Cobalamin-Binding Capacity of Haptocorrin and Transcobalamin: Age-Correlated Reference Intervals and Values from Patients

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Unsaturated cobalamin-binding capacity in plasma (P-UBBC) is determined by use of a silica gel (QUISO G32) to separate haptocorrin (P-ApoHC) and transcobalamin (P-ApoTC). The method is sensitive and precise: detection limit 13 pmol/L; interassay coefficients of variation 3% for P-UBBC (mean = 1080 pmol/L), 4% for P-ApoTC (mean = 700 pmol/L) (n = 30). Values for P-UBBC, P-ApoHC, P-ApoTC, and P-TBCC (P-UBBC plus P-cobalamin) determined in a population study of 228 individuals, ages 21–87 years, did not differ by sex. These values increased with age, whereas the cobalamin saturation (P-cobalamin as percentage of P-TBCC) decreased with age. However, these changes were statistically significant but marginal and thus not clinically important. We therefore suggest using combined reference intervals (central 95 percentiles) for all age groups: 500–1200 pmol/L for P-UBBC, 90–275 pmol/L for P-ApoHC, 400–850 pmol/L for P-ApoTC, 850–1600 pmol/L for P-TBCC, and 20–50% for cobalamin saturation. Results for 277 inpatients show high P-ApoHC in myeloproliferative disorders or acute nonlymphatic leukemia, whereas P-ApoTC concentrations are high in some patients with lymphoproliferative disorders or autoimmune diseases.

Additional Keyphrases: vitamin B₁₂ • age-related effects • cancer • autoimmune disease • myelo- and lymphoproliferative disorders • cobalamin-binding proteins • silica gel used for separation

Two cobalamin-binding proteins are found in plasma, haptocorrin and transcobalamin. The latter is responsible for the transport of cobalamin into cells, whereas the function of the former is virtually unknown (1). Both proteins circulate in blood partly saturated (holo- and partly unsaturated (apo-) with cobalamin. The clinical importance of determining the concentration of these proteins in plasma has been a subject of increasing interest during the last decades. Most often the substance concentrations of the cobalamin-binding proteins are estimated by determining the total unsaturated cobalamin-binding capacity in plasma (P-UBBC) (2), then separating haptocorrin and transcobalamin by various methods (3–14).

Few laboratories have developed radioimmunoassays for determining the total substance concentration of the two proteins (15–17), and antibodies to haptocorrin or transcobalamin are not easily available. Consequently, we decided to evaluate the method of Jacob and Herbert (10) for determining in plasma the unsaturated cobalamin-binding capacities of haptocorrin (P-ApoHC) and transcobalamin (P-ApoTC). We present data on age-correlated reference intervals for P-UBBC, P-ApoTC, P-ApoHC, total cobalamin-binding capacity (P-TBCC equal to P-UBBC plus P-cobalamin), and cobalamin saturation (P-cobalamin as percentage of P-TBCC). We also compared these reference intervals with the results for P-ApoTC and P-ApoHC in inpatients with various diseases.

Materials and Methods

Age-correlated reference group. The age-correlated reference group comprised 228 persons, 121 men and 107 women, ages 21–87 years, who were participating in a population survey (The Copenhagen City Heart Study).

Study sample of hospitalized patients. We also studied a group of 277 patients, 150 men and 127 women, ages 10–92 years (mean 60.5), who had been referred to the department of internal medicine and hematology as inpatients or as outpatients of the ambulatory care unit during a four-year period. The patients could be divided into five diagnostic groups:

- 35 patients had anemia—seven with treated and 14 with untreated pernicious anemia, and 14 with folate deficiency, iron deficiency, hemolysis, or anemia due to chronic infections;
- 56 patients with myeloproliferative disorders—43 were as yet untreated for polycythemia (n = 23), chronic myelogenous leukemia (n = 9), primary myelofibrosis (n = 8), or essential thrombocytosis (n = 1), and 13 were being treated for these diseases;
- 72 patients with lymphoproliferative disorders—30 were as yet untreated for chronic lymphocytic leukemia (n = 10), non-Hodgkin’s malignant lymphoma (n = 7), Hodgkin’s disease (n = 4), multiple myeloma (n = 4), hairy cell leukemia (n = 1), Waldenström’s macroglobulinemia (n = 1), acute lymphoblastic leukemia (n = 1), lymphocytic leukemia (n = 1), and angioimmunoblasticoma (n = 1), whereas 42 were being treated for chronic lymphocytic leukemia (n = 8), non-Hodgkin’s malignant lymphoma (n = 9), Hodgkin’s disease (n = 8), multiple myeloma (n = 7), hairy cell leukemia (n = 1), Waldenström’s macroglobulinemia (n = 1), acute lymphoblastic leukemia (n = 8);
- 33 patients with acute nonlymphocytic leukemia—27 untreated, of whom eight had had a previous myelodysplastic syndrome, and six who were receiving treatment;
- S2 with various non-hematological disorders, including autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, and scleroderma; infections; acute myocardial infarction; hepatic cirrhosis; and patients referred for further evaluation, who turned out to be healthy.

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² Nonstandard abbreviations: UBBC, unsaturated cobalamin-binding capacity; ApoHC, unsaturated cobalamin-binding capacity of haptocorrin; ApoTC, unsaturated cobalamin-binding capacity of transcobalamin; TBCC, total cobalamin-binding capacity, equal to the sum of UBBC and cobalamin concentration; and P-plasma.

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Preparation of samples. Plasma was prepared by centrifuging EDTA-treated blood within 2 h after sampling and kept frozen at -20 °C until analysis.

P-ApoHC and P-ApoTC by separating on silica gel. We used a modification of the method of Jacob and Herbert (10) as previously described (18). All specimens containing >1200 pmol/L were appropriately diluted with 0.15 mol/L NaCl and re-analyzed. The silica gel was QUSO G32 (obtained from Bie and Berntsen Ltd., Rødovre, Denmark).

Plasma cobalamin (P-cobalamin). We quantified P-cobalamin in 216 specimens obtained from the reference group, with use of pure intrinsic factor as the binder (19).

P-TBCC and cobalamin saturation. P-TBCC was calculated as the sum of P-UBBC and P-cobalamin from each specimen; cobalamin saturation indicated the fraction that the P-cobalamin was of P-TBCC.

Statistical methods. We used standard parametric and nonparametric statistical tests as indicated later in the text.

**Fig. 1.** Concentrations of the plasma cobalamin-binding proteins, and cobalamin saturation as a function of age in 228 apparently healthy subjects: (a) P-UBBC, (b) P-ApoHC, (c) P-ApoTC, (d) P-TBCC, (e) cobalamin saturation

The regression lines for each set of data are indicated.
Results

Reliability of the QUSO G32 Method

Charcoal precipitation of cyano-\textsuperscript{67}Co\textsuperscript{cobalamin-labeled plasma combined with QUSO G32 was used to determine the P-UBBC and P-ApoTC (adsorbed to QUSO G32). P-ApoHC was calculated as the difference between P-UBBC and P-ApoTC.

In EDTA-stabilized pooled plasma kept at \textasciitilde 20 \textdegree C for up to 12 months, we could demonstrate no time-related change in the P-ApoHC or P-ApoTC (P >0.5, n = 60).

The results for serial dilutions of P-UBBC with 0.15 mol/L NaCl to give concentrations in the range of 13 to 1250 pmol/L fell on a straight line (P \leq 0.0001). P-ApoTC could be detected when it constituted at least 0.5% of P-UBBC. The detection limit, defined as twice the SD at low concentrations, for the absolute amount of P-UBBC, P-ApoHC, and P-ApoTC was 13 pmol/L.

Analysis of pooled plasma 30 times during six months showed interassay coefficients of variation (CVs) of 3% for P-UBBC and 5% for P-ApoTC at mean respective concentrations of 1082 and 700 pmol/L. Intra-assay CVs were 2% for both P-UBBC and P-ApoTC. The CV was also 3% for P-UBBC (5069 pmol/L (mean)) in plasma with a high content of P-ApoHC (from patients with chronic myelogenous leukemia whose P-ApoHC concentrations were about 5000 pmol/L). The CV for P-ApoTC was \textasciitilde 15% at 605 pmol/L in plasma from patients with chronic myelogenous leukemia, in whom the P-ApoTC constituted between 11% and 23% of the P-UBBC (n = 10).

Intra-Individual Variation

Two healthy volunteers had blood sampled every week for 10 weeks. CVs were 4.2–12% for mean (and SD) P-ApoHC concentrations of 150(10.3) and 178(7.7) pmol/L, and for P-ApoTC of 585(73.6) and 601(33.6) pmol/L.

Interindividual Variation

Reference population. The interindividual variation of these analyses in the 228 individuals in the reference population, expressed as CV, was 27% for P-ApoTC (627, SD 139.6, pmol/L) and 32% for P-ApoHC (168, SD 54.0, pmol/L). For P-UBBC, P-ApoTC, P-ApoHC, and P-TBCC the concentrations significantly increased with age (P <0.001, Spearman rank correlation, \( r = 0.35, 0.38, 0.31, \text{ and } 0.28, \text{ respectively}). No sex-related difference could be demonstrated for P-UBBC, P-ApoTC, P-ApoHC, or P-TBCC, either for the population as a whole or divided into age decades (P >0.2). The observed ranges of values as a function of age are shown in Figure 1 and Table 1.

The cobalamin satiation significantly decreased with age (P <0.0001, Spearman rank correlation, \( r = -0.29 \) (Figure 1), again, with no significant sex-related difference (P >0.36). The age-related ranges observed are indicated in Table 1.

Patients. The distribution of results for the 277 patients is shown in Figure 2. More than half of the patients with myeloproliferative disorders had increased P-ApoHC and, within this group, all patients with chronic myelogenous leukemia had increased P-ApoHC. About a third of the patients with acute nonlymphocytic leukemia had high P-ApoHC, and a sixth had high P-ApoTC. The lymphoproliferative disorders showed a more variable picture, with about a fourth having increased P-ApoTC. In comparison, in the remaining patients, values were between 6% and 16% below or above the reference limits, with no characteristic diagnoses accompanying the data points outside the reference limits.

Discussion

Here we have evaluated a method for quantifying P-UBBC, P-ApoTC, and P-ApoHC (10), and found it to be precise and easy to use for analyzing a large number of
samples. The method involves a direct estimation of P-ApoTC and an indirect quantification of P-ApoHC.

We used samples from a large, unselected group of persons for the determination of reference intervals—a justifiable approach because major disturbances in the cobalamin-binding proteins are relatively rare. As shown, we found no difference in the values obtained for male and female individuals, either in the whole population examined, or in those younger than 50 years. Previous studies (20–22) have indicated that values for P-UBBC and P-ApoTC were higher in women than in men (younger than 50 years). In the largest of the studies (22), care was taken to ensure that the women were not pregnant and did not receive oral contraceptives, which are known to decrease P-UBBC (23,24). In the present study, we did not have information on how many of the women were taking oral contraceptives.

Cobalamin-binding proteins present in human plasma are partly saturated with cobalamin (HoloTC and HoloHC). This part of the proteins, quantified as P-cobalamin, was found (25) to decrease with age in the same population as we examined. The median value of P-cobalamin decreases by 65 pmol/L from age 20 to age 80. Over the same age span, P-ApoTC and P-ApoHC increase by 140 and 60 pmol/L, respectively, and the increase in P-TBBC is about 180 pmol/L. Whether this increase in the cobalamin-binding proteins in plasma with age indicates increased synthesis or decreased elimination is unknown.

Although the increase in P-ApoTC and P-ApoHC with age is highly significant, the absolute changes with age are marginal. Therefore we do not recommend use of age-related reference intervals for daily routine.

In the present study we were able to calculate the cobalamin saturation. The possible clinical importance of the cobalamin saturation of plasma in the diagnosis of a cobalamin-deficiency state remains to be proven.

Our results are important for the interpretation of the previous results concerning P-cobalamin (25). As we have shown here, the decrease of P-cobalamin with age is not induced by a decrease in the cobalamin-binding proteins, because P-ApoHC, P-ApoTC, and P-TBB all increase with age. This observation reinforces the questions of whether the elderly have a suboptimal concentration of P-cobalamin, and if so, whether this implies a latent cobalamin deficiency. Available data do not support the idea that elderly people with relatively low P-cobalamin have functional cobalamin deficiency. However, little is known concerning the natural history of persons with low P-cobalamin, and it is uncertain how many will eventually develop cobalamin deficiency.

As we saw, interindividual variation in the concentration of the cobalamin-binding proteins is small. Thus determination of the cobalamin-binding proteins may be potentially useful in the diagnosis and prognosis of various disorders. Also, because intra-individual variation is small, at least in healthy persons, the cobalamin-binding proteins in plasma may serve as "markers" of disease activity. The present paper confirms that quantification of the cobalamin-binding proteins may be of potential use in cases of myeloproliferative disorders (26) and acute nonlymphocytic leukemia, in which concentrations of haptocorrin are increased, and in lymphoproliferative and autoimmune disorders (17,27), in which concentrations of transcobalamin are increased. Our paper emphasizes that only a few patients with other diseases have concentrations of haptocorrin or transcobalamin outside the reference intervals. The increase in haptocorrin or transcobalamin is in most cases sufficiently pronounced to be reflected in an increased P-UBBC. However, longitudinal investigations of patients with the above-mentioned disorders are needed to decide whether quantification of P-UBBC alone is suitable for the clinical use of determinations of cobalamin-binding proteins in plasma.

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