010.

Table 3. Study period, inclusion criteria, number of units in the study and outcome variables in the different papers.

Paper	Study period	Inclusion criteria	Number	Outcome variable
I	1992–2000	Analyses at Tibro pHCC	Ca analyses: 7.364 PTH analyses: 105	Total and raised Ca analyses Total and raised PTH analyses
	1995–2000	Patients with elevated calcium at Tibro HCC	Patients: 142	Diagnoses
II	2008–2010	Examination of patients with elevated and normal calcium concentrations	Patients: 127 Patients: 254	Diagnoses, mortality Mortality
III	2008–2010	Examination of patients with elevated and normal calcium concentrations	Patients: 127 Patients: 254	Quality of life (SF-36) Hospitalisation Admissions to health care centres Medication Sick leave
IV	2005	All individuals attend any of the HCCs in Skaraborg	Patients: 154.629 Physicians: 457 HCCs: 24	Presence or absence of calcium analysis

3.2 Study I. Data from the medical records at Tibro health care centre

3.2.1 Study population

By using EpiInfo (EPI6, version 6.04d, Centre for Disease Control and Prevention, CDC, Atlanta, in collaboration with the WHO), all patients with an elevated calcium concentration (serum calcium $\geq$ 2.56 mmol/l), regardless of albumin concentration, at Tibro HCC between 1995 and 2000 were identified in the computerised medical records and 142 individuals were retrieved. Ionised calcium concentrations were not included in the study.

3.2.2 Outcome variables

A computer search of the medical records between 1992 and 2000 was performed to establish the total number of laboratory analyses, serum calcium, and PTH analyses in relation to the number of medical consultations. Parameters were extracted using EpiInfo from the ProfDoc database.

A manual evaluation of Tibro HCC's medical records of all patients with elevated calcium concentrations was carried out in March 2002 to identify underlying diagnoses. The results from consultant opinions, laboratory and radiological examinations between 1995 and 2000 were studied.

3.2.3 Statistical analysis

Only descriptive statistics were used in this study.

3.2.4 Additional analyses for this thesis

We thought it would be of interest to continue to investigate the number of calcium analyses. Data from Tibro HCC became available during the writing of this thesis and we therefore decided to analyse the number of calcium analyses in recent years. Creatinine and blood count concentrations were chosen for comparison, to determine whether a possible change in the number of calcium analyses could be attributed to a greater change in the number of analyses. The number of calcium analyses at Tibro HCC in the 2000s was retrieved by using EpiInfo in the Tibro HCC computerised medical records.

3.3 Study II and III. Examination of patients with elevated and normal calcium concentrations at Tibro health care centre

3.3.1 Study population

All patients with elevated calcium concentrations at Tibro HCC detected in study I were offered a follow-up visit after 9–15 years. At the examination, we found an additional 35 patients with elevated calcium concentration at baseline who were not included in the previous study because of technical registration problems, Figure 1. Two control patients, matched for age and sex, with calcium concentrations < 2.45 mmol/l were selected for each patient with an elevated calcium concentration from the Tibro HCC medical records. Thus, all controls were patients at the HCC. The age of the controls was matched to within two months, but for the oldest patient (n=2), the match was within three years. Live study subjects were invited by mail to participate until July 2011. A nurse interviewed and examined all the participants. Subjects who had moved were interviewed by phone and blood samples were taken at their local HCC.

3.3.2 Excluded individuals

Patients with calcium concentrations between 2.45–2.55 mmol/L were excluded from the control group. Forty-five patients with high calcium concentrations only had a single, initial high calcium concentration, but normal calcium concentrations at follow-up. Most of these samples were taken between January 1997 and April 1998, when the calcium analysis deviated above defined quality levels in several instances**.** We found no underlying diagnoses at follow-up in any of these 45 patients and they were therefore excluded from the analysis. Furthermore, we only wanted to study adult individuals and therefore excluded six individuals who were below 18 years of age at the time when the blood samples were taken.

3.3.3 Outcome variables

At the visit to the nurse the participants answered questions about their former and current disorders. Self-reported alcohol consumption and current medication were recorded. Weight and height were measured. Further blood samples were analysed: calcium, albumin, ionised calcium, blood count, sedimentation rate, albumin, creatinine, alkaline phosphates and serum-intact parathyroid hormone.

3.4 Study II. Diagnoses and mortality

3.4.1 Study-specific outcome variables

For patients with elevated calcium concentrations, results from laboratory and radiological examinations and consultant opinions, as detailed in their medical records for the years 1992–2011, were studied to find an underlying diagnosis associated with the elevated calcium concentrations.

Data from the Swedish Cause of Death Registry, updated in August 1, 2011, were derived both for patients with elevated and normal calcium concentrations. Furthermore, data on mortality for the background population in Tibro community from 1995 to 2010 were collected from the Swedish Cause of Death Registry.

3.4.2 Statistical analyses

Descriptive statistics were presented and comparisons were performed using the Chi-Square Test and T-test. A comparison of survival time between the groups was performed with a Kaplan-Meier survival analysis with log-rank test. The mortality among men and women with elevated calcium concentrations was also compared with the mortality among inhabitants in Tibro, using standardised (with respect to age group and time period) standardised mortality ratios (SMR), and was expressed as an odds ratio (OR) and presented with a 95 % confidence interval (CI). For mortality rates among inhabitants in Tibro, data from the Swedish Death Registry were used, and the comparisons and calculations of person-years at risk and SMR were performed using the PAMCOM software [45-48]. To analyse the impact of different levels of calcium on mortality, patients were divided into three groups according to their calcium concentration ($\leq$2.30, 2.31–2.46, $\geq$ 2.56 mmol/L). A p value < 0.05 was considered statistically significant. All statistical analyses except the SMR were performed using the SPSS 20 statistical package.

3.5 Study III. Quality of life and health care consumption

3.5.1 Study-specific outcome variables

The study participants also completed a QoL health survey, the SF-36. This survey allows comparison of the burden of illness [33] and has been validated for a variety of disorders [35]. Most of the SF-36 items originate from other

instruments that have been in use since the 1970s and 1980s [34]. The SF-36 has been used in its current form since 1990 [34].

The survey includes 36 simple questions, which are scored and aggregated. The survey defines eight separate and distinct areas or domains of health status: physical functioning, physical role functioning, bodily pain, general health, vitality, social role functioning, emotional role functioning and mental health. Three scales, physical functioning, physical role functioning and bodily pain, correlate with the physical component [34]. A further three scales, social role functioning, emotional role functioning and mental health, correlate with the mental component [34]. The number of questions that contribute to each domain ranges from 2 to 10. All scales are standardised from 0 to 100, with higher scores indicating better QoL. The SF-36 is suitable for people over 14 years of age [34].

SF-36 data from both comparison groups (patients with elevated calcium concentrations and patients with normal calcium concentrations) were compared with an age and gender-matched reference material (on average, 2.3 individuals) from Sweden (n=703) in the Swedish SF-36 national normative database [49]. We used 20-year intervals, (20−39, 40–59, 60–79, and 80–99 years) in the analyses.

Data regarding visits to general practitioners and psychosocial teams between January 1, 1998, and December 8, 2010, were collected from the HCC medical records. All sick leave notes issued at Tibro HCC were recorded directly in the medical records. No sick leave notes were written by hand on paper. It was therefore possible to record all certified sick leave instances between November 4, 2003, and December 27, 2010. Diagnoses at and the duration of all in-patient care instances between January 1, 1967, and December 31, 2010, were derived from The Swedish National Board of Health and Welfare.

3.5.2 Statistical analysis

Descriptive patient statistics were presented and comparisons were performed using the T-test, the Chi-Square Test and the Mann-Whitney U test, depending of the type of data included in the analysis. The SF-36 survey results were analysed with non-parametric tests, primarily the Mann-Whitney test. A p value < 0.05 was considered statistically significant. Data are given as mean ± SD and as mean, 10^{th} and 90^{th} percentile. All statistical analyses were performed using the SPSS 20 statistical package.

3.6 Study IV. Data from the medical records of 24 health care centres in Skaraborg

3.6.1 Study population

All individuals who attended any public HCC in Skaraborg during 2005, altogether 154 629 persons, were included in the analysis, together with all the physicians who worked for longer or shorter periods in Skaraborg public primary care during 2005, n=457.

3.6.2 Multilevel model

In this study we wanted to study factors that affect the individual patient's chance of having his/her calcium concentration analysed. Differences in clinical practice can be observed both between HCCs, but also between physicians within the same HCC, Figure 2.

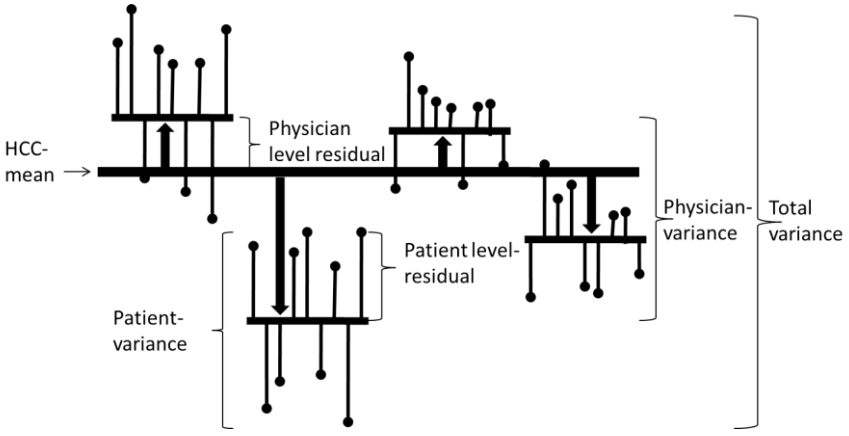

Figure 2. Explanation of differences in practice between different patients and different physicians at a health care centre.

To study these phenomena a model can be used, with patients nested within physicians who, in turn, are nested within HCCs, Figure 3. This type of hierarchical model is called a multilevel model.

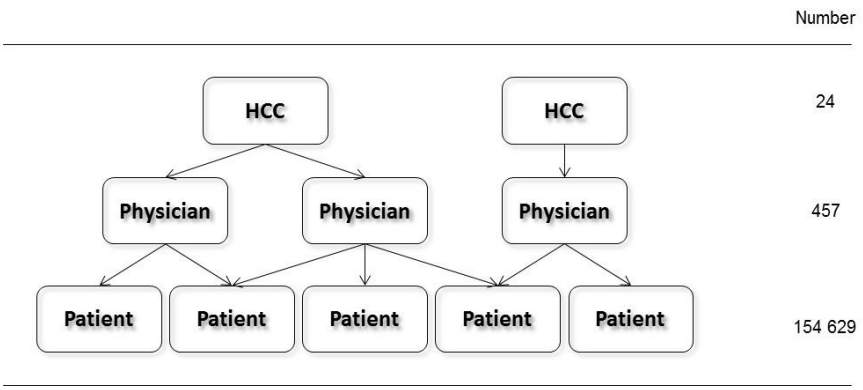

Figure 3. A multilevel model with 24 health care centres (HCC), 457 physicians and 154 629 patients.

3.6.3 Multilevel regression analysis

The fact that the variation can be seen at any of the different levels included makes the multilevel regression analysis techniques (MLRA) a suitable, robust tool to analyse these kinds of hierarchically organised data [50, 51]. The MLRA enables the inclusion of variables from both individual and higher levels in the same analysis and calculation of the total variation that can be attributed to each level.

The MLRA provides measures of the degree of association between the level-specific variables, the fixed effects, like any other regression analysis, dummy Table 4. In the fixed-effects part of the MLRA, we calculated the OR and their 95 % CI. The credible intervals were used as being equivalent to confidence intervals. In order to quantify the importance of the different levels in the analysis we calculated the second part of the MLRA, the random effects. Here, we obtain the variance at physician and HCC level, expressed as the Median Odds Ratio (MOR) [52, 53].

Table 4. Dummy table with an example of a three-level mulitlevel regression analysis with one emty model (A) and three models (B,C,D), including specific variables as fixed effects. The random effects are expressed as median odds ratios (MOR). Reproduced with permission from Per Hjerpe (thesis 2011).

	Model A	**Model B**	**Model C**	**Model D**
Fixed effects	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Patient				
Female	-	REF	REF	REF
Male	-	x.xx(x.xx–x.xx)	x.xx(x.xx–x.xx)	x.xx(x.xx–x.xx)
Physician				
Female	-	-	REF	REF
Male	-	-	x.xx(x.xx–x.xx)	x.xx(x.xx–x.xx)
HCC				
Finance form				
Public	-	-	-	REF
Private	-	-	-	x.xx(x.xx–x.xx)
Random effects	**Variance (95 CI%)**	**Variance (95 CI%)**	**Variance (95 CI%)**	**Variance (95 CI%)**
HCC	x.xx(x.xx–x.xx)	x.xx(x.xx–x.xx)	x.xx(x.xx–x.xx)	x.xx(x.xx–x.xx)
MOR_{HCC}	x.xx(x.xx–x.xx)	x.xx(x.xx–x.xx)	x.xx(x.xx–x.xx)	x.xx(x.xx–x.xx)
Physician	x.xx(x.xx–x.xx)	x.xx(x.xx–x.xx)	x.xx(x.xx–x.xx)	x.xx(x.xx–x.xx)
$MOR_{physician}$	x.xx(x.xx–x.xx)	x.xx(x.xx–x.xx)	x.xx(x.xx–x.xx)	x.xx(x.xx–x.xx)
HCC and physician	x.xx	x.xx	x.xx	x.xx
$MOR_{HCC+physician}$	x.xx	x.xx	x.xx	x.xx
DIC	xx.xx	xx.xx	xx.xx	xx.xx

The MOR translates the variance into the widely used OR scale, which has a consistent and intuitive interpretation. It can therefore be directly compared with other OR (associations) in the model. The MOR is defined as the median value of the odds ratio between the area at highest risk and the area at lowest risk. Stated otherwise, the MOR could be interpreted as the median odds of a calcium analysis being ordered for a patient increasing if this patient moved to a physician/HCC with higher odds of ordering a calcium analysis. A MOR of 1 indicates that there are no differences between physicians/HCCs with regard to the odds of their ordering a calcium analysis. The larger the difference between physicians/HCCs, the higher the MOR.

MLRA analyses are usually performed by adding one level of explanatory variable at a time to the model, Table 4. We developed four consecutive models. The empty model A, without any level-specific variables, was used to calculate the variance without any explanatory variables. In model B we added patient characteristics. Model C included both patient and physician characteristics and, finally, model D contained all the characteristics of patients, physicians and HCCs. In this way, we could investigate whether the contextual characteristics in the fixed-effects part of the model explained the residual variation at physician and HCC level.

As one patient could attend several physicians at a HCC, Figure 3, we used a multiple membership model [54]. Weights were constructed according to the individual patient's number of visits to a certain physician during our study period. It was impossible to identify whether physicians and patients attended more than one HCC, as all HCC records were encrypted at the local R&D department. This means that it is impossible to determine if a patient or a physician occurs in several HCCs.

The MLRA estimations were made using Markov chain Monte Carlo (MCMC) methods [55] with the MLwiN software (MLwiN 2.20, Centre for Multilevel Modelling, University of Bristol) [56].

3.6.4 The outcome variable

The outcome variable was the presence or absence of a calcium analysis during 2005.

3.6.5 Level-specific variations

To explain the variation in a phenomenon we identified specific characteristics at the different levels that may affect the variation.

Patients-level variables

The hypothesis was that different types of patients seek different physicians. Physicians caring for nursing homes may have more elderly and sick patients than doctors servicing the child welfare centre. To characterise the patients we created a list of ICD-10-coded diagnoses, symptoms and medications associated with calcium analyses. Subsequently, a risk score for a calcium analysis was created with stepwise logistic regression using these variables and the patient's age [57]. The risk score was divided into quintiles. Patients with the lowest chance of a calcium analysis (group 1) were used as reference. Some of the characteristics included in the risk score are listed in Table 5. We also included the sex of the patient and calcium analyses during 2004 as explanatory fixed-effects variables.

Table 5. All the patients in the risk score equation. Total number of patients 154 629.

Title	All positive diagnoses in the stepwise regression	ICD-10 codes	Odds ratio	95 % CI		Number of P-Ca analyses
Neoplasms	Neoplasm UNS of female genital organs	D39	4.0	1.3	12.6	16
	Neoplasm UNS of urinary organs	D41	10.5	0.6	174.5	7
	Neoplasm UNS of brain and CNS	D43	10.1	1.9	54.8	6
	Sacroidosis	D86	8.4	3.3	21.4	21
Endocrine disorders	Hypothyroidism	E03	1.5	1.3	1.7	1 631
	Nontoxic goitre	E04	3.1	2.2	4.5	177
	Thyrotoxicosis	E05	3.0	2.0	4.4	132
	Thyroiditis	E06	4.5	2.7	7.5	80
	Other disorders of thyroid	E07	3.6	2.0	6.4	63
	Non-insulin-dependent diabetes mellitus	E11.8	0.7	0.5	0.9	526
	Non-insulin-dependent diabetes mellitus	E11.9	0.6	0.5	0.7	1 798
	Unspecified diabetes melllitus	E14	0.8	0.7	0.9	3 751
	Hyperparathyroidism	E21	7.3	4.8	11.2	111
Mental disorders	Unspecified dementia	F03	2.5	2.0	3.0	523
	Bipolar affective disorder	F31	2.6	1.2	5.7	36
	Depressive episode	F32	2.3	2.0	2.5	3 196
	Unspecified mood (affective) disorder	F39	1.5	1.0	2.3	182
	Phobic anxiety disorders	F40	2.9	1.5	5.7	53
	Panic disorder	F41.0	2.3	1.7	3.0	414
	Anxiety disorder	F41.9	1.7	1.5	2.0	1 438
	Obsessiv compulsive disorder	F42	3.9	1.9	8.0	44
	Posttraumatic stress disorder	F43.1	3.3	1.9	5.7	76
	Reaction to severe stress, and adjustment disorder	F43.9	1.5	1.3	1.8	1 787
	Eating disorder	F50	7.9	2.4	26.2	15
	Bulimia nervosa	F50.2	21.2	4.9	92.0	9
	Eating disorder, unspecified	F50.9	16.7	9.4	29.7	52
	Nonorganic sleeping disorders	F51	1.5	1.3	1.7	1 636
	Mental disorder, not otherwise specified	F99	3.1	1.9	5.0	108
	Alzheimers's disease	G30	2.8	2.1	3.9	189

	Tension-type headache	G44.2	1.6	1.2	2.2	412
	Transient cerebral ischaemic attacks and related syndromes	G45	1.5	1.1	2.1	220
	Sleep disorders	G47	2.5	1.6	4.0	113
	Polyneuropathy, unspecified	G62.9	1.7	1.1	2.4	170
	Postviral fatigue syndrome	G93.3	2.4	1.5	3.9	139
	Other disorders of nervous system, not elsewhere classified	G98	2.5	1.4	4.3	74
Diseases of the ear and the mastoid process	Benign paroxysmal positional vertigo	H81.1	1.8	1.2	2.6	182
Diseases of the cirkulatory system	Essential hypertension	I10	1.8	1.7	1.9	12 867
	Hypertensive heart and renal disease	I13	1.4	1.1	1.8	383
	Angina pectoris, unspecified	I20.9	1.3	1.1	1.5	1 507
	Chronic ischaemic heart disease	I25	1.2	1.0	1.3	1 856
	Atrial fibrillation and flutter	I48	1.5	1.3	1.6	1 792
	Heart failure	I50	1.7	1.5	1.9	1 937
	Complications and ill-definated descriptions of heart disease	I51	1.9	1.3	2.9	140
	Other cerebrovascular diseases	I67	1.5	1.1	2.0	224
Diseases of the digestive system	Constipation	K59.0	1.7	1.4	2.0	730
	Coeliac disease	K90.0	3.2	2.0	5.0	112
Diseases of the musculoskeletal system	Pain in joint	M25.5	1.8	1.6	2.0	3 233
	Other artritis	M13	2.4	1.9	2.9	577
	Rheumatism, unspecified	M79.0	2.8	2.2	3.5	463
	Myalgia	M79.1	1.8	1.6	2.0	3 749
	Osteoporosis with pathological fracture	M80	3.4	2.3	5.0	124
	Osteoporosis without pathological fracture	M81	2.0	1.6	2.5	529
Disease of the genitourinary system	Unspecified renal failure	N19	3.3	2.4	4.6	188
	Calculus of kidney and ureter	N20	3.2	2.3	4.4	241
	Other disorders of kidney and ureter, not elsewhere classified	N28	2.7	1.5	4.9	57
Symptoms	Abnormal blood pressure reading witout diagnosis	R03.0	2.7	2.3	3.3	580
	Nausea and vomiting	R11	3.3	2.2	3.9	275

	Cramp and spasm	R25.2	4.9	5.9	7.2	138
	Other symptoms involving the nervous and musculoskeletal systems	R29.8	2.1	1.7	2.5	673
	Polyuria	R35	2.0	1.5	2.7	355
	Dizziness and giddiness	R42	2.5	2.2	2.8	1 907
	Other symptoms involving general sensations	R44	2.0	1.2	3.4	84
	Symptoms and signs involving emotional state	R45	2.2	1.2	4.2	56
	Headache	R51	2.5	2.2	2.9	1 392
	Pain, not elsewhere classified	R52	1.8	1.7	2.1	2 662
	Malaise and fatigue	R53	6.5	5.9	7.2	2 261
	Hyperhidrosis	R61	12.6	6.8	23.4	51
	Other symptoms concerning food and fluid intake	R63.8	4.7	2.3	9.6	47
	Other specificd general symptoms and signs	R68.8	2.9	1.4	6.0	45
	Elevated erythrocyte sedimentation rate	R70.0	3.9	2.2	6.8	62
	Abnormal finding of blood chemistry	R79	4.5	3.4	6.0	242
Contact with health services	General medical examination	Z00.0	3.3	3.0	3.7	1 911
	Worried well	Z71.1	2.5	2.2	2.8	1 732
Laboratory analysis	Calcium analysis 2004		1.7	1.6	1.9	14 698
Drug	Bisphosphonate		1.6	1.4	1.9	1 234
	Calcium and vitamin D supplements		3.3	2.1	5.1	2 938
	Thiazide diuretics		1.3	1.2	1.4	8 305
	Prednisolon		1.4	1.2	1.6	1 628

Physician-level variables

When deciding on the physician variables, the hypothesis was that the number of calcium samples ordered varies with the physician's years in the profession. The majority of physicians in education have recently finished university and older specialists have worked for many years. The physicians were therefore categorised according to gender and position. GPs and locums were also dichotomised at 46 years. As only six doctors among the house officers and preregistration house officers were above 45 years of age, they were not dichotomised. GPs 46 years or older were used as references in the analysis.

HCC-level variables

Many HCCs use standardised group analyses in which calcium is included; for instance, analyses of electrolytes, hypertension check-ups and diagnosing of dementia. Our hypothesis was that the differences in the number of standardised predefined group analyses including calcium affect the number of calcium analyses. We therefore categorised HCCs as having none, 1–2, and ≥ 3standardised groups including calcium. The HCCs having no group analyses were used as references.

3.7 Quality controls of laboratory analyses at Tibro health care centre

Between 1993 and 2006, the calcium analyses in whole blood at Tibro HCC were performed by a laboratory assistant with a spectrophotometric method on a Vision instrument. Internal quality controls were performed every week when a calcium sample was analysed both at the HCC and at the clinical chemistry department at the hospital in Skövde. In case of discrepancies between samples, the analysis at the HCC was calibrated. The analysis of calcium at Tibro HCC was suspended for a short period in March 1997, due to too large a discrepancy at the calibration. The analyses were only resumed when a new Vision instrument was purchased. Additional, national quality controls were performed by Equalis, an external quality assessment body for clinical laboratory investigations based in Uppsala, Sweden [58]. Their quality controls revealed that between January 1997 and April 1998, when parts of the analyses for this study were performed, the calcium analysis deviated above the defined quality levels in several instances, Figure 4.

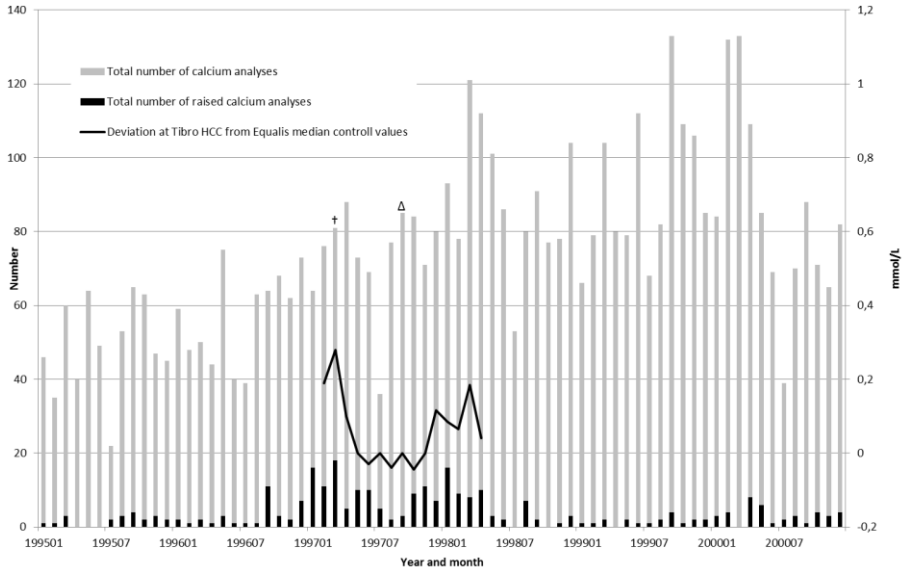

Figure 4. Number of total and elevated calcium concentrations at Tibro health care centre between January 1, 1995, and December 31, 2000. The external quality controls performed by Equalis revealed that between January 1997 and April 1998, the calcium analysis deviated above the defined quality levels.

All the laboratories of the HCCs in the area were certified by the Swedish Board for Accreditation and Conformity Assessment (SWEDAC), assuring adherence to the ISO 15 189 standard, in September 1998.

4 RESULTS

4.1 Study I. Data from the medical records at Tibro health care centre

4.1.1 Number of analyses

The number of serum calcium analyses at Tibro HCC increased during the 1990s, from 51 calcium analyses per 1 000 inhabitants per year in 1992 to 100 calcium analyses per 1 000 inhabitants per year in the year 2000. The total number of blood analyses also increased during the period, from approximately 70 000 to 100 000 per year. Therefore, roughly 1 % of all the analyses were calcium concentration analyses. Throughout the years, the number of analyses with elevated calcium concentrations (>2.55 mmol/l) remained constant, on average, 50 per year. The number of medical consultations at Tibro HCC also remained relatively constant at about 14 500/year. Between 1992 and 2000, 7 364 calcium concentrations were analysed and 424 elevated calcium concentrations were found in 276 patients. The number of PTH analyses increased during the study period from 1 per year to >25 per year, while the number of patients detected with elevated PTH concentrations (>65 ng/ l) increased from 0 to 10 per year. On average, one third of the PTH analyses were elevated.

4.1.2 Additional analyses for this thesis

The additional analysis showed that the number of calcium analyses at Tibro HCC reached a peak in 1999, when 1 100 calcium samples were analysed, Figure 5. Thereafter, the number of calcium analyses declined and remained at approximately the same level – around 540 calcium analyses per year – as in the early 1990s. A similar pattern was shown in the number of creatinine and blood count analyses during the 1990s and 2000s.

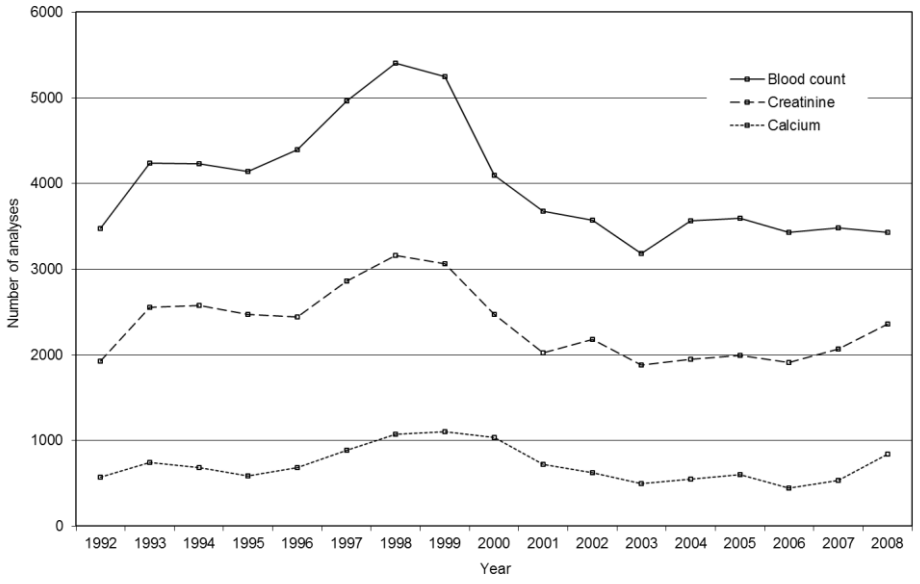

Figure 5. Number of analyses per year between 1992 and 2008 at Tibro Health Care Centre

4.1.3 Diagnoses

During 1995–2000, we retrieved 142 patients (96 females and 46 males) with elevated calcium concentrations in at least one analysis from the laboratory file in the patients' records. These patients came to the HCC with a multitude of symptoms and no single symptom dominated. The calcium analyses were repeated in 70 % of patients with elevated concentrations. One quarter of the patients with an elevated calcium concentration, especially those with the highest calcium concentrations, were referred to other specialists for further investigation.

pHPT was found in 22 (15 %) patients with elevated calcium concentrations. Forty per cent of the pHPT patients had just slightly elevated calcium concentrations, between 2.55 and 2.60 mmol/l. None of the patients with pHPT had calcium concentrations above 3.0 mmol/l. Almost 90 % of the females with pHPT were 60−80 years old while the majority of males were between 50 and 59 years old. Half of the patients with pHPT were treated surgically or were on a waiting list for surgery. The rate of pHPT was 0.22 % in the Tibro population during the time of the survey. The second most common diagnosis in patients with elevated calcium concentrations was skeletal disease, followed by kidney disease. Three per cent of the patients with an elevated calcium concentration had malignant disease, such as

myeloma, prostate cancer, small cell lung cancer and anaplastic thyroid cancer. In 70 % of the patients with an elevated calcium concentration, no underlying diagnosis was found in the medical records.

4.2 Study II and III. Examination of patients with elevated and normal calcium concentration at Tibro health care centre

During the follow-up period, 153 of the 381 participants died and 32 dropped out for other reasons, Figure 1. Thus, the number of subjects examined was 196, which was 86 % of the eligible population. For all 381 participants the median follow-up time was 10.1 years (range 0.0 to 16.6 years), resulting in 3 863 person-years.

The patient group with elevated calcium concentrations at baseline had normalised mean calcium concentrations at the time of the follow-up; however, it was still higher than in the group of patients with normal calcium concentrations at baseline. The two groups demonstrated similar values in the upper reference range of parathyroid hormone levels. Patients with elevated calcium concentrations had a higher mean corpuscular volume. This could not be explained by differences in alcohol consumption, as the patients with elevated calcium concentrations had significantly lower alcohol consumption.

At follow-up, patients with an elevated calcium concentration had a higher mean age than the patients with normal calcium concentrations; however, the difference was not significant. There was no difference in marital status, level of education, employment or in the number of smokers between patients with elevated and normal calcium concentrations. We found that individuals with elevated calcium concentrations had significantly fewer own teeth left, compared with patients with normal calcium concentrations. For women, the age at menarche and at menopause, and the number of child-births were similar between the two groups.

4.3 Study II. Diagnoses and mortality

4.3.1 Diagnoses

In all patients with elevated calcium concentrations, including deceased patients and dropouts, underlying diagnoses were sought in the Tibro HCC

medical records. Many diagnoses increased between the two observations, the most common being pHPT. Forty-three per cent of the patients with pHPT had undergone surgery. In 12 % of the patients, the cause of the elevated calcium concentration remained unknown. The majority of these individuals had one single, unconfirmed elevated calcium concentration.

4.3.2 Mortality

Total mortality was not significantly different between patients with elevated and normal calcium concentrations. Men with elevated calcium concentrations had a tendency towards higher mortality compared with male patients with normal calcium concentrations. This was most pronounced between 7 and 14 years of follow-up. There was an increase in cancer mortality (p=0.039) in men. A comparison with the background population revealed a 2.3 x increased mortality rate (SMR 2.3, 95 % CI 1.3–3.8) in men, but not in women with elevated calcium concentrations.

We found significantly higher mortality (p=0.004) in patients with low calcium concentrations, but not in high calcium concentrations (p=0.08), when compared to the middle calcium category of 2.31–2.46 mmol/L. The pattern of mortality diagnoses in patients with low calcium concentrations was the same as that of the background population.

4.4 Study III. Quality of life and health care consumption

4.4.1 Quality of Life

The group with elevated calcium concentrations had lower scores in all SF-36 domains compared with the group with normal calcium concentrations, Figure 6. In five domains, the scores were at least 10 points lower in the group with elevated calcium concentrations compared with the patients with normal calcium concentrations. The differences were significant in all domains, except emotional functioning, both in the overall group and for women. In the male subpopulation, the numerical differences between the groups with elevated and normal calcium concentrations were greater in many domains compared with women; however, significant in just one domain. Compared with the Swedish norm group, patients with elevated calcium concentrations had significantly lower scores in all SF-36 survey domains except bodily pain.

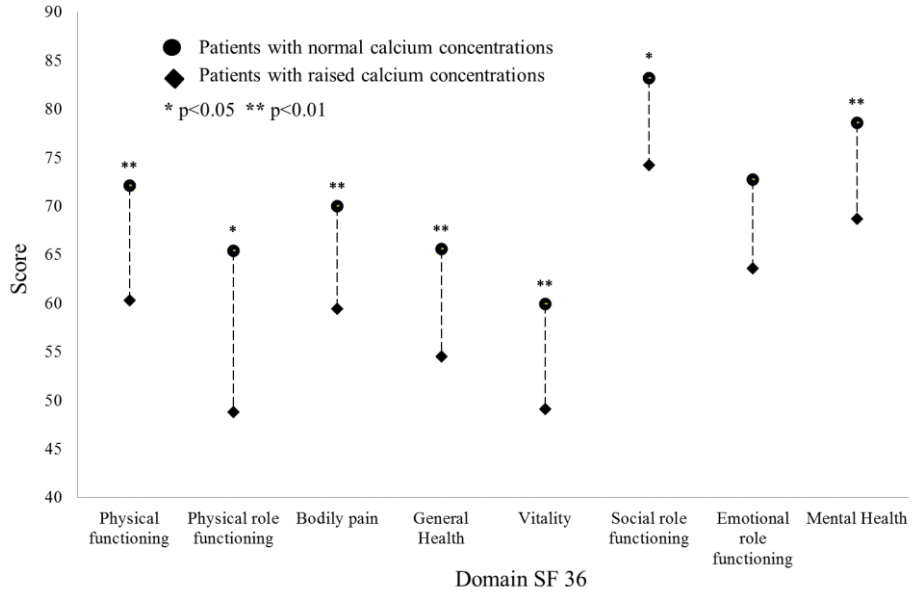

Figure 6. Quality of life studied with SF-36 survey in patients with elevated and normal calcium concentrations at Tibro Health Care Centre, Sweden. Higher scores indicate better quality of life.

4.4.2 Health care consumption

At the time of follow-up in July 2011, only 21 of the patients with elevated calcium concentrations were of working age, the rest had retired or died. For the whole group, there were significantly more days in in-patient care compared with the normal calcium group. For the male subpopulation, the difference was not significant, although numerically, the group with elevated calcium concentrations had twice as many hospital days and care occasions as the group with normal calcium concentrations. Patients with elevated calcium concentrations had significantly more cancer diagnoses associated with hospitalisation than patients with normal calcium concentrations. We could not find any differences between the groups in the number of visits to the HCC physician; however, men with elevated calcium concentrations had more contacts with the psychosocial team.

Overall, patients with elevated calcium concentrations had significantly more sick leave and drug treatment than patients with normal calcium concentrations.

4.5 Study IV. Variations in calcium test ordering among 24 health care centres

Overall, 5.8 % of the inhabitants in Skaraborg and 9 % of the patients (11 % of the women and 8 % of the men) attending the HCCs had a calcium analysis in 2005. The mean age of the patients with calcium analyses was 62 years compared with 45 years for patients with no calcium analyses. At the different HCCs, the number of standardised group analyses including calcium analyses varied from zero to seven. The locums were the most numerous physicians, Table 6. There was substantial variation in the number of calcium analyses, both between HCCs and between physicians. Some physicians and HCCs seem to order significantly fewer calcium analyses, while others seem to order significantly more calcium analyses.

Table 6. Staffing of physicians at health care centres, number of patients visits and frequency of plasma calcium analyses in the county of Skaraborg during 2005.

	Physicians		**Patients visits**	
	Total number	% women	Total number	With P-Ca test %
Preregistration house officer	51	39	10 373	11
House officer	68	69	20 706	11
GP ≤ 45 years	39	41	34 692	10
GP > 46 years	85	32	66 134	8
Locum ≤ 45 years	112	17	8 624	7
Locum > 45 years	102	17	14 100	8
Total	457	32	154 629	9

Table 7. Multilevel logistic regression analysis of plasma calcium analyses in primary care in the county of Skaraborg, Sweden.

		Model A	Model B	Model C	Model D
Fixed effects		**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Patient					
Female		-	REF	REF	REF
Male		-	*0.80 (0.77–0.83)*	*0.80 (0.77–0.83)*	*0.80 (0.77–0.83)*
P-Ca test 2004		-	*1.44 (1.37–1.51)*	*1.44 (1.36–1.51)*	*1.44 (1.37–1.51)*
Risk score		-			
	Group 1	-	REF	REF	REF
	Group 2	-	*2.40 (2.15–2.70)*	*2.43 (2.18–2.71)*	*2.40 (2.14–2.68)*
	Group 3	-	*4.51 (4.08–5.04)*	*4.56 (4.13–5.06)*	*4.51 (4.08–4.97)*
	Group 4	-	*8.92 (8.11–9.87)*	*9.01 (8.14–9.96)*	*8.91 (8.12–9.78)*
	Group 5	-	*25.8 (23.5–28.5)*	*26.1 (23.7–28.8)*	*25.8 (23.5–28.4)*
Physician					
Female		-	-	REF	REF
Male		-	-	0.93 (0.78–1.09)	0.95 (0.78–1.24)
Preregistration house officer		-	-	1.48 (1.00–2.00)	*1.51 (1.07–2.05)*
House officer		-	-	*1.69 (1.35–2.24)*	*1.57 (1.26–2.09)*
GP < 46 years		-	-	*1.30 (1.02–1.76)*	1.16 (0.93–1.60)
GP ≥ 46 years		-	-	REF	REF
Locum < 46 years		-	-	0.84 (0.61–1.08)	0.78 (0.58–1.03)
Locum ≥ 46 years				0.73 (0.57–0.94)	0.69 (0.51–0.89)

HCC					
Number of groups include P-Ca					
	Group 1	-	-	-	REF
	Group 2	-	-	-	*2.59 (1.56–3.53)*
	Group 3	-	-	-	*2.79 (1.25–5.09)*
Random effects		**Variance (95 CI%)**	**Variance (95 CI%)**	**Variance (95 CI%)**	**Variance (95 CI%)**
HCC (Intercept)		0.28 (0.15–0.58)	0.32 (0.16–0.67)	0.32 (0.18–0.66)	0.36 (0.16–0.80)
MOR_{HCC}		1.65 (1.44–2.07)	1.71 (1.47–2.18)	1.72 (1.49–2.17)	1.77 (1.48–2.34)
Physician (Intercept)		0.49 (0.41–0.59)	0.59 (0.50–0.71)	0.52 (0.43–0.62)	0.52 (0.43–0.63)
$MOR_{physician}$		1.95 (1.85–2.08)	2.09 (1.96–2.24)	1.98 (1.87–2.12)	1.99 (1.88–2.13)
HCC and physician		0.77	0.91	0.84	0.88
$MOR_{HCC+physician}$		2.31	2.48	2.4	2.45
DIC		89 550	76 438	76 427	76 427

Figure in italics are significant at 0.05 level
P-Ca = plasma calcium
95 % CI = 95 % credible interval
MOR = median odds ratio. OR = odds ratio
DIC=Deviance Information Criterion

4.5.1 Multilevel model

Fixed effects

All variables that were analysed are shown in Table 7. The patient variables showed that male sex was associated with a lower likelihood for a calcium analysis (OR 0.80). Those patients who had a calcium analysis the previous year had an increased likelihood of a new calcium analysis (OR 1.44). As expected, patients with an increased number of diagnoses and a higher risk score had a greater probability of a calcium analysis, Group 5 (OR 25.8).

The physician variables show that preregistration house officers (OR 1.48), house officers (OR 1.69) and younger GPs (OR 1.30) ordered more calcium analyses than older GPs. Locums ordered fewer calcium analyses (OR 0.73). There were no differences between male and female physicians.

Analyses of the HCC variable demonstrate that a high number of standardised group analyses were associated with a high number of calcium analyses (three or more group analyses compared to none (OR 2.79)).

Random effects

The four models used in the analyses are shown in Table 7. In the empty model A, the $MOR_{physician+HCC}$ indicated that for a patient changing both his/her GP and HCC to a GP and HCC with higher odds, the median increase in the odds for a calcium analysis was 2.31. The physician level, $MOR_{physician}$= 1.95, contributed somewhat more than the HCC level, MOR_{HCC} = 1.65.

Variables have been added to the subsequent models, to try to explain the variation between physicians and HCCs; however, none of the variables and confounders that we have chosen could explain the variation at the higher levels, as the MOR value was substantially unchanged in model A compared with model D; despite the fact that many of the variables included were associated themselves with the increased frequency of calcium analyses.

5 DISCUSSION

Elevated calcium concentration, is it dangerous?

The main question in this thesis was to examine, though long-term follow-up, whether elevated calcium concentrations measured are primary care is dangerous. A proportion of those who had an elevated calcium concentration only had a single instance of elevated calcium and no diagnosis at follow-up. Elevated calcium concentrations appeared to be dangerous to men, as shown by their increased mortality. Both men and women with elevated calcium concentrations had impaired quality of life and increased health care consumption.

5.1 Strengths of the thesis

In longitudinal studies, it is essential to have a high participation rate at follow-up. The investigation was confined to the only HCC in the municipality with a staff with good knowledge of the population attending the HCC. This could probably explain the high participation rate (86 %) in the follow-up. Furthermore, virtually all patients with a need of specialised care were referred to one hospital in the vicinity. The studies from Tibro and from the whole Skaraborg region are all based on data from ordinary clinical practice. This was made possible, thanks to the Tibro R&D unit and later the R&D unit in Skaraborg, which could help retrieve data from medical records. This is an advantage, both in cross-sectional and prospective studies, as the results may be skewed if the staff is aware of the on-going sampling of data. These studies also fill a gap, as there are few studies in this field in primary care.

5.2 Limitations of the thesis

The strengths of the study, thanks to its being confined to one HCC, are counteracted in part by the small study sample; hence, the results should be interpreted with caution, especially for the subgroups of patients. The focus during the follow-up was the diagnoses identified in patients with elevated calcium concentrations and the mortality compared with patients with normal calcium concentrations. We did not compare morbidity in the two groups in this part of the study. Another limitation was that the study was based on just one elevated calcium concentration and that the quality of the calcium test

was dependent on the accuracy of the quality system in the point-of-care analyses.

5.3 Validity of the results

How valid are the results from Tibro HCC for primary care in Sweden? During the first study period around three times as many calcium analyses were performed in Tibro than, for instance, in the capital of Stockholm [59, 60]. There was also a large variation between different HCCs in Skaraborg. The high frequency of analyses was probably due to the fact that Tibro HCC was well staffed with physicians with a special interest in internal medicine. Despite the high rate of analyses, no increase in high calcium concentrations was found. There seemed to be saturation in the number of calcium analyses and there is little likelihood that a further increase would reveal more cases with elevated calcium concentrations. Moreover, the same prevalence of pHPT was found at Tibro HCC as in screening studies when the entire population was examined. Consequently, we believe that the results from Tibro and Skaraborg are valid for Swedish primary care.

5.4 Discussion of the methods

5.4.1 Elevated calcium concentration in contrast to hypercalcaemia

The inclusion criterion in the study was at least one elevated calcium concentration. The risk is that single elevated concentrations are included in the study. However, in study I, we wanted to investigate what happened after a high calcium concentration had been detected. Other studies have also been based on a single increased calcium level, which Leifsson and Ahrén investigated in a population-based study including 33 346 individuals. In line with our study II, they found increased mortality among participants with a single increased calcium concentration in Malmö, Sweden [25].

5.4.2 Quality of electronic data

When retrieving data from a clinical database, the quality of the data are very important. At follow-up, we found patients with elevated calcium concentrations with analysis results to which an asterisk had been added by the laboratory staff in order to pay attention to a high concentration that they had re-checked. These tagged values were not automatically retrieved in the first extraction of data for study I. This emphasises the importance of good quality control of the recorded data.

5.4.3 Quality controls in the laboratory at Tibro Health Care Centre

The quality of the analyses is important. An elevated concentration may be erroneously high. During the work with study II, validation of the quality controls revealed that calcium analyses deviated above defined quality levels in several instances. This highlights the importance of rechecking laboratory tests, especially pathological results [61]. The consequence for study II and III was the exclusion from the analysis of 45 patients with one single elevated calcium concentration. Another way would have been to recheck using ionised calcium, which is a more accurate estimate of the calcium concentration [62].

5.4.4 Selection of controls for the studies

For the prospective study we would have chosen healthy, normocalcaemic controls from the background population. As this was a retrospective study at baseline this was not possible. Our controls were also patients with various disorders, who had come to the HCC with symptoms that occasioned a calcium analysis. The effect, if any of using this ”imperfect” control group would rather be an underestimation of the group difference. To check this possible effect, we compared the mortality among patients with elevated calcium concentrations with the background population in Tibro using SMR.

5.4.5 Advantages and drawbacks of the multilevel regression analysis

In order to study the associations between different factors and the outcome, single-level, statistical models are usually used. However, when the data are hierarchically structured, multilevel models allow for more robust analyses to be performed. The MLRA allows characteristics from different levels in hierarchical systems to be included in the same analysis. Ignoring a multilevel structure and using single-level statistical models may cause problems; for example, making assumptions at an individual level (in this case the patient) based on information aggregated to a higher level (in this case the HCC) leads to the risk of ”the ecological fallacy” [51, 63]. One example is when HCCs with many locums differ in some respect from HCCs with no locums, this does not have to mean that locums per se contribute to this difference at the individual level. There may be some other factor at the HCCs employing locums that causes the difference. On the other hand, disaggregating data from the HCC level to the patient level can lead to an increased sample size and the risk of type I errors (finding an association where, in fact there is none) [51].

A drawback of the MLRA is that only variables included in the model can be evaluated. In our analysis, none of the observed variation could be explained by the variables. There appears to be so far unidentified factors that contribute to the variation in the calcium analyses. In previous studies, other characteristics of the physicians, such as attitude to risk-taking and involvement in the development of guidelines, explained parts of the higher level variance [64]. The education in internal medicine and the interest in pHPT may vary between universities in Sweden. It is also possible that the HCC's distance to the nearest hospital can explain some of the variation in the number of calcium analyses, but we have not investigated this aspect as the number of HCCs would have been too small.

5.5 Discussion of the results

5.5.1 Number of calcium analyses

Already in 1994, the number of calcium analyses was high at Tibro HCC. In the following years, the number of analyses increased and reached a peak in 1999, but then fell to previous numbers. In 2005, when we performed the MLRA analyses, the number of calcium analyses had fallen at Tibro HCC. There was, however, a substantial variation in the number of calcium analyses between the different HCCs in Skaraborg in study IV [65]. Group analyses including calcium may increase the number of calcium samples analysed in primary care. The HCC in Tibro had no such group analyses that included calcium.

It is not certain that the number of calcium analyses affects the number of patients found with pHPT. The number of patients with elevated high calcium concentrations was unchanged when the number of calcium analyses increased. It appears that an increased number of calcium analyses resulted in the inclusion of a broader spectrum of patients with less risk of having pHPT.

Creatinine and blood count showed a similar pattern during the 1990s and 2000s. Creatinine and blood count represents the total number of samples when we perceive the frequencies of these analyses as stable over time and not sensitive to trends. We decided not to use the total number of analyses, as there has been great variation in what has included in the data journal laboratory list over the years, for instance waist circumference and smoking have sometimes been included. From the autumn of 2000, recruiting doctors to Tibro HCC became more difficult. These problems were accentuated during the early 2000s. The number of analyses decreased during this period, with staffing problems and more locums and fewer physicians in education

working at the HCC. This is in line with the results in multilevel study IV, where the locums ordered fewer calcium analyses and physicians in education ordered more calcium analyses.

In the investigation in 2008–2010, the mean calcium concentration in the group with elevated calcium concentrations at baseline had normalised. The highest calcium concentrations at baseline were found among those who died during the observation period. Furthermore, almost half of the 46 patients with pHPT had undergone parathyroidectomy (and in most cases became normocalcaemic after surgery) [66]. This contributes to the decline in calcium concentrations at group level. It is reasonable to assume that patients who did not participate in the follow-up may have been in the poorer spectrum of health, as many drop-outs reported disease as a reason for not participating in the follow-up.

5.5.2 Number of parathyroid hormone analyses

We also found that, during the 1990s, an increase in parathyroid hormone (PTH) analyses resulted in a corresponding increase in the number of high PTH concentrations that was detected. Analysis of PTH was a new and expensive analysis in the 1990s. For this reason, most primary care physicians at that time ordered a limited number of PTH analyses. We believe that this has changed with time and that the number of PTH analyses is higher nowadays.

5.5.3 Underlying diagnoses in patients with elevated calcium concentrations

In as many as 70 % of the patients with at least one elevated calcium concentration in the first investigation, it was not possible to find an underlying diagnosis in the patients' records. Among those were the 45 patients with one single elevated calcium concentration who were still healthy at follow-up. On the other hand, repeated high calcium concentrations were associated with different diseases and prognoses in men and women, with pHPT being the most common in women and cancer in men. Elevated calcium concentrations should not be ignored, as the investigation showed that at least 88 % of the patients turn out to have an underlying disorder during a ten year follow-up period. This underscores the importance of further careful investigation of patients with increased calcium concentrations. A delay in the pHPT diagnosis may be due to vague symptoms, a calcium concentration just above the upper reference interval or to physicians being influenced by the debate about the minor significance of slightly deviating calcium concentrations [67, 68]. Some calcium analyses

may have been ordered as a part of a group analysis, and not through the active choice of the physician which may influence the decision to investigate an elevated concentration further.

5.5.4 Primary hyperparathyroidism

Almost half of the patients diagnosed with pHPT in the first study had an only marginally raised calcium concentration (2.55–2.60 mmol/l). This is in line with the lowering of the upper reference limits for the serum calcium concentration to 2.50 mmol/l, following the Nordic Reference Interval Project (NORIP) [44]. Analysing PTH in patients with only slightly raised calcium concentrations seemed to detect more cases of pHPT. The prevalence of pHPT seen at Tibro HCC was the same as the prevalence in observed population screenings in Tromsö, Gävle and Stockholm [22, 24, 69]. A limitation of this study was that the true prevalence could not be calculated, as this is a clinical sample. The figure from Tibro probably represents a mixture of prevalence and incidence during the six years included in this study.

5.5.5 Mortality in patients with elevated calcium concentrations

In the first study in Tibro we found that 2 % of the patients with elevated calcium concentrations had cancer, which is comparable to the figure found in a health survey in Stockholm [22]. This frequency seems low; nevertheless, we found an increased mortality among men, especially from cancer [66] during the follow-up in Tibro. The total mortality in men with elevated calcium concentrations was also increased compared with patients with normal calcium concentrations and the background population. In women, who were more numerous in this study, no excess mortality was found. The most common underlying disorder was pHPT, for which a substantial proportion of the patients were surgically treated. Against this background, it appears that elevated calcium concentrations are not dangerous to women, as part of them were surgically treated for their pHPT.

5.5.6 Low calcium concentrations

This study showed that the calcium concentration had an impact on total mortality. Low calcium concentrations were associated with higher mortality. Several studies from intensive care units have shown that a low calcium concentration is associated with increased mortality without any specific underlying cause [40-42]. In primary care, a low calcium concentration may be due to vitamin D deficiency, malnutrition, kidney and intestinal disease, or

a malignancy-dependent low albumin concentration. This was an unexpected finding, as the studies aimed at investigating increased calcium concentrations. The whole range of calcium concentrations was not included. We omitted patients with calcium concentrations between 2.45 and 2.55 mmol/l, as we wanted to contrast patients with elevated and normal concentrations. Further studies in mortality of patients should include patients with the whole range of calcium concentrations.

5.5.7 Quality of life

To our knowledge, this is the first study showing that patients in primary care with elevated calcium concentrations per se have significantly reduced QoL compared with patients with normal calcium concentrations. This reduction was even more pronounced when compared with the Swedish norm population [49]. Our interpretation is that the differences found in the SF-36 scores may, in fact, underestimate the reduction in QoL in patients with elevated calcium concentrations. Many of the patients with the highest calcium concentrations had already died during the follow-up period. A SF-36 survey at baseline would have resolved this matter.

5.5.8 Health care consumption

In this study, we only have access to reports on sick leave, ordered by the physicians at Tibro HCC. We have no access to statistics on sick leave ordered by hospital physicians or company doctors. Patients with elevated calcium concentrations had significantly more contacts with the psychosocial team, more sick leave, more medication and more hospitalisations than patients with normal calcium concentrations. Their impaired QoL seems to result in high health care consumption. The cost to society is likely to be considerable in terms of numerous visits to the health service, more hospitalisation, increased cost of medication and sick leave. More knowledge about elevated calcium concentrations and QoL among general practitioners would hopefully lead to a search for underlying diagnoses in these patients.

5.5.9 Multilevel analysis

Females had a greater chance of having calcium analysis, probably due to their higher risk of pHPT. Test ordering varied between physicians, with the oldest and most experienced physicians ordering fewer tests, which is in line with an earlier study [70]. There was no difference between male and female physicians. At HCC level, the number of group analyses was associated with more calcium analyses. None of the associations in the fixed effects could explain the variation between physicians or HCCs (random effects).

6 CONCLUSIONS

Study I. A doubling of the number of calcium analyses did not increase the detection rate of elevated calcium concentrations.

A similar rate of pHPT as in earlier population screenings was found in the Tibro study.

In 70 % of patients, no underlying cause of the elevated calcium concentration was found.

Study II. One decade after the detection of a single elevated calcium concentration, the number of patients without an underlying disorder fell to 12 %.

Elevated calcium concentrations were mainly associated with primary hyperparathyroidism in female patients and with malignancies and increased mortality in male patients, compared with patients with normal calcium concentrations.

It is important to recheck elevated calcium concentrations as the levels may fluctuate.

Study III. Elevated calcium concentrations are associated with significantly reduced QoL.

The patients with elevated calcium concentrations had higher frequencies of sick leave, drug prescription and visits to HCCs and admissions to HCCs and hospitals.

Study IV. There was substantial variation in the number of calcium analyses, both between physicians and HCCs.

Female gender of the patient and an increasing number of diagnoses was associated with a greater likelihood of a calcium analysis.

Physicians in education order the most calcium analyses and locums the least, but the gender of the physician has no influence.

The variables included at patient, physician and health care centre level could not explain the variation in the numbers of calcium analyses.

7 FUTURE PERSPECTIVES

We plan to follow morbidity and mortality rates among patients with elevated calcium concentrations and include patients with normal calcium concentrations by using national registers.

The finding that low calcium concentrations were also associated with increased mortality was surprising. It would be interesting to follow this up in a future study, using data from primary care in Skaraborg.

In conjunction with the investigation we conducted bone density measurements. These data have not yet been analysed and we plan to study bone density in the patients in relation to calcium concentration and various markers of skeletal disorders.

As the variation in calcium test ordering could not be explained in the multilevel analysis, it would be interesting to conduct interviews with physicians, for instance, in focus groups, about their thoughts and attitudes towards calcium analyses. Knowledge from such studies can be used in future analyses of variations in calcium test ordering.

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