

Calcitonin (serum, plasma)

1 Name and description of analyte

1.1 Name of analyte
Calcitonin

1.2 Alternative names
None

1.3 Not used

1.4. Function(s) of analyte
Calcitonin is a 32 amino acid peptide derived from the larger precursor, procalcitonin. However, calcitonin is functionally distinct from procalcitonin, which is used as a marker of bacterial infection and sepsis. Calcitonin is a hormone secreted from the parafollicular C cells of the thyroid gland in response to a rise in serum calcium concentration.

Calcitonin has been shown to interact with G-protein-coupled receptors on the cell surface of differentiated osteoclasts. However, patients who have excess of or are deficient in calcitonin show no change in bone or mineral metabolism. Thus, the physiological role of calcitonin remains unknown. Plasma [calcitonin] is increased during pregnancy, lactation and in infants, which supports a role in protecting the skeleton when the body is under calcium stress.

Calcitonin is licensed for the treatment of Paget's disease, prevention of acute bone loss due to immobility and hypercalcaemia of malignancy. At therapeutic doses, a decrease in plasma $[Ca^{2+}]$ is brought about via inhibition of bone resorption and a decrease in renal calcium reabsorption.

Immunoassays have been developed to detect monomeric calcitonin, which is the biologically active form. Polymeric forms may occur naturally but are of unknown significance.

2 Sample requirements and precautions

2.1 Medium in which measured
Heparanised plasma or serum. This should be confirmed with the manufacturer's kit insert. For example K-EDTA tubes are compatible with the Elecsys® calcitonin assay but not the Siemens Immulite® 2000 calcitonin assay.

2.2 Precautions re sampling, handling etc.
The British Thyroid Association recommends that calcitonin samples should be kept on ice, separated within 30 mins of collection and serum or plasma frozen until required. The manufacturer's kit insert may specify alternative requirements.

3 Summary of clinical uses and limitations of measurements

- 3.1 Uses
1. Diagnosis of medullary thyroid carcinoma (MTC) – a malignant tumour of thyroidal parafollicular C cells.
 2. Measuring response to treatment for MTC and the likelihood of tumour recurrence.
 3. Pentagastrin/calcium stimulation test to investigate familial MTC, multiple endocrine neoplasia 2 A and B (MEN2A,B) or a mildly raised [calcitonin].

3.2 Limitations

- An elevated [calcitonin] is not sufficient for the diagnosis of MTC; ultrasound and fine needle aspiration cytology (FNAC) should be performed. Abnormally high [calcitonin] may be observed in non-thyroidal disorders or in response to certain drugs (section 7.1.1). [Calcitonin] should therefore be interpreted in light of the clinical presentation and history.
- Patient samples collected during the pentagastrin/calcium stimulation test should be analysed using the same calcitonin method. Due to the lack of pentagastrin availability a calcium infusion is often used.
- MTC monitoring should be performed using the same calcitonin method.

4 Analytical considerations

4.1 Analytical methods

Two-site two-step immunometric assays have now largely replaced competitive radioimmunoassays. A number of non-competitive immunoassays, with variable performance, have now been developed for the detection of monomeric calcitonin:

1. chemiluminescent immunometric assay (CMIA) Siemens Immulite®, DiaSorin LIAISON®
2. enzyme-linked immunosorbent assay (ELISA) DIAsource ImmunoAssays®
3. electro-chemiluminescence immunoassay (ECLIA) Roche Elecsys®
4. immuno-radiometric assays (IRMA) DIAsource ImmunoAssays®.

4.2 Reference method

There is currently no agreed reference method for calcitonin.

4.3 Reference materials

WHO reference material for human calcitonin. 2nd International Reference Preparation. NIBSC code: 89/620. One ampoule contains approximately 92 µg synthetic human calcitonin

4.4 Interfering substances

Grossly haemolysed, icteric or lipaemic samples may cause interference. Heterophilic antibodies have been shown to produce positive interference with immunoassay methods, leading to falsely elevated results. Positive interference due to macrocalcitonin (calcitonin-immunoglobulin aggregates) has also been described in three post-operative MTC patients.

4.5 Sources of error

- Delay in sample handling (see 2.2).

- Previously, some IRMA methods were affected by the hook effect. However current methods appear to be unaffected by the hook effect at calcitonin concentrations of 16,500 ng/L or less (this is manufacturer dependent).
- Inter-laboratory measurement or change of method. Reference intervals are method and manufacturer dependent. The same method should be used pre and post thyroidectomy.

5 Reference intervals and variance

5.1.1 Reference interval (adults)

There is currently no defined reference range or criteria. The reference interval will be method dependent and should be determined by the individual laboratory. The reference interval may be gender specific i.e. higher for men due to the increase in parafollicular C cell mass compared to women (*see: Reference values for calcitonin in men, women and children. Basuyau JP, Mallet E, Leroy M, Brunelle P. Clin Chem 2004;50:1828–1830*). Typical values: are:

- females <5 ng/L
- males <12 ng/L.

Many laboratories use <10 ng/L as recommended by the French Calcitonin-Secreting Tumours Study Group.

5.1.2 Reference intervals (others)

Age, growth, pregnancy and lactation may affect calcitonin concentrations in healthy individuals though no reference interval has been defined. Calcitonin is raised in infants up to six months of age. However, there are few indications for measuring calcitonin in this age group (*see: Basuyau JP et al, above*).

5.1.3 Extent of variation

5.1.3.1 Interindividual CV – no data

5.1.3.2 Intraindividual CV – no data

5.1.3.3 Index of individuality – no data

5.1.3.4 CV of method – Typically < 20% though this will be method dependent and should be determined by the individual laboratory.

5.1.3.5 Critical difference – no data

5.1.4 Sources of variation

- Sample handling (see section 2.2)
- MTCs produce more calcitonin per gram of tumour than non-thyroidal malignancies (see 7.1.1)
- Thyroidal/non-thyroidal disorders and drugs may increase plasma [calcitonin] (section 7.1.1)

6 Clinical uses of measurement and interpretation of results

6.1 Indications and interpretation

1. Diagnosis of medullary thyroid carcinoma (MTC).

MTC is a malignant tumour of the thyroid parafollicular C cells. The circulating calcitonin concentration correlates well with MTC tumour volume. A raised calcitonin (>90 ng/l) is consistent with a diagnosis of MTC. Calculating the doubling time of [calcitonin] also has clinical utility in monitoring the progression of MTC, disease recurrence and prognosis.

2. MTC response to treatment.

The treatment for MTC is total thyroidectomy and central compartment node dissection. Post thyroidectomy, serum [calcitonin] can provide a measure of response to treatment. Calcitonin should be measured 15 days post surgery (at the earliest) and will gradually decline over a 2-month period. Sustained hypercalcaemia warrants further investigation and imaging.

3. Pentagastrin/calcium stimulation test.

MTC can occur sporadically (75% of cases), as part of familial MTC or MEN2A/B. When screening for familial MTC or MEN2A/B, if the serum calcitonin is normal and the individual is negative for the *RET* gene variant, a calcium or pentagastrin stimulation test may be performed. Delivery of calcium/pentagastrin promotes release of calcitonin from the parafollicular C cells, which is measured at 0, 2, 5 and 7–10 min intervals. There is no exact threshold however calcitonin >250 ng/L in women and 500 ng/L in men is suggestive of MTC.

6.2 Confounding factors

- Gender
- Age
- Pregnancy and lactation
- Renal insufficiency

7 Causes of abnormal results

7.1 High values

7.1.1 Causes

Thyroidal disorders:

- C-cell hyperplasia
- MTC
- Autoimmune thyroiditis

Non-thyroidal disorders:

- Acute or chronic renal failure
- Hypercalcaemia (hyperparathyroidism)
- Small cell or large cell lung carcinoma
- Hypergastrinaemia
- Neuroendocrine tumours

Drugs:

- Proton pump inhibitors e.g. Omeprazole
- Glucocorticoids
- Beta-blockers
- Glucagon

7.1.2 Investigation

Diagnosis of MTC involves ultrasound of the thyroid, fine needle aspiration cytology and measurement of circulating calcitonin. A raised calcitonin (>60–100 ng/L) is consistent with a diagnosis of MTC. Rarely, a pentagastrin/calcium stimulation test may need to be performed for the investigation of familial MTC or mildly raised [calcitonin] (see section 6.1).

The treatment for MTC is total thyroidectomy and central compartment node dissection. However, prior to surgery the following investigations should be performed:

- serum calcitonin and carcinoembryonic antigen (CEA)
- serum calcium and parathyroid hormone to exclude hyperparathyroidism
- 24 h urine sample for the analysis of catecholamines and normetanephrines (or plasma normetanephrines) to rule out pheochromocytoma
- imaging of the neck to guide surgery
- genetic analysis to identify *RET* oncogene variants in patients with confirmed MTC. This will assess whether there is a genetic basis to the disease
- prophylactic thyroidectomy should be made available to family members who are *RET*-positive.

7.2 Low values

7.2.1 Causes

- Athyroid individuals
- No calcitonin deficiency syndrome has been described.

7.2.2 Investigation

Not applicable

7.3 Notes

The laboratory should be made aware of patients who have received monoclonal antibody therapies due to the potential for interference in the calcitonin assay.

Biochemical cure for MTC is defined as the normalisation of calcitonin levels post surgery. Biochemical cure is only observed in 40-66% of patients and further resection may be performed. Note that a low [calcitonin] does not exclude metastatic disease.

8 Performance

8.1 Sensitivity, specificity etc. for individual conditions

In the absence of a reference method and standardised reference interval for calcitonin, calculations of the sensitivity and specificity for individual conditions will vary.

One literature review revealed calcitonin screening for the detection of MTC had a sensitivity of 70–80% and a specificity of 96–100%. (Cheung K, Roman SA, Wang TS *et al.* Calcitonin measurement in the evaluation of thyroid nodules in the United States: a cost-effectiveness and decision analysis. *J Clin Endocrinol Metab.* 2008;93(6):2173–80.)

Another study assessed the positive predictive value (PPV) of calcitonin for MTC. Basal calcitonin >100 ng/L had a PPV of 100%. However, basal calcitonin 50–100 ng/L had a PPV of only 25% and basal calcitonin 20-50 ng/L had a PPV 8.3%. (Constante G, Meringolo D, Durante C *et al.*

Predictive value of serum calcitonin levels for preoperative diagnosis of

medullary thyroid carcinoma in a cohort of 5817 consecutive patients with thyroid nodules. *J Clin Endocrinol Metab.* 2007;92(2):450–5.

9 Systematic reviews and guidelines

9.1 Systematic reviews

The following provide an overview of the diagnostic strategies and management of MTC:

1. Rowland KJ, Linda XJ, Moley JF. Biochemical cure after reoperations for medullary thyroid carcinoma: A meta-analysis. *Ann Surg Oncol* 2015; 22(1):96–102
2. Grande E, Santamariá SJ, Capdevila J *et al.* Consensus on management of advanced medullary thyroid carcinoma on behalf of the Working Group of Thyroid Cancer of the Spanish Society of Endocrinology (SEEN) and the Spanish Task Force Group for Orphan and Infrequent Tumors (GETHI). *Clin Transl Oncol.* 2016;18(8):769–75.
3. Risteli J, Winter WE, Kleerekoper M, Risteli L. Bone and Mineral Metabolism. In Burtis CA, Ashwood ER, Bruns DE, Border BG. (eds), *Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics.* 5th edn. St Louis USA: Elsevier Health Sciences; 2012.
4. Ahmed SR, Ball DW. Incidentally discovered medullary thyroid cancer: diagnostic strategies and treatment. *J Clin Endocrinol Metab.* 2011; 96(5):1237–1245.

9.2 Guidelines

1. Perros P, *et al.* Management of thyroid cancer. *Clin Endocrinol (Oxf).* 2014; 81:1–122. *These guidelines recommend measuring serum calcitonin for the diagnosis of suspected MTC. Calcitonin levels measured 6 months post-thyroidectomy should be used to assess response to treatment and likelihood of recurrence of MTC. Guidance on method selection, analytical requirements and clinical interpretation is also provided.*

1. Mitchell AL, Gandhi A, Scott-Coombes D, Perros P. Management of thyroid cancer: United Kingdom National Multidisciplinary Guidelines. *J Laryngo Otol.* 2016; 130(S2):S150–S160. *Summary of the British Thyroid Association guidance.*
2. Wells SA Jr, Asa SL, Dralle H *et al.* Revised American Thyroid Association Guidelines for the Management of Medullary Thyroid Carcinoma. *Thyroid.* 2015; 25(6):567–610. *This document provides guidance on the diagnosis and treatment of patients with sporadic and hereditary MTC specifically.*

9.3 Recommendations

None identified

10 Links

10.1 Related analytes

Procalcitonin

10.2 Related tests

CEA is also secreted by the parafollicular C cells and provides a useful tumour marker for MTC progression. Serum calcitonin and CEA

concentrations are proportional to the C-cell mass and thus tumour volume. However, CEA is not recommended for the early investigation of suspected MTC.

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