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### Case Report

# Spurious laboratory alterations in pernicious anemia



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### Introduction

The authors report a case of pernicious anemia presenting with normocytosis, “nonthyroidal illness syndrome” (NTIS) and elevated cancer antigen 15.3 (CA 15.3).

Megaloblastic anemia is caused by defects in DNA synthesis and typically presents with macrocytosis. One of its causes is low serum vitamin B12 (cobalamin) levels, caused in 20–50% of the cases by a decrease in intrinsic factor due to atrophy of the gastric mucosa and consequently loss of parietal cells – an autoimmune condition known as pernicious anemia.<sup>1,2</sup>

CA 15.3, a serum tumor marker, is a mucinous antigen of the Mucin 1 (MUC-1) glycoprotein that is expressed by epithelial cells (mainly breast epithelial cells) and erythroid precursors (it is absent in mature erythrocytes).<sup>3</sup> Its elevation is mainly associated with breast adenocarcinoma, but it can also be found in other cancers and in some benign diseases.<sup>3–5</sup>

NTIS is a condition in which there are abnormal thyroid function tests in a patient without thyroid disease due to an

adaptive response an adaptive response in order to minimize to minimize the catabolism rate during high-stress periods. It usually presents with decreased free triiodothyronine (FT3) and thyroid-stimulating hormone (TSH) and normal free thyroxine (FT4).<sup>6,7</sup>

### Case report

Herein, the case of a 44-year-old female patient admitted to the Emergency Room with complaints of asthenia and dyspepsia for three months is reported. There were no other associated symptoms, such as fever, weight loss or hemorrhage. The patient denied alcohol, tobacco or substance abuse, regular medications, dietary restrictions, prior blood transfusions or any other relevant medical or surgical event. Epidemiological and family data were irrelevant.

On admission, she was hemodynamically stable, with pale mucosa and icteric sclera; the remaining physical examination, including cardiac and pulmonary auscultation, and abdominal and cervical palpation, was unremarkable.

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**Table 1 – Evolution of analytical parameters.**

	Reference values	At admission	6 months later
Erythrocytes ( $\times 10^6/\mu\text{L}$ )	4.2–5.4	2.05	4.71
Hematocrit (%)	37.0–47.0	20.2	38.4
Hemoglobin (g/dL)	12.5–16.0	6.8	13.1
MCV (fL)	78.0–100.0	98.4	81.5
MCH (pg)	27.0–31.0	33.1	26.4
RDW (%)	11.5–14.0	29.9	15.2
Leucocytes ( $\times 10^3/\mu\text{L}$ )	4.0–10.5	3.72	6.06
Platelets ( $\times 10^3/\mu\text{L}$ )	150–450	136	264
Ferritin (ng/mL)	4.63–204	117.2	123.5
Vitamin B12 (pmol/L)	141–489	40.0	740.9
TSH ( $\mu\text{IU/mL}$ )	0.27–4.2	0.02	1.92
FT3 (pg/mL)	2.5–4.3	0.81	3.67
FT4 (ng/dL)	0.93–1.7	1.37	1.57
CA 15.3 (IU/mL)	<26.4	126.5	17.4

MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; RDW: red blood cell distribution width; TSH: thyroid-stimulating hormone; FT3: free triiodothyronine; FT4: free thyroxine; CA 15.3: cancer antigen 15.3.

The blood count revealed a normocytic normochromic anemia [hemoglobin (Hb): 6.8 g/dL; mean corpuscular volume (MCV): 98.4 fL], with reduced leukocyte ( $3.72 \times 10^3/\mu\text{L}$ ), platelet ( $136 \times 10^3/\mu\text{L}$ ) and reticulocyte ( $26.2 \times 10^3/\mu\text{L}$ ; index 0.63%) counts. The peripheral blood smear showed anisopoikilocytosis, hypersegmented neutrophils and no blasts.

The serum level of total vitamin B12 was markedly decreased (40.0 pmol/L), with normal folic acid, and iron stores (ferritin: 117.2 ng/mL; erythrocyte sedimentation rate: 10 mm/h).

The patient had unconjugated hyperbilirubinemia, elevated lactate dehydrogenase (LDH) (4788 IU/L; normal: 240–480), and low haptoglobin (0.3 mg/dL; normal: 30–200). No changes in liver profile enzymes, kidney function or electrolyte balance were detected. The direct Coombs test was negative (Table 1).

A bone marrow aspirate smear revealed hypercellularity, with hyperplasia and immaturity of erythroid lineage, megaloblastosis, giant metamyelocytes and hypersegmented neutrophils, findings consistent with megaloblastic anemia.

FT3 and TSH were decreased, while FT4 and thyrotropin-releasing hormone (TRH) were normal. The investigations for infections by cytomegalovirus, Epstein Barr virus and parvovirus B19 were negative. Nuclear, anti-dsDNA, and anti-thyroid autoantibodies were also negative.

Available tumor markers for the most probable tumors for gender and age (carcinoembryonic antigen, CA-125 and CA 15.3) were assessed, identifying an increase in CA 15.3 (126.5 U/mL; normal: <26.4). In order to exclude an occult malignancy, low-radiation radiological studies (chest radiography, mammography and thyroid, abdominal, pelvic and breast ultrasounds) were performed, that revealed no anomalies.

Gastric parietal cell antibodies were positive and anti-intrinsic factor antibodies were negative. Gastric endoscopy and biopsies showed an atrophic pangastritis, without *Helicobacter pylori* infection, corroborating the diagnosis of pernicious anemia.

The patient's previous blood counts had revealed persistent microcytosis (MCV 80 fL), Hb (11.5–12.5 g/dL), and normal ferritin. High-performance liquid chromatography (HPLC) of Hb was normal (Hb A<sub>2</sub>: 2.9%; Hb F: 0.3%) excluding  $\beta$ -thalassemia. We did not perform other Hb studies, namely  $\alpha$ -thalassemia.

The patient received 2000  $\mu\text{g}$  of intramuscular cyanocobalamin plus two units of packed red blood cells (due to marked complaints of tiredness) and was referred to the Internal Medicine Outpatient Clinic, with monthly administrations of 1000  $\mu\text{g}$  of cobalamin.

At the control appointment, six months after the beginning of the treatment, a complete resolution of symptoms had occurred, and laboratory abnormalities had normalized. However, MCV and mean corpuscular hemoglobin decreased to the lower limit, which suggests a concomitant cause of microcytosis (Table 1).

## Discussion

Although the diagnosis of megaloblastic anemia is usually easy to suspect after reviewing the complete blood count and peripheral blood smear, the absence of macrocytosis, usually due to a concomitant cause of microcytosis (such as thalassemia or iron deficiency), could delay the diagnosis.<sup>1,8,9</sup>

In this case, ineffective erythropoiesis was suspected due to the unconjugated hyperbilirubinemia, elevated LDH, and decreased haptoglobin; the negative direct Coombs test excluded an autoimmune etiology for this hemolytic anemia. Low vitamin B12 levels, positivity for gastric parietal cell antibodies and stomach biopsy results (which we only assessed later) corroborated the hypothesis of pernicious anemia.<sup>1,2</sup> Because of a constitutional history of microcytosis with normal iron stores, an Hb disorder ( $\alpha$ -thalassemia or Hb variants not assessed by HPLC after excluding  $\beta$ -thalassemia) was highly suspect. Meanwhile, because these tests are not available in our institution and as they would not significantly affect the therapeutic approach, they were not performed. In many cases of hypoproliferative anemia and pancytopenia, it is also essential to exclude hematopoietic diseases, bone marrow infiltration and inflammatory causes.<sup>1</sup>

Pernicious anemia is most frequent in people with other autoimmune diseases, mainly thyroid, with anti-thyroid antibody positivity in about half of patients. So, the onset of autoimmune diseases should be monitored during the follow-up.<sup>1,2</sup>

In this case, the initial study of thyroid function revealed changes in the thyroid hormone profile. However, as there were only slight decreases in TSH and FT3, the FT4 and TRH were normal, anti-thyroid antibodies were negative and there were no changes in thyroid ultrasound, a diagnosis of NTIS was established.<sup>5,7</sup> Although this entity is usually associated with serious or critical illness, it can also be found in less serious diseases as was demonstrated by this case. In fact, the pathophysiological significance of the alterations detected in NTIS remains controversial, since it is not known whether the hormone responses represent an adaptive and normal, physiologic response to stress which just needs to be followed up, or whether it is a maladaptive response contributing to a worsening of the disease which needs appropriate treatment.<sup>6,7</sup> In

this case, the TSH level normalized after the acute stress, being concordant with an adaptive response, highlighting the lack of usefulness of assessing thyroid hormones in stress.

CA 15.3 is an immunodominant epitope in the extracellular portion of the membrane bound mucin. The mucin protein, encoded by the *MUC1* gene, is a cell surface transmembrane glycoprotein, expressed at the apical surface of most epithelia (such as the mammary gland) in normal tissue, and seems to be involved in erythroid differentiation during erythropoiesis.<sup>5</sup> In tumors, the mucin protein undergoes proteolytic cleavage, and releases the extracellular segment, which has been identified as CA 15.3, into the bloodstream.<sup>5</sup> Likewise, when erythroblasts become apoptotic, the CA 15.3 levels increase due to the release of the mucin fragment and Lewis antigen, which is also recognized by the antibody that usually binds CA 15.3. Therefore, CA 15.3 levels increase without an underlying tumor.<sup>3,5,10</sup> Studies have shown that serum CA 15.3 levels are associated with the degree of peripheral hemolysis and ineffective erythropoiesis in vitamin B12 deficiency and in other causes of hemolysis, such as thalassemia or sickle cell anemia. CA 15.3 is not related with an increase in the incidence of breast cancer or other neoplasms over time, on the contrary, it is one of the tumor markers more frequently found increased in non-neoplastic diseases.<sup>3,10</sup>

Present data do not recommend using tumor markers for screening and diagnosis due to low sensitivity and specificity; the finding of an increased marker level mandates imaging studies in order to exclude cancer, which can lead to inappropriate and unnecessary investigations.<sup>3,4</sup>

The treatment of pernicious anemia is based on 4–6 weekly administrations of intramuscular vitamin B12 at a dose of 1000 µg (cyanocobalamin quarterly; hydroxocobalamin monthly) after a loading dose. Oral replacement therapy has not yet been validated in pernicious anemia and the transfusion of packed red blood cells is usually not indicated.<sup>6,7</sup>

Besides the replacement of vitamin B12 stores, it is important to be vigilant for the co-occurrence of gastric adenocarcinoma, neuroendocrine tumors and marginal non-Hodgkin lymphoma by endoscopic examination, because they are more frequent in patients with pernicious anemia.<sup>1-3</sup>

## Conclusion

This case illustrates spurious laboratory findings of megaloblastic anemia. Moreover, an extensive laboratory investigation sometimes can be more confounding than clarifying, and laboratory abnormalities found in the acute phase of the disease may lead to unnecessary exams.

## Conflicts of interest

The authors declare no conflicts of interest.

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