Artefactual serum hyperkalaemia and hypercalcaemia in essential thrombocythaemia

M R Howard, S Ashwell, L R Bond, I Holbrook

Abstract

Aim—To investigate possible abnormalities of serum potassium and calcium levels in patients with essential thrombocythaemia and significant thrombocytosis.

Methods—24 cases of essential thrombocythaemia with significant thrombocytosis (platelet count $> 700 \times 10^9/\text{litre}$) had serum potassium and calcium estimations performed at the time of maximum thrombocytosis before treatment, and at the time of low platelet count after treatment with cytoreductive drugs. Selected patients were further investigated with plasma sampling and estimation of ionised calcium and parathyroid hormone.

Results—At the time of maximum thrombocytosis six patients had serum hyperkalaemia ($> 5.5 \text{ mmol/litre}$) and five had serum hypercalcaemia ($> 2.6 \text{ mmol/litre}$). Following treatment and reduction of the platelet count, hyperkalaemia resolved in all cases and hypercalcaemia in four of the five cases. Mean serum potassium and calcium concentrations were raised ($p < 0.0001$) at maximum thrombocytosis compared with the values when the platelet count was low. Serum potassium and calcium values were significantly correlated at all stages. Measurements on plasma consistently corrected the hyperkalaemia but not the hypercalcaemia. Serum hypercalcaemia was associated with raised ionised calcium and normal parathyroid hormone concentrations.

Conclusions—Essential thrombocythaemia with significant thrombocytosis is associated with serum hyperkalaemia and hypercalcaemia. The probable mechanism of hypercalcaemia is the secretion of calcium in vitro from an excessive number of abnormally activated platelets. It is thus likely that the hypercalcaemia is an artefact, as is the hyperkalaemia.

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Essential thrombocythaemia is a chronic myeloproliferative disorder characterised by a persistently raised platelet count.¹ It has previously been reported that the marked thrombocytosis that is often associated with essential thrombocythaemia may lead to spurious laboratory abnormalities including pseudohyperkalaemia² and pseudohypoxaemia.³ Serum hyperkalaemia appears to result from

the release of potassium ions from platelets which are entrapped within the clot in a serum sample. This phenomenon generally occurs with platelet counts in excess of $600 \times 10^9/\text{litre}$, with a roughly predictable increment in serum potassium for every further increase in platelet count. The potassium level is normalised if the estimation is made using plasma rather than serum.^{4 5}

Abnormalities of other ions have not been well described in essential thrombocythaemia. Despite the presence of calcium in platelet-dense granules and its secretion from platelets during activation, there has been no systematic study of serum calcium levels in patients with essential thrombocythaemia and significantly increased platelet counts. There has been a single case report of serum hypercalcaemia associated with essential thrombocythaemia. In this case the hypercalcaemia rapidly resolved following reduction of the platelet count.⁶ We have investigated a group of patients with essential thrombocythaemia and significant thrombocytosis to further characterise the nature of pseudohyperkalaemia and also to establish whether there are similar alterations in serum calcium levels.

Methods

PATIENTS

Thirty three patients with essential thrombocythaemia were identified from clinical records and 24 were eligible for the study. To be eligible, patients had to have a presentation platelet count in excess of $700 \times 10^9/\text{litre}$ and measurements of serum calcium and serum potassium made at the time of maximum platelet count before treatment and at the time of a low platelet count after appropriate treatment of thrombocytosis. Where relevant biochemical tests were available at more than one normal platelet count after treatment, the lowest platelet count was used.

The mean age of the patients was 66 years (range 17-84 years). The male:female ratio was 11:13. The mean pretreatment platelet count was $1144 \times 10^9/\text{litre}$ (range 736-2291). The patients were treated with either oral hydroxyurea ($n = 20$) or busulphan, anagrelide, or interferon alfa ($n = 4$).

Following cytoreductive treatment the platelet count fell to a mean level of $288 \times 10^9/\text{litre}$ (range 61-526). Two patients with poor compliance with treatment and fluctuating levels of severe thrombocytosis (cases 1 and 2) associated with serum hyperkalaemia and hypercalcaemia had serial monitoring of serum potassium and serum calcium during their clinical course, and also the following

Department of
Haematology, York
District Hospital,
Wigginton Road, York
YO31 8HE, UK
M R Howard
S Ashwell
L R Bond

Department of
Biochemistry, York
District Hospital
I Holbrook

Correspondence to:
Dr Howard.

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Figure 1 Platelet count and serum potassium concentrations in 24 patients with essential thrombocythaemia. Serum potassium values are shown at the highest (○) and low (●) platelet counts. Broken lines indicate the upper limit (UL) and lower limit (LL) of normal reference ranges for potassium (k) and platelet count (p).

investigations: plasma sampling in addition to serum sampling for potassium and calcium concentrations, and measurement of serum phosphate, ionised calcium, and parathyroid hormone.

LABORATORY METHODS

Blood count

All blood counts were performed using a Sysmex automated cell counter. The normal platelet reference range was defined as $150\text{--}400 \times 10^9/\text{litre}$. Blood films were routinely inspected to confirm the degree of thrombocytosis.

Serum and plasma potassium estimation

Potassium was measured on a Hitachi 917 analyser (Roche Diagnostics, Lewes, E Sussex, UK) using an indirect ion selective electrode method. The reference range was 3.5–5.5 mmol/litre.

Serum and plasma calcium estimation

Calcium was measured on a Hitachi 917 analyser using an o-cresolphthalein complex-one method. The reference range was 2.10–2.60 mmol/litre.

Serum phosphate estimation

Phosphate was measured on a Hitachi 917 analyser using an ammonium molybdate method, the reaction being monitored at an ultraviolet wavelength. The reference range was 0.8–1.4 mmol/litre.

Ionised calcium estimation

Serum ionised calcium was measured on a Ciba Corning 634 Ca^{2+}/pH analyser (Beckman Instruments, High Wycombe, Buckinghamshire, UK) using a calcium ion selective and pH electrodes. The reference range was 1.18–1.38 mmol/litre.

Parathyroid hormone (PTH) estimation

Intact PTH was analysed on a Nichols Advantage analyser (Nichols Institute Diagnostics, Newport, Gwent, UK) using a chemiluminescent immunoassay method. The reference range was 10–60 ng/litre.

STATISTICAL METHODS

Possible differences in the mean serum calcium and serum potassium levels at the time of maximum thrombocytosis and low platelet count were analysed using the paired *t* test. Possible correlations between serum calcium and serum potassium levels were detected using the Pearson correlation coefficient test.

Figure 2 Platelet count, serum potassium concentration, and serum calcium concentration in two patients with poorly controlled essential thrombocythaemia (cases 1 and 2). Broken lines indicate the upper limits of the normal range.

Figure 3 Platelet count and serum calcium concentration in 24 patients with essential thrombocythaemia. Serum calcium is shown at the time of highest (○) and low (●) platelet counts. Broken lines indicate the upper limit (UL) and lower limit (LL) of normal reference ranges for calcium (c) and platelet count (p).

Results

SERUM AND PLASMA POTASSIUM ESTIMATIONS

Serum potassium

Serum hyperkalaemia (> 5.5 mmol/litre) was observed in six patients at the time of maximum thrombocytosis and in no patients at the time of low platelet count (fig 1). The mean serum potassium level was significantly raised at the time of maximum thrombocytosis compared with the value when the platelet count was low (5.29 v 4.57 mmol/litre; $p < 0.0001$).

Plasma potassium

In two cases of combined serum hyperkalaemia and hypercalcaemia (cases 1 and 2), there was normalisation of the potassium level on a simultaneously measured plasma sample (case 1; serum potassium 6.5 mmol/litre, plasma potassium 4.5 mmol/litre; case 2; serum potassium 6.3 mmol/litre, plasma potassium 4.2 mmol/litre).

Correlation of serum potassium with the platelet count in two patients with poorly controlled thrombocythaemia

In these patients (cases 1 and 2) there was a close correlation between the degree of serum hyperkalaemia and the fluctuating degree of thrombocytosis (fig 2).

SERUM AND PLASMA CALCIUM ESTIMATIONS

Serum calcium

There was serum hypercalcaemia (> 2.6 mmol/litre) in five patients at time of thrombocytosis and in one patient at the time of low platelet count (fig 3). In the single patient with persisting hypercalcaemia at low platelet count, the low platelet count was still above the normal range, at 413×10^9 /litre. In the remaining four patients, serum hypercalcaemia entirely resolved following normalisation of the platelet count with oral cytoreductive treatment. No

patients developed clinical symptoms of hypercalcaemia. The mean serum calcium was significantly raised at the time of maximum thrombocytosis compared with the value when the platelet count was low (2.52 v 2.33 mmol/litre; $p < 0.0001$).

Plasma calcium

In two patients with coexisting serum hyperkalaemia and hypercalcaemia (cases 1 and 2), the degree of hypercalcaemia was not significantly altered by the collection and analysis of a plasma sample in case 1, but there appeared to be a small correction in the plasma sample in case 2 (case 1: serum calcium 2.79 mmol/litre, plasma calcium 2.72 mmol/litre; case 2: serum calcium 2.64 mmol/litre, plasma calcium 2.51 mmol/litre).

Correlation of serum calcium with the platelet count in two patients with poorly controlled thrombocythaemia

In these patients (cases 1 and 2) there was a close correlation between the degree of serum hypercalcaemia and the degree of thrombocytosis (fig 2).

CORRELATION BETWEEN SERUM CALCIUM AND SERUM POTASSIUM

There was a significant correlation between serum potassium and serum calcium, both at the time of maximum thrombocytosis ($p = 0.02$) and at the time of low platelet count ($p = 0.02$) (fig 4).

FURTHER INVESTIGATIONS IN TWO PATIENTS WITH SERUM HYPERCALCAEMIA

In the two patients with most marked serum hypercalcaemia (cases 1 and 2) there was an associated increase in ionised calcium (case 1, 1.43 mmol/litre; case 2, 1.42 mmol/litre) but normal parathyroid hormone concentrations (case 1, 41.3 ng/litre; case 2, 20.2 ng/litre). The

Figure 4 Correlation of serum potassium and serum calcium at time of highest (○) and low (●) platelet counts in 24 patients with essential thrombocythaemia. Broken lines indicate the upper limit (UL) and lower limit (LL) of normal reference ranges for potassium (k) and calcium (c).

serum phosphate was normal in case 1 (1.11 mmol/litre) but raised in case 2 (1.80 mmol/litre). In both of these cases bone marrow trephine samples had been collected at diagnosis and there was no morphological evidence of bone resorption in these samples.

Discussion

An increase in serum potassium, commonly termed "pseudohyperkalaemia," has been well described in patients with essential thrombocythaemia, correlating with the raised platelet count. There is a similarly documented but lesser effect in reactive thrombocytosis. The finding of a normal potassium concentration in a simultaneously collected plasma sample suggests that the high serum potassium is an artefact. It is presumed that potassium leaks into the serum sample from the numerous platelets which are trapped within the clot. Although this is considered to be an artefact, it is nevertheless of clinical significance as in our experience patients may be wrongly investigated or even treated for hyperkalaemia owing to ignorance of the association with thrombocytosis. Our study has confirmed the relatively high frequency of serum hyperkalaemia in this patient group, with the presence of frank hyperkalaemia in six of the 24 patients. It is noteworthy that, in contrast to some other studies, there was not a very close correlation between the magnitude of the increase in the platelet count and the degree of hyperkalaemia, and also that significant hyperkalaemia (> 6 mmol/litre) occurred in patients with a platelet count of less than 1000×10^9 /litre.

The high incidence of serum hypercalcaemia observed in this series of patients with essential thrombocythaemia has not been described before. As with hyperkalaemia, the increased serum calcium appears to be related to thrombocytosis as the hypercalcaemia quickly resolved following the normalisation of the platelet count with cytoreductive chemotherapy. The cause of hypercalcaemia in these patients is conjectural. Hypercalcaemia has been described in other myeloproliferative disorders and has been attributed to several possible mechanisms, including bone destruction mediated by leukaemic cells, ectopic production of parathyroid hormone, and tumour derived transforming growth factors stimulating osteoclastic bone resorption.⁷⁻⁸ In our series the two patients with most marked hypercalcaemia had normal parathyroid hormone levels, making ectopic production of this hormone an unlikely cause. Bone marrow trephine samples in these patients showed no morphological evidence of leukaemic transformation or bone resorption.

The close correlation between calcium and potassium concentrations in these patients suggests that the mechanism of hypercalcaemia may be similar to that of hyperkalaemia. Various qualitative defects have been described in the platelets in essential thrombocythaemia. It is often difficult to establish whether these abnormalities arise because of the production of intrinsically abnormal platelets from neoplastic megakaryocytes, or whether the plate-

lets acquire abnormalities within the circulation or in vitro. Certainly there is evidence to suggest that these platelets are abnormally activated and may become "exhausted" within the circulation or in vitro, with release of their granule contents.⁹⁻¹⁰ Specific findings to support this are decreased levels of α granule β thromboglobulin associated with raised plasma concentrations of this platelet specific protein,¹¹⁻¹² and the finding of a low platelet calcium content in myeloproliferative disorders in comparison with normal controls.¹³ We suggest that the most likely cause of hypercalcaemia in essential thrombocythaemia is the release of calcium ions in vitro from increased numbers of abnormally activated platelets. It is of interest that, although serum hyperkalaemia was not reproducible in a plasma sample, significant hypercalcaemia occurred in both serum and plasma samples in one case, and in a second case there was only a slightly lower calcium concentration in the plasma sample. This is presumably because potassium is only released slowly from degranulating platelets within a clot, whereas the release of calcium ions will occur promptly into the sample as an early stage of the platelet secretory response. This mechanism of in vitro hypercalcaemia, as opposed to a more long standing in vivo abnormality in calcium haemostasis, is further supported by the lack of any clinical symptoms of hypercalcaemia and by the very close correlation between calcium and platelet levels over time in two of our patients with poorly controlled thrombocythaemia owing to lack of compliance with treatment.

Our observations in these patients confirm that serum hyperkalaemia is a common complication of essential thrombocythaemia with significant thrombocytosis. In addition we have documented for the first time that there is a significant incidence of serum hypercalcaemia in this disorder. It is likely that these biochemical abnormalities are artefactual, but it is important that clinicians understand these associations to avoid unnecessary investigation and treatment.

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