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Medical Policy Manual

Topic: Serum Holo-Transcobalamin as a Marker of Vitamin B12 (i.e., Cobalamin) Status

Date of Origin: July 5, 2005

Section: Laboratory

Approved Date: February 2014

Policy No: 44

Effective Date: May 1, 2014

IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

Holotranscobalamin (holo-TC) is a transcobalamin-vitamin B12 complex which has been investigated as a diagnostic test for vitamin B12 deficiency in symptomatic and at-risk populations, as well as an assay for monitoring response to therapy.

Vitamin B12 (cobalamin) is an essential vitamin that is required for DNA synthesis affecting red blood cell formation and methionine synthesis affecting neurologic functioning. Cobalamin deficiency can result from nutritional/dietary deficiencies (most common among the vegetarian and the elderly), malabsorption of vitamin B12 (seen after gastrectomy or associated with autoantibodies [e.g., pernicious anemia]), or other relatively uncommon gastrointestinal conditions (e.g., Whipple's disease, Zollinger Ellison syndrome). Clinical signs and symptoms of cobalamin deficiency include megaloblastic anemia, paresthesias and neuropathy, and psychiatric symptoms such as irritability, dementia, depression, or psychosis. While the hematologic abnormalities disappear promptly after treatment, neurologic disorders may become permanent if left untreated.

The diagnosis of cobalamin deficiency has traditionally been based on low levels of total serum cobalamin, typically less than 200 pg/ml in conjunction with clinical evidence of disease. However, this laboratory test has been found to be poorly sensitive and specific. Therefore, attention has turned to measuring metabolites of cobalamin as a surrogate marker. For example, in humans only two enzymatic

reactions are known to be dependent on cobalamin: the conversion of methylmalonic acid (MMA) to succinyl-CoA, and the conversion of homocysteine and folate to methionine. Therefore, in the setting of cobalamin deficiency, serum levels of MMA and homocysteine are elevated, and have been investigated as surrogate markers.

There has also been interest in the direct measurement of the subset of biologically active cobalamin. Cobalamin in serum is bound to two proteins, transcobalamin and haptocorrin. Transcobalamin-cobalamin complex (called holo-transcobalamin, or holo-TC) functions to transport cobalamin from its site of absorption in the ileum to specific receptors throughout the body. Less than 25% of the total serum cobalamin exists as holo-TC, but this is considered the clinically relevant biologically active form. Serum levels of holo-TC can be measured using standard laboratory immunoassay techniques (i.e., radioimmunoassay or enzyme immunoassay). In the first step, holo-TC in the serum sample is separated by magnetic microspheres coated with monoclonal antibiotics to human transcobalamin. The cobalamin bound to the holo-TC is then released and measured by a competitive binding radioimmunoassay or by fluorescence, depending on the device used.

Regulatory Status

The Axis-Shield HoloTC RIA is an example of a radioimmunoassay for holo-TC that was cleared for marketing by the U.S. Food and Drug Administration (FDA) through the 510(k) process with the following labeled indication for use:

> "The Axis-Shield HoloTC RIA is an in vitro diagnostic assay for quantitative measurement of the fraction of cobalamin (vitamin B12) bound to the carrier protein transcobalamin in the human serum or plasma. Measurements obtained by this device are used in the diagnosis and treatment of vitamin B12 deficiency."

In November 2006, the device Axis-Shield HoloTC Assay, an enzyme immunoassay for holo-TC, was cleared for marketing by the FDA through the 510(k) process. The FDA determined that this device was substantially equivalent to existing devices for use in "quantitative determination of holotranscobalamin…in human serum and plasma on the AxSym® System. HoloTC is used as an aid in the diagnosis and treatment of vitamin B12 deficiency."

MEDICAL POLICY CRITERIA

Measurement of holo transcobalamin is considered **investigational** in the diagnosis and management of Vitamin B12 deficiency.

SCIENTIFIC EVIDENCE[1]

Validation of the clinical use of any diagnostic test focuses on 3 main principles:

1. the technical feasibility of the test;
2. the diagnostic performance of the test, such as sensitivity, specificity, and positive and negative predictive value in different populations of patients and compared to the gold standard; and
3. the clinical utility of the test, i.e., how the results of the diagnostic test will be used to improve the

management of the patient.

Technical Feasibility

The technical feasibility of serum holotranscobalamin (holo-TC) measurement has been established. As noted in the Description section, serum measurements of holo-TC involve the use of standard laboratory immunoassay techniques.

Diagnostic Performance

The diagnostic performance must be compared to the established gold standard for measuring cobalamin deficiency. This is particularly problematic, since there is currently no established gold standard. As noted in the Description section, serum levels of total cobalamin are considered poorly sensitive and specific, and there have been several reports proposing serum measures of methylmalonic acid (MMA) and homocysteine as an alternative gold standard.[2-4] One possible strategy would be to develop diagnostic parameters for holo-TC (i.e., the establishment of cut-off points for normal vs. low values) based on a known population, followed by remeasuring holo-TC after treatment. In a second step, the established diagnostic parameters could be applied to an independent population (representative of U.S. population and diet) with suggestive symptoms. One population of interest is composed of asymptomatic patients who are considered at risk for cobalamin deficiency, such as those with high-risk nutritional factors (i.e., elderly patients or those with restrictive diets), or those with a predisposing disease or condition, such as gastrectomy or autoimmune disease. It is thought that identification of subclinical disease can prompt early treatment such that clinical symptoms do not develop. Given the absence of a definitive gold standard, confirmation of a diagnosis of subclinical disease is problematic.

Systematic Reviews

In 2013 Dullemeijer et al. reported on a systematic review and meta-analysis of studies on biomarker responses to B12 supplementation.[5] The authors found doubling the intake of B12 increased serum or plasma levels of B12 by 11% and decreased MMA levels by 7%. However, only 2 small RCTs with 3 holo-TC estimates were identified which showed B12 supplementation significantly increased serum or plasma holo-TC levels. However, the small size of the RCTs precluded meta-analysis. The authors cautioned the heterogeneity of studies limited the interpretation of the results reported.

O'Leary and colleagues in 2012 reported on a systematic review of B12 status and its relationship to cognitive decline and dementia.[6] The authors evaluated 35 cohort studies and found serum B12 levels were not associated with cognitive decline or dementia, though 4 studies found increased risks of cognitive decline or dementia were associated with MMA and/or holo-TC levels. Nevertheless, the use of underpowered cohort studies of short duration limits interpretation of these results.

In April 2009, Hoey and colleagues published a systematic review of the response of various biomarkers to treatment with vitamin B12.[7] Only one RCT[8] utilizing holo-TC was identified for the review; therefore the authors concluded that data were insufficient to draw conclusions about the effectiveness of serum holo-TC as a biomarker for vitamin B12 status.

Randomized Controlled Trials

In 2013, Hill and colleagues reported on a double-blind, placebo-controlled, randomized study of 100 elderly patients with poor B12 status.[9] Patients were treated for 8 weeks with vitamin B12 supplements

of 10 µg/d, 100 µg/d, or 500 µg/d. Compared to placebo, all B12 dosages had an effect on holo-TC levels ($p< 0.01$). However, even after receiving 500 µg/d B12 for 56 days, 12% of patients had below threshold (>200pmol/L) plasma B12 levels and 56% still had elevated plasma and urine MMA levels suggesting continued metabolic insufficiency despite supplementation.

In a double-blind trial to determine the effects of B12 supplementation on cognitive functioning in older adults, Eussen and colleagues measured holo-TC at baseline, 12, and 24 weeks in 195 subjects randomized to three groups: cobalamin, cobalamin plus folate supplementation, or placebo. The primary outcome measure was cognitive improvement.[10] The results did not support a significant difference in cognitive functioning. The authors noted a significant time-treatment interaction after 12 weeks in both treatment arms of holo-TC for all biomarkers measured (vitamin B12, MMA, holo-TC, homocysteine, and red blood cell folate [$p<0.0002$]). Specifically for holo-TC, in the vitamin B12 group, mean levels increased from 58 +/- 21 to 183 +/- 124 ($p<0.05$ for difference from baseline). In the folate and vitamin B12 supplementation group, holo-TC increased from 68 +/- 33 to 222 +/- 133 ($p<0.05$ for difference from baseline). Comparatively, the placebo group's levels did not significantly change, from 70 +/- 39 to 65 +/-43 ($p<0.05$ for difference from treatment groups). Further changes did not occur between 12 and 24 weeks of supplementation.

Eussen and colleagues published a smaller trial in 2008.[11] Once again, patients were randomly assigned to cobalamin, cobalamin plus folate, or placebo supplementation in subjects with known mild cobalamin deficiency. Along with serum cobalamin and MMA levels, holo-TC was utilized to assess deficiency status and did rise in response to therapy.

Nonrandomized Trials

In a study by Loikas et al, participants included 226 normal elderly subjects and 80 normal, non-elderly adult Finnish subjects.[12] Patients were excluded from the trial if they had hyperhomocysteinemia, evidence of a possible cobalamin deficiency. In addition, patients in the lowest one third of holo-TC results underwent additional testing with MMA; those with elevated MMA levels were also excluded. In the normal reference population, the holo-TC range was 25–254 pmol/L with a 95% central reference interval of 37–171 pmol/L. Therefore, the cut-off value for a low result was established at 37 pmol/L. This cut-off value was then applied to the results of 107 patients with presumed cobalamin deficiency, as evidenced by different combinations of an increased plasma homocysteine or MMA level, or a low total serum cobalamin level, defining patients with potential, possible, or probable cobalamin deficiency. A total of 48% of those with presumed deficiency had a holo-TC below 37 pmol/L. The frequencies of low holo-TC levels increased with increasing pretest probability of cobalamin deficiency. For example, among the sixteen patients thought to have the highest pretest probability of cobalamin deficiency, based on elevated levels of homocysteine and MMA, 100% had low levels of holo-TC. Therefore, this study used levels of homocysteine and MMA as the gold standard. Based on this standard, the sensitivity of the test was only 48% among those with potential, possible, or probable cobalamin deficiency. The authors conclude that further studies are needed to confirm the clinical utility and specificity of holo-TC in diagnosis of subclinical cobalamin deficiency. Also, these values for a homogeneous population of Finnish subjects with a diet high in fish might not be able to be extrapolated to the heterogeneous American population and diet. Furthermore, these cut-off points require confirmation in a larger population of patients whose cobalamin status is unknown.

Hvas and Nexo reported on a study of 143 subjects who were divided into four groups, those with a confirmed diagnosis of cobalamin deficiency based on a decreased total serum cobalamin (<200 pmol/L) and increased MMA (>0.70 µmol/L), a second group thought to be normal based on normal

values of total serum cobalamin and MMA, and finally two additional groups with an uncertain diagnosis due to conflicting values of total cobalamin and MMA.[13] Although these authors used the reference interval established in the above study (i.e., 24-157 pmol/L), the cut-off for a low result was set at 50 pmol/L. Using this cut-off point, measurements of holo-TC had a sensitivity of 1.00 and specificity of 0.89 in classifying patients very likely to be, or not be, cobalamin deficient. Among the 73 patients with conflicting levels of MMA and total cobalamin, 39 had low holo-TC levels. Without a gold standard, it is difficult to interpret the results in this group with an uncertain diagnosis. As noted by the authors, it is not possible to determine whether or not holo-TC correctly classified the individual as deficient or not.

Hermann and colleagues[14] reported on another series of patients using the same 37 pmol/L cut-off established by Loikas[12]. This study included 93 omnivorous German controls, and several other groups of patients considered at risk for cobalamin deficiency: 111 German and Dutch vegetarians, 122 apparently health Syrians, 127 elderly Germans, and 92 patients with renal failure. In addition to holo-TC, MMA, total serum cobalamin, and homocysteine were measured. A total of 72%, 50%, and 21% of vegetarians, Syrians, and the elderly respectively had holo-TC levels of less than 35 pmol/L. Similar to the study above, these low levels of holo-TC were associated with either normal or high levels of MMA. Conversely, high levels of MMA were associated with normal holo-TC levels in other patients. Again, it is difficult to interpret the clinical significance of these conflicting laboratory values.

Valente and colleagues reported on the diagnostic accuracy of holotranscobalamin, MMA, serum cobalamin, and other indicators of tissue vitamin B12 status in an elderly population.[15] Elderly subjects (n=700), age range 63-97 years, were recruited from an ongoing observational cohort study to collect data on 2,000 individuals older than 60 years with mild to moderate cognitive impairment. A separate reference population of 120 healthy volunteers, age 18-62 years, was used to determine a reference interval for the red cell cobalamin assay. The cut-offs for deficiency were defined as 20 pmol/L for holo-TC, 123 pmol/L for serum cobalamin, and less than 33 pmol/L for red cell cobalamin. The red cell lower limit of 33 pmol/L packed red cells was used to dichotomize the concentrations into deficient and nondeficient vitamin B12 status for the construction of receiver operating characteristic (ROC) plots. The areas under the curve (AUC) showed that serum holo-TC was the best predictor with AUC 0.90 (95% confidence interval [CI]: 0.86-0.93), and this was significantly better ($p<0.0002$) than the next best predictors serum cobalamin 0.80 (95% CI: 0.75-0.85), and MMA 0.78 (95% CI 0.72-0.83). For these 3 analytes, the authors constructed a 3-zone partition of positive and negative zones and a deliberate indeterminate zone between. The boundaries were values of each test that resulted in a posttest probability of deficiency of 60% and a posttest probability of no deficiency of 98%. The proportion of indeterminate observations for holo-TC, cobalamin, and MMA was 14%, 45%, and 50%, respectively.

Heil and colleagues evaluated usefulness of holo-TC as an initial screening assay for metabolic vitamin B(12) deficiency in a mixed patient population.[16] Three hundred and sixty blood samples were collected by five Dutch hospitals, and vitamin B12 and holo-TC in serum were measured. MMA in serum was measured by tandem mass spectrometry. Receiver-operating-curve analysis demonstrated a greater area under the curve for holo-TC than for vitamin B12 in detecting vitamin B12 deficiency characterized by three predefined cut-off levels of MMA. A cut-off value of 32 pmol/L of holo-TC resulted in the highest sensitivity (83%) with acceptable specificity (60%) in detecting MMA concentrations above 0.45 μmol/L. The combination of vitamin B(12) and holo-TC did not improve diagnostic accuracy at this cut-off level. The authors concluded that holo-TC has a better diagnostic accuracy than vitamin B12 and could replace the existing vitamin B12 assay as a primary screening test in patients suspected of vitamin B12 deficiency. Further randomized, controlled studies are necessary to validate the 32 pmol/L cut-off value established in this study across differing populations. In addition,

questions concerning the value of holo-TC testing to improve clinical management need to be answered.

Fragasso and colleagues conducted a small (n=22) study of serum cobalamin (Cbl) levels in alcoholics who can have falsely increased values of Cbl caused by alcoholic liver disease.[17] A significant positive correlation was found between serum Cbl and holo-TC levels however this study is limited by small sample size which restricts conclusions as to the usefulness of holo-TC as a measurement for assessing B12 status in alcoholics.

Other recent studies have utilized holo-TC as one of a number of measures of cobalamin status.[9,18-23] However, these studies do not attempt to assess the independent predictive capacity of the test and therefore do not add to the evidence base for this policy.

Clinical Utility

Advocates of holo-TC testing posit that this laboratory test can identify early subclinical stages of cobalamin deficiency, permitting prompt initiation of treatment, specifically supplementary cobalamin dietary supplementation. This hypothesis was not directly tested in any of the identified published literature. In the absence of an established gold standard, the clinical significance of subclinical cobalamin deficiency must be further studied by understanding the natural history of this condition. Does subclinical deficiency inevitably progress to clinical deficiency? Does cobalamin supplementation normalize the values? How variable are cobalamin levels within patients? These clinical issues have not been well addressed in the literature. Finally, for all patients at risk (e.g., vegetarians, the elderly, post-gastrectomy patients), the recommended treatment of subclinical disease is further dietary supplementation of cobalamin. This recommendation is appropriate, regardless of the level of measured cobalamin.

Clinical Practice Guidelines and Position Statements

Many societies have recommended vitamin B12 supplementation for specific clinical conditions or evaluation for vitamin B12 deficiency in the workup for clinical indication without specifying a methodology. An exception is in a 2009 practice parameter (reaffirmed July 2013) for peripheral neuropathy by the American Academy of Neurology (AAN) that has specified a methodology (evidence level C): “serum B12 level with metabolites (methylmalonic acid with or without homocysteine)” in the evaluation for vitamin B12 deficiency.[24] Measurement of serum holo-TC is not included in this methodology.

Summary

There are inadequate data to establish serum holotranscobalamin (holo-TC) testing as an alternative to either total serum cobalamin, or levels of methylmalonic acid (MMA) or homocysteine in the diagnosis and management of vitamin B12 deficiency. Holo-TC testing is technically feasible and likely to have diagnostic performance that approaches that of currently utilized tests. However, no evidence has been demonstrated on how this testing may be used to improve health outcomes (clinical utility) when used as a screening tool in the general or at-risk population, as a diagnostic tool in symptomatic individuals, or as an assay for monitoring response to therapy. Therefore, holo-TC testing is considered investigational for all indications.

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CROSS REFERENCES

None

CODES	NUMBER	DESCRIPTION
CPT	0103T	Holotranscobalamin, quantitative
HCPCS	None	