acid decarboxylase (GDC) test in 25% of cases, but it reacted with three (60%) of the *F. necrophorum* isolates in this study. This suggests that the majority of strains present in the Leicestershire and Rutland area are GDC-positive.

Breakdown of prevalence in cases of persistent sore throat samples were: 4.5% positive for *F. necrophorum*, 83% were negative, and 11% were identified as group A streptococci. Unexpectedly, 4.5% of non-PST samples were *F. necrophorum*. Group C streptococci and *Candida* were identified but in much smaller numbers (<1%) compared to *F. necrophorum* and group A streptococci. One *F. necrophorum* isolate was found to be present with a β-haemolytic group A *Streptococcus*, while another was present with a group C *Streptococcus*.

It was noted in a previous study that *F. necrophorum* has been found in co-infection with groups A and C streptococci.[3] Given that erythromycin (first-line treatment for streptococci) resistance is present in 15% of *F. necrophorum* isolates, patients may have unresolved infections.

The results of this study suggest that immediate inoculation of a swab on an agar plate is necessary to obtain the optimum number of viable colonies. However, it was demonstrated that the sample could be stored (i.e., 24 h at 4°C or 48 h at 22°C) prior to inoculation and still produce a viable colony.

In conclusion, this study found that the prevalence of *F. necrophorum* in samples from the East Midlands is consistent with historical data from the UK and Europe.[3,4] The organism was not found outside the 17–24 age group, which is also consistent with the findings of previous studies.[5,9–12] However, as *F. necrophorum* was found unexpectedly in reported non-persistent sore throat samples, further study or surveillance is warranted to monitor the prevalence of *F. necrophorum* in these cases and in persistent sore throat cases. □

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# Misleading hypercalcaemia in a patient with Waldenstrom's macroglobulinaemia

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Hypercalcaemia is a common clinical condition and is often associated with primary hyperparathyroidism or malignancy. Waldenstrom's macroglobulinaemia, a rare haematological neoplasm, is characterised by high serum monoclonal IgM, elevated serum viscosity, bone marrow lymphoplasmacytic infiltration and end-organ damage.[1] It is rarely associated with hypercalcaemia. This study reports a case of Waldenstrom's macroglobulinaemia with pseudohypercalcaemia due to calcium assay interference by IgM-κ paraprotein, resulting in unnecessary investigations and treatment.

An 85-year-old Chinese woman presented in August 2008 with poor appetite and significant weight loss over two months. Apart from splenomegaly, physical examinations were unremarkable. Investigations showed normochromic, normocytic anaemia with haemoglobin (Hb) 9–10 g/dL (reference interval 12–15), low albumin (26 g/L, reference interval 32–46), high globulin (58 g/L, reference interval 23–36), and increased erythrocyte sedimentation rate (ESR) to 59 mm/h. On further investigation, serum IgM concentration was grossly elevated to 38.20 g/L (reference range 0.46–3.04) with normal IgG and IgA levels. Serum and urine protein electrophoresis revealed the presence of a paraprotein band in the gamma region, typed as IgM-κ. Bone marrow biopsy revealed no lymphoplasmacytic infiltration but the picture was compatible with

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Waldenstrom's macroglobulinaemia. Skeletal survey did not show any osteolytic lesion.

Adjusted calcium level was 2.9 mmol/L (reference interval 2.05–2.60), with normal phosphate level (1.2 mmol/L, reference interval 0.8–1.5). Her serum parathyroid hormone (PTH) concentrations measured during hypercalcaemia were all within the reference range (5.4, 5.8, 3.5, 4.9, 5.0 pmol/L, reference interval 1.6–6.9). Intact PTH assay (Elecsys E170, Roche Professional Diagnostics, Indianapolis, US) was normal for her hypercalcaemia. Urine calcium:creatinine excretion ratio was 0.0126. Serum magnesium and thyroid function tests were all normal.

A provisional diagnosis of co-existing hyperparathyroidism was made. Sestamibi parathyroid scan showed a suspicious hyperfunctioning parathyroid adenoma lying slightly posterior to the interpolar region of the right thyroid gland. Ultrasound failed to show a definitely enlarged parathyroid gland. Although asymptomatic, she was hospitalised repeatedly because of persistent hypercalcaemia. Intravenous fluids and palmidronate infusion were given during hospitalisation and normocalcaemia was usually restored one to two days later, but levels crept up soon after discharge. Subsequently, she was referred to the endocrine team for further management.

Further investigation of her hypercalcaemia showed that her ionised serum calcium levels were normal (1.14, 1.27, 1.21 mmol/L, reference interval 1.12–1.30) despite increased serum adjusted total calcium levels (up to 3.72 mmol/L). All the previous total calcium levels were measured by the Arsenazo III dye binding method on the Beckman DxC analyser (Beckman Coulter, Fullerton, CA, US). As interference with calcium measurement was a possibility, samples were sent to another laboratory using the o-cresolphthalein complexone method (Modular Analytics, Roche Professional Diagnostics). Using this method, total calcium levels were all within the normal range (2.20, 2.21, 2.24 mmol/L) while simultaneous samples tested using the Arsenazo III dye method were raised (3.30, 3.80, 3.00 mmol/L).

Retrospective review of the case record showed that the patient had normal serum calcium levels prior to the diagnosis of Waldenstrom's macroglobulinaemia. Moreover, total calcium level tended to mirror the trend in serum IgM level (Fig. 1).

Serum PTH level is the most important test to differentiate the two major causes of hypercalcaemia (i.e., primary hyperparathyroidism and malignancy). It is either elevated or inappropriately normal for the prevailing hypercalcaemia in hyperparathyroidism, while it is suppressed in most cases of malignancy-mediated hypercalcaemia. Familial hypocalciuric hypercalcaemia, a rare but benign hereditary condition characterised by reduced urinary calcium excretion, can mimic primary hyperparathyroidism. Ectopic PTH secretion is observed rarely in malignancy in which elevated or inappropriately normal PTH level is found. However, pseudohypercalcaemia should be ruled out by measuring ionised calcium level before checking serum PTH.

Pseudohypercalcaemia occurs when persistently elevated total serum calcium in found in the presence of a normal ionised serum calcium level. It may be encountered in conditions associated with high serum albumin, thrombocythaemia and calcium binding to M-protein.[2] In patients with elevated serum albumin, slight

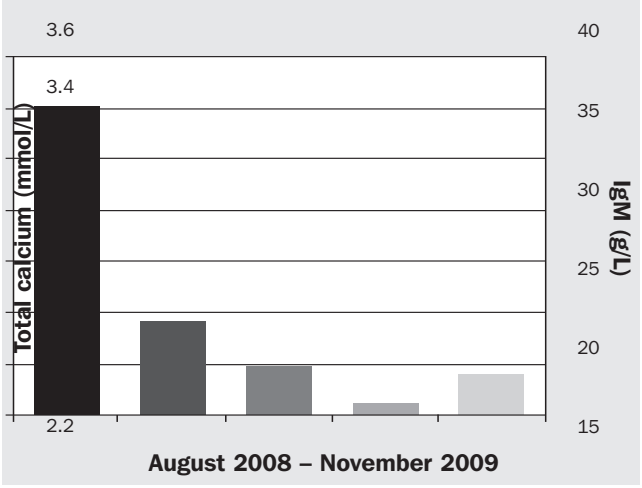


**Fig. 1.** Serum total calcium (●) and IgM (■) concentration between August 2008 and November 2009.

hypercalcaemia may be found because approximately 50% of the serum calcium is bound to albumin. It is also reported that hypercalcaemia can fluctuate directly with changes in platelet count in patients with essential thrombocythaemia.[3] Research suggests that *in vitro* release of calcium from activated platelets results in hypercalcaemia.

In the setting of an M-protein disorder, myeloma is frequently associated with hypercalcaemia as a result of increased bone resorption. Therefore, serum PTH level is usually suppressed. However, hypercalcaemia is an unusual finding in Waldenstrom's macroglobulinaemia. A case of pseudohypercalcaemia due to abnormal calcium binding by the IgM-κ paraprotein in a patient with Waldenstrom's macroglobulinaemia has been reported previously[4] The author used gel filtration chromatography to demonstrate a direct relationship between serum calcium and paraprotein concentration. A similar phenomenon has also been seen in IgA-κ, IgG-κ and IgG-λ in patients with multiple myeloma.

The cause of the pseudohypercalcaemia in the present case was due to paraprotein interference in the calcium assay. Interference is likely to be due to increased turbidity resulting from the interaction of the IgM paraprotein with the acidic medium of the Arsenazo III reagent.[5] In contrast, the o-cresolphthalein complexone method uses an alkaline medium and hence does not result in interference. Two patients with IgM-κ paraproteinaemia in Waldenstrom's macroglobulinaemia were found to have falsely elevated hypercalcaemia due to assay interference.[6]

Hypercalcaemia is not uncommon and it is important to identify genuine cases, especially in patients who have Waldenstrom's macroglobulinaemia, a condition in which hypercalcaemia is rare. Measurement of ionised calcium with or without urinary calcium excretion should be performed in suspicious cases. Any interference due to paraprotein concentration can be avoided by using a different assay method, as it is important to rule out the possibility of pseudohypercalcaemia in order to avoid unnecessary investigations, including neck exploration, and treatment. □

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