



The investigation of hypercalcaemia

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Introduction

Hypercalcaemia occurs in up to 0.3% of a healthy population and is even more prevalent among hospital patients where it may occur in up to 3%. This makes hypercalcaemia one of the most common metabolic disorders encountered in clinical practice. It is therefore important to have well developed strategies for its investigation and management.

Aetiology and pathogenesis

A large number of diseases can lead to hypercalcaemia (table). In most instances the underlying cause is either primary hyperparathyroidism or malignant disease; the many other causes are rarely encountered even within specialist units. The prevalence of primary hyperparathyroidism and hypercalcaemia of malignancy varies with the population sampled. In ambulant subjects in the community who were found to be hypercalcaemic as part of a screening programme, 86% were ultimately shown to have primary hyperparathyroidism and only 2% malignant disease.¹ The findings in hypercalcaemic inpatients are different: 58% of patients have malignant disease and 33% primary hyperparathyroidism.² Indeed, primary hyperparathyroidism is one of the most common endocrine diseases with a prevalence of around 1.3 per thousand, exceeded only by diabetes mellitus and thyroid disease.

In patients with primary hyperparathyroidism hypercalcaemia is the direct result of an increased circulating concentration of parathyroid hormone (PTH) which leads to hypercalcaemia through a variety of different mechanisms. In most cases the most important mechanism is an enhanced renal tubular reabsorption of calcium brought about through the direct effect of PTH on the distal convoluted tubule. PTH also acts on the skeleton and stimulates bone resorption and thereby the movement of calcium into the extracellular space. PTH also stimulates the renal production of calcitriol, the active metabolite of vitamin D, which in turn promotes the intestinal absorption of calcium and also bone resorption.

Some malignant processes, especially myelomatosis, lead to the local production of cytokines which stimulate bone resorption. In most patients with malignancy and hypercalcaemia this is brought about by a humoral factor with actions which closely resemble those of PTH. This has been identified and called parathyroid hormone-related peptide (PTHrP).³ True "ectopic" PTH production by malignant tumours is rare. PTHrP is considerably larger than PTH, but it shares a close sequence homology at the amino terminal, which is responsible for much of the biological activity of PTH. It is not surprising, therefore, that PTHrP can mimic the effects of PTH, and it is now clear that many cases of hypercalcaemia of malignancy are related to the production of this compound by tumour cells. This is particularly the case in squamous cell carcinoma of the lung and breast carcinoma, including those with metastatic disease.⁴

Once hypercalcaemia is established, the serum calcium concentration can remain stable for many years and this is commonly the case in many patients with mild primary hyperparathyroidism. In other patients the serum calcium can rise progressively mainly as a result of the deleterious effects of hypercalcaemia on renal function. Hypercalcaemia causes losses of sodium and water through effects on the renal tubules which lead to decreased glomerular filtration and filtered

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Causes of hypercalcaemia

Common:

Primary hyperparathyroidism
Malignant disease: Solid tumours
: Myeloma and other haematological
tumours

Less common:

Sarcoidosis and other granulomatous diseases
Thyrotoxicosis
Vitamin D intoxication
Familial hypocalcaemic hypercalcaemia

Rare:

Addison's disease
Immobilation
Thiazide diuretics
Recovery from renal failure
Following renal transplantation
Milk-alkali syndrome
Lithium treatment
Hypothyroidism
Vitamin A toxicity

load of calcium. These combine to reduce the renal excretion of calcium and exacerbate the hypercalcaemia still further. This situation of "disequilibrium hypercalcaemia" is a potential threat to life.⁵

Clinical features

The clinical features of hypercalcaemia are largely non-specific and it is seldom, if ever, possible to be confident of the diagnosis on clinical grounds alone.⁶ When the serum concentration of calcium is less than 3.0 mmol/l symptoms are rare; above this value there is an increasing likelihood of symptoms—notably thirst and polyuria, constipation, nausea and anorexia; as the concentration rises further (above 3.5 mmol/l) impaired consciousness, coma, and even death may occur.

In some patients with malignant disease it can be difficult to decide on clinical grounds whether the presence of these symptoms relates to the malignancy itself (or its treatment) or signals the onset of hypercalcaemia. In these circumstances the laboratory clearly has an important role in diagnosis and management. Patients can also present with the symptoms of conditions known to be caused directly or indirectly by the hypercalcaemia—examples of these include abdominal pain associated with acute pancreatitis, ureteric colic secondary to renal stones, or pseudogout associated with chondrocalcinosis.

Investigation of hypercalcaemia

CONFIRMATION OF DIAGNOSIS

The laboratory has a central role in establishing the diagnosis of hypercalcaemia. Although the "gold standard" method for the estimation of plasma calcium concentration is atomic absorption spectrophotometry, the cresolphthalein complexone based colorimetric methods, used by multichannel and discrete analysers, give results which are just as reliable for clinical purposes. In both cases the between batch precision is similar at less than 0.5%.⁷ As the variation within subjects in plasma calcium concentration is also small it can be estimated that a change in plasma calcium concentration of 5% is likely to be clinically important.⁸ In the past it has frequently been stated that blood samples for the estimation of calcium concentration should be taken in the fasting state and without the use of a tourniquet. These conditions minimise the risk of spurious hypercalcaemia, but the clinical importance of both has been grossly overstated. There is no evidence that fasting has any relevant effect on measured plasma calcium concentrations and even prolonged venous stasis only increases it by 0.15 mmol/l at the most, an effect largely offset by correcting for albumin concentration.⁹ Thus while it is advisable to avoid prolonged venous stasis, the failure to observe either of these precautions is unlikely to perturb the measured concentration of calcium to any clinically important degree.

About 45% of the calcium in serum is

bound to protein (albumin and globulins) and hence unavailable for biological activity. Relatively recently ion specific electrodes have been developed which measure the actual activity of the calcium ion in biological fluids. Although these techniques provide a measure which is much more closely related to the biological activity of calcium, their clinical usefulness is limited in the routine laboratory. Measurement of the plasma concentration of ionised calcium may be of value in the investigation of the occasional patient with mild or intermittent hypercalcaemia, and in patients with abnormal plasma proteins who may develop spurious hypercalcaemia. In most clinical situations measurement of the total plasma calcium is much simpler and provides a reasonable estimate of the ionised fraction. However, it is usually recommended that the measured calcium concentration be adjusted for the prevailing concentration of plasma proteins although this is unlikely to be of major clinical consequence. An appropriate formula to apply is:

$$\text{adjusted calcium} = \text{measured calcium} + 0.02 * (\text{mean normal albumin} - \text{measured albumin})$$

[calcium measured in mmol/l and albumin in g/l]

It is important to emphasise that this adjustment should be carried out to the mean of the normal range for albumin in the laboratory concerned using the normal method for plasma albumin rather than a standard albumin concentration of 40 g/l as previously suggested. Calcium is also bound to plasma globulins although with only a quarter of the affinity of the albumin binding so that, in patients with abnormal globulin concentrations (such as myeloma¹⁰), a correction for this should be considered.¹¹

Establishing the cause of hypercalcaemia

Having established that the plasma calcium concentration is raised, it is important to determine the underlying cause. In most instances this will be either primary hyperparathyroidism or malignant disease, but the other rarer causes of hypercalcaemia must be borne in mind (table). Although we will concentrate on the laboratory procedures which may be helpful in this situation, it is important to highlight those areas of the clinical history and examination which can shed light on the diagnosis.

Perhaps the most important information to be obtained is to establish whether or not there has been any evidence of hypercalcaemia in the past. Unfortunately the symptoms of hypercalcaemia (thirst, polyuria, constipation, tiredness, mental changes, etc.) are non-specific and do not usually allow this diagnosis to be made. However, a careful review of the case notes will often reveal a raised plasma calcium concentration some years before which has passed apparently unnoticed. Such a finding going back over several years is a very strong indicator that the underlying diagnosis is primary hyperparathyroidism. A similar inference can also be

drawn from a long history of renal stones especially if the hypercalcaemia was stable and of moderate degree. Conversely, the recent development of hypercalcaemia in a patient with the clinical features of malignant disease would strongly suggest that the hypercalcaemia was the result of that process. A detailed drug history is also important. Thiazide diuretics, although said to produce mild hypercalcaemia in their own right, are more likely to unmask hypercalcaemia from other causes such as primary hyperparathyroidism.¹² Vitamin D intoxication is more likely to produce severe hypercalcaemia; in many instances the offending agent has not been prescribed but taken as a proprietary medication available over the counter for conditions such as chilblains. In order to produce clinically important hypercalcaemia it is necessary to consume at least 250 µg (10 000 IU) of calciferol daily, unless an associated disorder of vitamin D metabolism, such as sarcoidosis, is also present.

Hypercalcaemia does not lead to any pathognomonic clinical signs, but in severe cases the patient is invariably salt and water depleted and will have the clinical features of this. Specific searches should also be made for any clinical pointers to the underlying cause of hypercalcaemia. If such features are found they are likely to point towards malignant disease. On the other hand, it is important to remember that certain benign causes of hypercalcaemia, such as thyrotoxicosis, can produce distinctive clinical features.

A variety of different investigations are likely to be helpful at this stage. As the primary differential diagnosis is between hyperparathyroidism and malignancy the most important single investigation is estimation of the serum PTH concentration which should be suppressed in the face of non-parathyroid driven hypercalcaemia. On the other hand, there are a variety of other investigations which should be undertaken at this stage. These fall into two groups: those aimed at determining the cause of the hypercalcaemia; and those aimed at monitoring the state of the patient and providing a baseline from which to judge response to treatment. The former include thyroid function tests, liver function tests, angiotensin converting enzyme activity, serum and urine protein electrophoresis, and, in male patients, acid phosphatase or prostate specific antigen (PSA). Other investigations which may also be considered in this category include chest x ray picture, skeletal radiology, isotope bone scan, abdominal ultrasonography, blood film and, in selected cases, bone marrow examination. The latter group include renal function and serum electrolytes, blood count and markers of bone turnover such as alkaline phosphatase activity.

Parathyroid hormone assay

As the major curable cause of hypercalcaemia is parathyroid overactivity it is imperative that this be sought in all cases of hypercalcaemia.

The early assays for PTH were immunoassays directed at the carboxyl terminal of the molecule. This is well separated from the major area of biological activity and these assays detected many biologically inactive fragments, especially in patients with impaired renal function. This explains the considerable overlap between the results obtained in patients with hyperparathyroidism and those with hypercalcaemia from other causes, particularly malignant disease.¹³ These assays were also very time consuming and required long preincubation and reaction times. They were therefore rarely available outside specialist centres, and if they were not performed there were notoriously unreliable.

Attempts have been made to obviate these problems by developing assays directed at the bioactive amino terminal of PTH, the middle of the PTH molecule, and most recently the intact molecule.

Amino terminal PTH assays make use of antibodies directed at the 1–34 portion of the molecule which is responsible for its biological activity. It is believed that the plasma half life of this moiety is so short that the assays really measure intact PTH concentration. Although good discrimination between hyperparathyroid patients and others can be achieved using such an assay,¹⁴ their use has largely been superseded by intact PTH assays.

Mid-molecule assays are directed at a variety of sites in the 44–68 amino acid range of the molecule. This is biologically inactive but has a relatively long plasma half life, allowing its concentration to be used as an index of PTH secretion. These assays have been reported to have varying success in discriminating between primary hyperparathyroidism and other causes of hypercalcaemia.^{15 16} In any case the use of such assays, like that of N-terminal PTH, has been rendered obsolete by the development of reliable intact PTH assays.

Measurement of intact PTH is undertaken using immunoradiometric assays (IRMAs) in which PTH is immobilised by an antibody directed at a site close to one end of the molecule and then detected by the use of an antibody directed at an epitope close to the opposite end. These assays are both sensitive and specific^{17–19} and some of the more recent methods use a chemiluminometric method of detection rather than radioiodine which is of considerable benefit.^{19 20} Some of these assays are now available as commercial kits and offer a reasonably robust and sensitive option for general use. Typical PTH values obtained using these assays give a normal range of 1–6 pmol/l (10–60 pg/ml).

Measurement of PTH using one of these specific methods is the single most important investigation in the determination of the cause of hypercalcaemia. It has been the usual practice to obtain blood for the estimation of PTH concentration on a fasting specimen in the early morning; using modern assays for intact PTH, a circadian rhythm for PTH secretion can be shown. This suggests

that the best time to sample to discriminate between normal and hyperparathyroid patients is the late morning or early afternoon.²¹ Whether this theoretical consideration has any major relevance for clinical practice is yet to be determined.

PTHrP ASSAY

The precise value of PTHrP measurement in the evaluation of patients with hypercalcaemia is still under investigation. Measurement of PTHrP is expensive and very careful sample collection and preparation is required. Although kits for the estimation of PTHrP concentrations are available, it is not yet possible to recommend their use in the routine investigation of hypercalcaemic patients. No doubt this will become clearer in the near future as experience with the use of these assays increases. Early reports indicating that the circulating concentration of PTHrP is increased in many patients with hypercalcaemia and malignant disease, in some with primary hyperparathyroidism, and in patients with renal insufficiency have been reviewed.²² More recently highly sensitive immunoradiometric assays of PTH and PTHrP have been applied to the investigation of patients with hypercalcaemia.^{4,23,24} In one such study PTHrP was undetectable in most patients (92%) with primary hyperparathyroidism. In contrast, most patients (88%) with solid tumours had detectable plasma PTHrP, and in the absence of coexisting primary hyperparathyroidism, undetectable or measurable (subnormal or normal) serum concentrations of PTH.⁴ In some patients with hypercalcaemia the finding of detectable PTHrP preceded the discovery of malignant disease and was an important clue to the underlying diagnosis.

Although the discovery of PTHrP was as a result of a biological assay system, this is unlikely to be of routine clinical use. A variety of immunoassays have accordingly been developed. Most of these are directed towards the amino terminal where the major biological activity resides. As this is also where there is most homology with PTH it is important that such assays are capable of differentiating between these two compounds. Currently available amino terminal assays include a radioimmunoassay which used components very similar to those in the Incstar kit²³; this gave good discrimination between normal subjects in whom 37 out of 38 had undetectable (< 2 pmol/l) PTHrP and patients with humoral hypercalcaemia of malignancy whose values ranged from 2.8 to 51.2 pmol/l. However, the kit itself still awaits formal independent assessment. Immunoradiometric assays have also been developed, the use of which will tend to obviate any worries there may be as to the seat of biological activity within the molecule. Such assays are capable of good discrimination between normal subjects and those with hypercalcaemia of malignancy.^{25,26} One of these has recently been developed as a commercial kit²⁷ which is produced by the Nichols Institute. An assess-

ment of this²⁸ failed to give as good separation between the diagnostic groups as had been seen in earlier studies. At present the use of assays for PTHrP must still be considered to be experimental, but once the problems with assay methodology and sample collection have been overcome the combination of sensitive PTHrP and PTH assays is likely to become extremely valuable in the diagnosis and management of patients with hypercalcaemia.⁴

INDIRECT MARKERS OF PARATHYROID ACTIVITY

Before the more specific assays for PTH were developed and became readily available, considerable time was devoted to establishing other indicators of PTH activity which might help to discriminate between hyperparathyroidism and other causes of hypercalcaemia. Among the most popular of these measures were the renal production of cyclic adenosine monophosphate (nephrogenous cAMP) and the renal tubular reabsorption of phosphate (most commonly expressed as per cent TRP or as a theoretical tubular maximum for reabsorption—TmP/GFR). As it is now known that many cases of hypercalcaemia of malignancy are mediated by PTHrP, which has similar renal effects to PTH, it is not surprising that none of these measures has any great discriminant power.²⁹ These indirect measures of parathyroid activity still have their use in a research setting, but they are unnecessary for the routine investigation of hypercalcaemia.

Similarly, biological assays of parathyroid activity do not discriminate between PTH and PTHrP, and so have little value in the investigation of hypercalcaemia.

OTHER HORMONE ASSAYS

Although uncommon, vitamin D intoxication is an important treatable cause of hypercalcaemia. If vitamin D intoxication is suspected in patients not receiving large doses of vitamin D for therapeutic purposes the concentration of 25 hydroxyvitamin D (25OHD) should be measured; grossly increased concentrations would support the diagnosis. Measurement of 25OHD in the serum can be measured by a variety of different techniques including competitive protein binding,³⁰ radioimmunoassay,³¹ and high performance liquid chromatography (HPLC).³² Widely differing results have been reported between laboratories.^{33,34} Serum 25OHD concentrations of up to 160 pmol/l have been reported as the result of sunlight exposure³⁵ and hence concentrations below this are unlikely to be of clinical importance. In general, patients with vitamin D intoxication have 25OHD concentrations in excess of 500 nmol/l.³⁶

Intoxication with the active metabolites of vitamin D (calcitriol and alfacalcidol) is not associated with increased concentrations of 25OHD but with increased concentrations of 1,25 dihydroxyvitamin D (1,25OHD₂; calcitriol). Measurement of 1,25OHD₂ can also be of use in the investigation of hypercalcaemia because of granulomatous diseases,

particularly sarcoidosis, in which there is autonomous production of this metabolite in the granulomata.³⁷ 1,25OHD₂ can be measured by either radioreceptor assay³⁸ or radioimmunoassay,³⁹ although in each case considerable sample preparation is necessary. The former technique is utilised in the commercially available kits for the measurement of 1,25OHD₂. A recent study suggests that these methods compare favourably with the more time consuming competitive binding assay following HPLC purification.⁴⁰ Although radioimmunoassay following HPLC purification permits quantification of the vitamin D₂ and vitamin D₃ components separately, this is seldom if ever required on a clinical basis and so this technique is largely confined to research applications.

Certain endocrine disorders can result in hypercalcaemia and these need to be considered if there is any clinical suspicion of their presence or that the hypercalcaemia is not PTH dependent. Of these, the most important is thyrotoxicosis but adrenal insufficiency and, very rarely hypothyroidism, can also lead to clinically important hypercalcaemia. Measurement of thyroxine and thyroid stimulating hormone (TSH) concentrations will usually be sufficient to confirm or refute the diagnosis of thyroid dysfunction. If Addison's disease is seriously being considered a short adrenocorticotrophic hormone stimulation test should be performed at the same time that blood is drawn for cortisol estimation. Sometimes endocrine diseases (particularly hyperthyroidism) and sarcoidosis co-exist with primary hyperparathyroidism. The same is true for primary hyperparathyroidism and malignant disease.

Components of hypercalcaemia

Calcium infusion studies in normal subjects have clearly shown that there is a relatively constant relation between the plasma calcium concentration (or more precisely the ultrafiltrable calcium concentration) and the amount of calcium excreted in the urine, expressed as calcium excreted per litre of glomerular filtrate (CaE)⁴¹ calculated as:

$$\frac{\text{Urine calcium} \times \text{plasma creatinine}}{\text{urine creatinine}}$$

This association can be examined for any patient by plotting their plasma calcium and CaE against the normal curve and deriving components of hypercalcaemia.⁴² In order to simplify this approach a variety of nomograms and computer programs to ease the calculations have been produced. Although clinically interesting, this approach cannot distinguish between the hypercalcaemia induced by PTH or PTHrP and is of only limited clinical usefulness, especially with the advent of more discriminating PTH assays. None the less this analysis of the components of hypercalcaemia can still prove helpful in the investigation of "difficult" cases of hypercalcaemia, and may provide useful clues as to the best approach to treatment.

Measurement of urine calcium excretion is valuable in the diagnosis of hypocalciuric hypercalcaemia. This condition "benign familial hypercalcaemia" is inherited as an autosomal dominant.⁴³ The hypercalcaemia is usually mild and arises through the avid reabsorption of calcium from the glomerular filtrate which leads to very low urine calcium excretion (CaE < 0.015 mmol/l glomerular filtrate). It is imperative that patients are not receiving thiazide diuretics while this investigation is performed because these drugs produce an identical biochemical picture. Familial hypocalciuric hypercalcaemia is important to recognise because it seldom causes symptoms and calls for no specific treatment; parathyroid surgery is generally contraindicated.

DISCRIMINANT FUNCTION ANALYSIS

Several authors have developed a variety of so-called discriminant functions in which the results of different investigations are weighted and combined to yield a value which provides an indication of the cause of hypercalcaemia. On the whole such approaches have not been particularly successful and their use has been rendered obsolete by the newer PTH assays.

STEROID SUPPRESSION TEST

Hydrocortisone 40 mg given eight hourly for 10 days was initially described as a means of differentiating primary hyperparathyroidism, in which there is little change in calcium concentration, from other causes of hypercalcaemia, in which the calcium concentration moves towards normal.⁴⁴ Since its introduction it has been the subject of much controversy, although many of its detractors have given insufficient corticosteroid for too little time in their modification of the test. The steroid suppression test is rarely needed these days. It may occasionally be a useful investigation in the diagnosis of those difficult cases of hypercalcaemia where the PTH and other assays have given equivocal results. If the test shows a positive response, even if no specific cause for the hypercalcaemia is determined, it does point to the possible use of steroids as a therapeutic measure.

OTHER INVESTIGATIONS

The investigation of hypercalcaemia is not confined to the biochemistry laboratory and it must be emphasised that successful management of this potentially life threatening condition calls for a multidisciplinary approach in which there is full cooperation between clinicians and the full range of clinical support services. Although it is likely that biochemical studies will give the most useful information, it will be apparent from the foregoing discussion that haematological and radiological services also have an important role in the establishment of underlying diagnosis.

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