Measuring and Interpreting Holo-Transcobalamin
(Holo-Transcobalamin II)

At one time, diagnosing cobalamin deficiency was fairly simple, usually involving a patient with clinical problems, and was usually settled by determining whether the serum cobalamin concentration was low or not. As reviewed elsewhere (1), things began to change after sensitive metabolic tests were introduced and as attention extended to the cobalamin status of asymptomatic persons. Metabolic studies confirmed that most low cobalamin concentrations in asymptomatic patients and seemingly healthy persons represented subclinical cobalamin insufficiency, but ~30–40% of these cobalamin concentrations did not represent insufficiency and thus could be considered “falsely low”. The diagnostic reliability of serum cobalamin was further challenged by metabolic demonstrations of “falsely normal” cobalamin concentrations; these too occurred most often, but not exclusively, in asymptomatic persons.

The search continues for the optimal test to diagnose deficiency because the metabolic tests also have disadvantages. Increased plasma total homocysteine is too nonspecific; methylmalonic acid determination, although posing fewer problems of specificity, is complex and expensive; and the deoxyuridine suppression test is too unwieldy for practical use.

Interest has been drawn to the possible benefit of measuring only the cobalamin attached to transcobalamin (TC; also called TC II; the TC-cobalamin complex is called holo-TC or holo-TC II) rather than the total cobalamin content of plasma. The concept, suggested by Lindemans et al. in 1983 (2), is simple and attractive: holo-TC contains the biologically available cobalamin because only TC promotes specific uptake of its cobalamin by all cells. The much larger fraction of serum cobalamin carried by haptocorrin (HC; also called TC I, R binder, or sometimes cobalophilin) is considered metabolically inert because no cellular receptors exist for holo-HC (also called holo-TC I).

However, the physiologic cycle of holo-TC is quite complex: (a) ileal holo-TC enters the portal circulation, carrying cobalamin absorbed from the gut (3); (b) some of the portal holo-TC is cleared by hepatocytes, with holo-TC concentrations decreasing by 26–72% in the hepatic vein (4); (c) the uncleared holo-TC then circulates systemically, where an unknown proportion is delivered to other cells; (d) most of the peripheral holo-TC clearance occurs via glomerular filtration followed by tubular uptake in the kidney, which is very rich in cobalamin and whose tubular epithelium is rich in TC receptors (5); (e) animal data suggest that renal tubules also synthesize TC, which emerges in the blood stream as new holo-TC carrying the reabsorbed cobalamin (6). The pathologic influences on most of these individual phases in the cycle are still unknown.

Many things affect the holo-TC concentration found in antecubital vein blood. These include not only the amount of absorbed cobalamin but also the rates of hepatic and renal uptakes of holo-TC, the production and release of ileal and possibly renal holo-TC, tissue requirements for cobalamin, and perhaps other unknown factors such as qualitative and quantitative variations in TC. It also seems worth pondering why 5’-deoxyadenosylcobalamin makes up more of the cobalamin in holo-TC than in holo-HC (7).

It is not surprising, therefore, that what low holo-TC concentrations really tell us remains elusive. The favored hypotheses have been that low holo-TC is either an early sign of general cobalamin insufficiency or specific evidence of decreased absorption of cobalamin. The distinction between these two very separate explanations has blurred, particularly as advocacy became more enthusiastic, but it is not an idle distinction. This central issue and many other questions need resolution.

Malabsorption and deficiency are not identical, nor are their clinical implications. Patients can have cobalamin deficiency without having malabsorption and can have malabsorption without deficiency. If holo-TC concentrations reflect absorption alone, then even the transient malabsorption caused, for example, by temporary exposure to drugs, such as colchicine or omeprazole, or by alcohol abuse should depress holo-TC. Moreover, if holo-TC concentrations depend primarily on the influx of absorbed cobalamin, temporary dietary restriction might also depress holo-TC, although indirect evidence from two old studies suggests otherwise (2, 8). Either of these types of temporary influences could limit the clinical and metabolic value of the test because it takes years to deplete cobalamin stores.

Does the evidence favor cobalamin deficiency or impaired absorption as the determinant of low holo-TC? Convincing evidence for either explanation does not exist today. Support for low holo-TC as a marker of cobalamin malabsorption has rested on data from only four patients with AIDS (9) and a troubling study in which gastric and, even more speculatively, duodenal histology was used instead of, and sometimes in conflict with, direct absorption testing to define a state “compatible with malabsorption” (10). Moreover, 20 of the 53 low holo-TC concentrations (38%) occurred in patients who were not in such a state (10). New evidence of low holo-TC concentrations in Indian vegetarians (11) suggests that a popular characterization of holo-TC assay as a surrogate Schilling test is incorrect. However, studies equating low holo-TC with

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cobalamin insufficiency are also open to question. Future study models must be selected carefully to allow the influences of deficiency and malabsorption to be distinguished clearly. Both malabsorption and metabolic status must be defined rigorously and demonstrated directly rather than by proxy measures.

Specificity of the low holo-TC concentration. Wickramasinghe and Ratnayaka (12) concluded that low holo-TC was not specific for cobalamin abnormality. Nine of their 24 patients with low holo-TC (38%) had normal cobalamin status, as determined with the sensitive deoxyuridine suppression test; the diagnoses in the 9 included myelodysplasia, congenital dyserythropoietic anemia, and alcohol abuse. Another study mentioned low holo-TC concentrations in patients with folate deficiency (10).

Clinical utility of holo-TC measurement. Utility will depend ultimately not just on the diagnostic meaning and technical performance characteristics of holo-TC measurement but also on how often and when holo-TC determination clinically outperforms the simple and inexpensive measurement of total cobalamin in the blood. Demonstrations of significant correlations between holo-TC and cobalamin status or absorption will not suffice without also comparing them directly with total cobalamin concentrations. Furthermore, if the preponderant diagnostic advantage of holo-TC testing turns out to be in persons with subclinical cobalamin deficiency, that too must inform judgment about the assay’s utility.

Methodologic issues. Technical problems have been major impediments to the resolution of the previously mentioned issues. Plasma contains ~1.5 times as much TC as HC, but only a small fraction of the TC carries cobalamin. Holo-TC was 20–220 pmol/L in a study using immunoadsorption techniques, whereas total TC (apo and holo forms combined) was 422–1086 pmol/L (2). In comparison, holo-HC was 87–491 pmol/L in a total HC of 154–750 pmol/L. Widely quoted estimates are that 6–20% of cobalamin is carried in plasma as holo-TC, although reported values have varied between 0% and >50%.

Holo-TC measurement involves two sequential steps: separation of total TC from total HC [and other minor cobalamin-carrying proteins (4)], and determination of the cobalamin content in the TC fraction. Each step has been dogged by technical problems. Separation of TC from HC has relied most often on physical adsorption differences between the two proteins, using adsorbents such as microfine silica (9, 10, 12), which however adsorbs only 85–90% of the TC and adsorbs some HC (12). Other approaches have used immunoadsorption (2, 8) or liquid chromatography (4), which is unwieldy. The difficulty of the second step, measuring cobalamin in holo-TC, has been the need for assays to distinguish minute concentrations of cobalamin in holo-TC that are often beyond their precision; a difference between 20 and 25 pmol/L may be diagnostically crucial. That laboratories and assay methods often cannot reliably quantify serum total cobalamin concentrations <50 ng/L (37 pmol/L), instead reporting them all as <50 ng/L, illustrates the difficulty of applying such assays to holo-TC measurement. The usual indirect resort by holo-TC methods to quantifying the larger holo-HC fraction instead and subtracting it from the separately measured total cobalamin value is just as problematic because the CV of the cobalamin assay often exceeds the small difference between the two values.

The two new holo-TC assay methods reported in this issue of Clinical Chemistry have addressed many of the technical problems. Both assays use specific anti-TC antibody rather than imprecise physicochemical methods to separate TC from HC and other holoproteins. Ulleland et al. (13) also reduced the cobalamin measurement imprecision by concentrating the final sample eightfold, so that the amount of holo-TC cobalamin presented for assay is greater. Nexo et al. (14) have used the ingenious approach of reversing the order of manipulations and avoiding the cobalamin assay entirely. They first separated holoproteins from apoproteins by use of cobalamin-coated magnetic beads and then directly assayed the TC fraction of the holoproteins by ELISA. It may be that they thus also avoided the interference by very high apo-TC concentrations in quantifying holo-TC that Ulleland et al. (13) found. It is not clear why the assay of Nexo et al. (14) measured higher holo-TC concentrations than that of Ulleland et al. (13). Future studies should clarify the performance characteristics of these two methods at the critical juncture between low and normal holo-TC values. It also appears that separate reference intervals will be needed for serum and plasma.

Armed with these new methods, investigators can now address the many questions surrounding holo-TC and what it means, and carefully designed clinical surveys can be undertaken. Until the answers emerge, we must all take very seriously the American commercial distributor’s warning that the newly released reagent set, described here by Ulleland et al. (13), is for research purposes only.

To paraphrase the psychiatrist’s invitation in the final line of Philip Roth’s Portnoy’s Complaint: And now, perhaps, we may begin?

References


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