Cobalamin (Vitamin B₁₂) Deficiency Detection by Urinary Methylmalonic Acid Quantitation

By Eric J. Norman, O. J. Martelo, and M. Drue Denton

A study was made to assess the value of cobalamin deficiency detection through quantitation of urinary methylmalonic acid (MMA). Urinary MMA was measured in 1118 patients suffering from megaloblastic anemia, other anemias, elevated red cell mean corpuscular volume, or unexplained neurologic disorders. Patients without proven cobalamin deficiency had urinary MMA levels <20 μg/ml. All patients (n = 27) confirmed to have cobalamin deficiency showed MMA levels >20 μg/ml. Data are presented showing the Schilling test results, the comparison of serum cobalamin to urinary MMA levels, and other basic hematologic data. MMA levels are a good indication of cobalamin distribution and function since they are directly related to a cobalamin-dependent metabolic pathway. With rapid, reliable quantitation by mass spectrometry, urinary MMA can now be a useful clinical test.

Furthermore, serum cobalamin levels may be normal or high despite a functional cobalamin deficiency. Serum cobalamin and cobalamin-binding proteins have been shown to be abnormally high in some hematologic malignancies such as acute promyelocytic leukemia and chronic myeloid leukemia. In addition, a study of nonhematologic malignancy showed approximately 50% of these patients had abnormalities of cobalamin and its binding protein. In situations where the serum cobalamin level is artificially elevated due to abnormalities in cobalamin-binding protein, measuring cobalamin status requires either a tissue biopsy for cobalamin content or a laboratory test for normality of function of a cobalamin-dependent pathway, such as measurement of MMA, or the deoxyuridine (du) suppression test. Excretion of elevated MMA has been reported to be the first indication of cobalamin deficiency since it is a reflection of the tissue availability of the vitamin.

Low serum cobalamin levels in the absence of tissue deficiency of the vitamin have been reported in a variety of conditions, sometimes as an artifact of the assay procedure, sometimes because of abnormality of a cobalamin-binding protein, and sometimes without clear explanation.

To help avoid problems in the diagnosis of cobalamin deficiency, quantitation of urinary MMA may be very helpful. We report data on 1118 patients who were assayed for urinary MMA.

MATERIALS AND METHODS

Specimen Storage

Urine specimens were stored without preservative in plastic tubes at −20°C. Although urines were frozen upon receipt, no special handling was required. No significant changes in MMA levels were noted in several urines stored at room temperatures for 6 mo containing both high and normal MMA levels.

MMA Measurements

The method of assaying for urinary MMA has been previously described in detail. Urine (50μl) was derivatized to form the dicyclohexyl ester of MMA and quantitated using a Finnigan 9500
gas chromatograph interfaced to a Finnigan 3200 mass spectrometer with selected ion-monitoring capability.

**Serum Cobalamin Levels and Schilling Test**

These results were obtained from the patients' charts, and radioassay tests were performed by the Radiobiology Divisions of Cincinnati’s General Hospital, Jewish Hospital, Veteran’s Administration Hospital, Good Samaritan Hospital, and Christ Hospital. Current radioassay kits in use were obtained from Diagnostic Products, Los Angeles, Calif. or Becton Dickinson, Salt Lake City, Utah.

**RESULTS**

Table 1 illustrates data obtained on 1118 patients who were assayed for urinary MMA. The number of patients for each range of concentration is specified. Most patients gave levels <5.0 μg MMA/ml urine. We have arbitrarily established this value as our normal range. A few patients showed slightly elevated levels between 5.0 and 20.0 μg MMA/ml urine. In most cases, the increased values can be attributed to more concentrated specimens. We have recently made creatinine measurements on urines with slightly elevated levels of MMA for a better evaluation. Nearly all patients in this study were also assayed for levels of serum cobalamin. In cases where there was a discrepancy between serum cobalamin and urinary MMA levels, cobalamin malabsorption was tested with a Schilling test. To date, no patient with <20 μg MMA/ml has been reported by their physician to have pernicious anemia.

Table 2 lists 27 patients with clearly elevated levels of urinary MMA (>20 μg MMA/ml). Table 3 lists the patient’s neurologic abnormalities and their pertinent history. Patients 1 through 14 may have been assayed for serum cobalamin using older kits containing impure intrinsic factor. However, these patients were shown to have defective cobalamin absorption through the Schilling test or below normal serum cobalamin levels by the radioassay. The strikingly abnormal elevated levels of urininary MMA have proven to be more diagnostic of cobalamin deficiency than the serum cobalamin levels. The method of MMA quanti-
Urine and serum (notation gave a day-to-day specimens were usually taken on the same day always prior to cobalamin therapy.

...explained by an extremely elevated creatinine of 6.7 mg/ml urine. Creatinine measurements allow a better urinary MMA obtained from a pernicious anemia patient was 26 ug MMA/ml with a creatinine at 0.38 mg/mI urine. On the other hand, a low level of urinary MMA was considered normal was 14 ug MMA/ml and was...

Table 3. Neurologic Abnormalities and Pertinent Past History

<table>
<thead>
<tr>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
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of inadvertent cobalamin injection immediately prior availability of the vitamin.8 Accordingly, the problem dures for MMA quantitation.9'2 The gradual reduc-
tion in urinary MMA after cobalamin therapy (Table 4) illustrates that MMA is a measure of the tissue availability of the vitamin.9 Accordingly, the problem of inadvertent cobalamin injection immediately prior to venipuncture (estimated 2.5%) would not negate the MMA assay as it would the serum radioassay.

Table 4. Reduction of Urinary MMA After the Initiation of Cobalamin Therapy: An Intramuscular Injection of 1000 μg of Cobalamin Was Given on Day 0

<table>
<thead>
<tr>
<th>Day</th>
<th>μg MMA/ml Urine</th>
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<tbody>
<tr>
<td>0</td>
<td>105</td>
</tr>
<tr>
<td>1</td>
<td>53</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>4</td>
<td>5.6</td>
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<td>6</td>
<td>8.7</td>
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The patients listed in Table 2 all showed 4 to 300 times normal levels of MMA. These elevated values have proven to be highly specific for the detection of cobalamin deficiency. Patients 2, 11, 14, and 19 may have been missed by less sensitive and specific procedures for MMA quantitation.19,20 The gradual reduction in urinary MMA after cobalamin therapy (Table 4) illustrates that MMA is a measure of the tissue availability of the vitamin.9 Accordingly, the problem of inadvertent cobalamin injection immediately prior to venipuncture (estimated 2.5%)9 would not negate the MMA assay as it would the serum radioassay.

Three patients previously discussed with levels of 38, 2000, and 2900 μg MMA/ml urine were not included in Table 2. Patients 12, 17, and 18 had high serum folate (Table 3), indicating folic acid supplements may mask anemia manifestations. Patients 3, 6, 16, 20, and 21 were discharged in a wheelchair, patients 15 and 27 were mentally incapacitated, and patient 22 expired in the hospital.

The mass spectrometric procedure used for MMA quantitation has proven to be rapid, sensitive, specific, and reliable. This routine clinical test has been highly beneficial to physicians in the detection of cobalamin deficiency and in the exclusion of cobalamin deficiency in complex clinical presentations. It provides a means for the wide-scale, noninvasive screening of patients for cobalamin deficiency, allowing the prevention of permanent neurologic disability through the early detection of pernicious anemia. MMA levels are a good indication of cobalamin distribution and function, since they are directly related to a cobalamin-dependent metabolic pathway.

ACKNOWLEDGMENTS

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REFERENCES

Cobalamin (vitamin B12) deficiency detection by urinary methylmalonic acid quantitation

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