The Value of the Serum Vitamin B₁₂ Level in Diagnosing B₁₂ Deficiency

By H. Irving Pierce and Robert S. Hillman

Serum vitamin B₁₂ levels of 2523 individuals were measured using the hemoglobin-coated charcoal assay. In one hospital, the test was performed on 1698 patients under hematologic evaluation for erythrocytic abnormalities of all types, while 825 patients from five other hospitals were studied because of suspected B₁₂ deficiency. The incidence of low serum vitamin B₁₂ was surprisingly low for both groups (2.3% and 3.5%, respectively). The value of the test as a diagnostic tool was further reduced by two characteristics of physician performance. First, with those patients who demonstrated a clear-cut macrocytic anemia and were suspects for intrinsic factor deficiency (pernicious anemia), extensive evaluations for B₁₂ malabsorption were usually carried out prior to the receipt of the low B₁₂ level. Second, when a deficiency state was not suspected, the return of a low value did not reliably stimulate physicians to begin evaluation or institute therapy. This was true despite the presence of clinical evidence of a possible gastrointestinal abnormality in many of the patients.

Measurement of the serum vitamin B₁₂ level has gained wide acceptance as a valuable tool in the diagnosis of vitamin B₁₂ deficiency states. While several methods are available, the most popular at present is the hemoglobin-coated charcoal radioassay of Lau et al. In fact, the introduction of this reliable technique in 1965 has encouraged an even wider usage of serum vitamin B₁₂ levels in diagnosis; most commercial laboratories and major medical centers now offer the test on a routine basis.

In view of the popularity and ready availability of the serum vitamin B₁₂ level, it is of some interest to analyze its actual value as a diagnostic tool for the identification of vitamin B₁₂ deficiency in patients under evaluation for a hematologic abnormality. To this purpose, we have undertaken a retrospective study of the circumstances surrounding a large number of vitamin B₁₂ measurements carried out in a centralized laboratory serving both the University of Washington School of Medicine affiliated hospitals and a number of private hospitals in Seattle, Wash. The results provide an insight into the value of the technique according to the characteristics of the several hospitals, the specialty interests of the staff physicians, and the disease characteristics of various population groups.

Materials and Methods

Over a period of 3½ yr, the hematology laboratory of Harborview Medical Center measured the serum vitamin B₁₂ levels of 2523 individuals who were under medical evaluation at any one of
six Seattle area hospitals and their associated outpatient clinics or private physician’s offices. For the purpose of analysis, the patients were divided into two major categories: Group A, consisting of 1698 patients, admitted to or evaluated at Harborview Medical Center, and Group B, consisting of 825 patients admitted to the University Hospital (357 patients), VA Hospital (390 patients), USPHS Hospital (49 patients), and two private hospitals, Swedish Hospital and Providence Hospital (29 patients). The patients in Group A came from a lower socioeconomic population group and often were admitted for the medical complications of alcoholism. Serum vitamin B₁₂ levels were measured in all patients referred by the house staff to the hematology service for evaluation of an erythrocytic hematologic abnormality such as anemia, abnormal mean cell constants (Wintrobe Indices), abnormal morphology on peripheral smear (including increased lobation of granulocytes), or an elevated reticulocyte index. In contrast, Group B individuals were admitted to hospitals serving middle class economic groups, patient populations where alcoholism was of lower incidence. Vitamin B₁₂ levels from these hospitals were drawn at the request of either an internist or a specialist hematologist after an initial hematologic work-up indicated possible vitamin B₁₂ deficiency.

All vitamin B₁₂ levels were determined by the hemoglobin-coated charcoal radioassay method of Lau et al., as modified by Hillman et al. Determinations were controlled over the 3-year period by simultaneously measuring a serum standard curve. All values below 200 μg/ml were repeated at least twice prior to accepting the low value for this report. However, the finding of a low vitamin B₁₂ level was reported to the care physician within 4-14 days after submission of the specimen. Additional hematologic tests were performed in the individual hospital laboratory, except for serum folic acid levels, which were determined in the same central laboratory. It was considered common knowledge by all physicians ordering the vitamin B₁₂ measurement that patients suspected of having vitamin B₁₂ deficiency or demonstrating B₁₂ values below 200 μg/ml should have a full hematologic evaluation including mean cell constants (Wintrobe Indices), bone marrow aspiration, reticulocyte count, repeat serum vitamin B₁₂ level, serum folic acid level, estimation of gastric acidity by either the Diagnex blue test or intubation with measurement of free acid after histamine stimulation, and/or a Schilling test with and without intrinsic factor. In addition, it was considered common knowledge that an abnormal Schilling test with intrinsic factor should be followed by further evaluation of small bowel function, looking for a specific malabsorption disorder. While the number of tests and the several institutions involved would seem to make it difficult to attain accurate agreement for all measurements, it should be appreciated that the above techniques were well standardized throughout the Seattle area by virtue of close cooperation between each hospital laboratory, the practicing hematologists, and the School of Medicine.

A careful study of the hematologic work-up, including review of case records, blood and bone marrow morphology, and tissue specimen histology, was carried out on those patients with vitamin B₁₂ levels below 200 μg/ml. Special attention was directed towards (1) the type and incidence of the red cell abnormality, (2) the cause of the vitamin B₁₂ deficiency state, (3) whether the vitamin B₁₂ deficiency and/or intestinal malabsorption was initially suspected and confirmatory studies initiated prior to the receipt of the vitamin B₁₂ level, and (4) whether the return of a low vitamin B₁₂ level altered the evaluation or treatment regimen. Items 3 and 4 were based on the authors’ evaluation of the physician’s apparent recognition of suspected or confirmed B₁₂ deficiency by way of notations in the hospital chart or outpatient medical record.

*Harborview Medical Center (Group A) serves the Seattle area in general, but draws most heavily on the lower-economic areas of the city. Between 1968 and 1972, yearly admissions ranged between 10,500 and 8500; 60% were female; mean patient age ranged between 40 and 50 yr, and racial distribution was 86% white, 13% black, and 1% Oriental. During the 1970-1972 period both the Obstetrics and Pediatric services were relocated, limiting the inpatient population to adults with major, acute medical and surgical illness.

*Other than the obvious sexual preference of the VA Hospital, Group B hospitals draw equally on more middle income populations according to the racial distribution of the Seattle area—87% white, 7% black, and 5% Oriental. While 5.6% of the white population in Seattle is of Scandinavian descent (national frequency, 0.86%), this group received most of their medical care from other hospitals and clinics more convenient to their neighborhood areas.
or his subsequent evaluation and choice of therapy over a period of several months. The lack of any such notation or action was taken as an indication that nothing further was done about the result.

RESULTS

During the measurement of serum vitamin B\textsubscript{12} levels from 2523 patients admitted to six different hospitals, 68 individuals were found to have values below 200 \( \mu \text{g/ml} \), an incidence of 2.9\%. The 68 patients included 39 males and 29 females, ranging in age from 18 to 93 yr (mean 57.8 yr). As shown in Table 1, 39 of the abnormal patients were from Group A, while 29 came from Group B hospitals. The respective frequencies of values below 200 \( \mu \text{g/ml} \) was, therefore, 2.3\% and 3.5\%, a difference which was not considered significant \((p > 0.05)\). As for values below 100 \( \mu \text{g/ml} \), three individuals were in Group A (0.18\%), while eight occurred in Group B (0.9\%).

Detailed review of the hematologic evaluation of these 68 patients revealed a definite red cell abnormality in all but nine (five of these did not have a hematologic evaluation). A breakdown of the type and frequency of observed abnormalities based on a careful review of all historical and laboratory data and peripheral smear and bone marrow morphology is shown in Table 2. The majority of patients demonstrated anemias unrelated to B\textsubscript{12} deficiency; in only 17 patients was the deficiency of vitamin B\textsubscript{12} clearly responsible for the erythrocytic abnormality. This included 13 patients with intrinsic factor deficiency (i.e., pernicious anemia) and four individuals with small intestinal disease. On the other hand, when medical records were analyzed for potential causes of vitamin B\textsubscript{12} deficiency, a total of 15 patients (eight from Group A and seven

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<th>Table 1. Incidence of Vitamin B\textsubscript{12} Deficiency</th>
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<td>Intrinsic factor deficiency with anemia (pernicious anemia)</td>
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<th>Table 2. Type of Hematologic Abnormality (59 patients) *</th>
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<td>Hypoproliferative anemia</td>
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<td>Iron and folate deficiency</td>
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<td>Acute blood loss</td>
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*Classification according to the criteria described by Hillman and Finch.\(^4\)
Group B) were felt to have a deficiency of intrinsic factor on the basis of either an abnormal Schilling test which corrected with exogenous intrinsic factor (13 patients), severe gastric atrophy on endoscopy (one patient), and/or histamine-fast achlorhydria (one patient) (Table 3). Another 15 patients, three of whom were from Group B, demonstrated an abnormality in gastric or small bowel anatomy, and, finally, 14 patients were greater than 69 yr of age (range 69–93, mean 80.0 yr) and might be expected to show some degree of gastric atrophy and decreased intrinsic factor secretion, although none were specifically evaluated for the presence of achlorhydria. No cause was apparent in 24 patients.

At the same time, the range and mean serum B₁₂ values were not considerably different for any of the categories, including the patients without an apparent etiology.

Case records were analyzed as to the relative importance of the vitamin B₁₂ level in the physician’s approach to the diagnosis of a suspected B₁₂ deficiency state. The extent of the evaluation in those individuals suspected of having classical pernicious anemia as compared to the remainder of the patients is shown in Table 4. Eleven of the 13 pernicious anemia patients had additional
studies performed, including bone marrow aspirate, serum folate assay, Schilling test, and a test for gastric acidity, within the first 2-3 days of admission (Table 4). In virtually all instances, these additional studies yielded abnormal results, and therapy was given almost immediately, thus tending to negate the diagnostic usefulness of the serum B₁₂ level.

In contrast, the remaining 55 individuals not suspected of having pernicious anemia had fewer diagnostic tests performed. While marrow aspirates and folate levels were obtained in about 60% of cases, studies of B₁₂ absorption were performed in less than 20% of the patients. Moreover, the receipt of a low vitamin B₁₂ level had little effect toward stimulating further evaluation or a trial of vitamin B₁₂ therapy. In only 11 individuals was vitamin B₁₂ prescribed alone or in combination with folic acid.

DISCUSSION

A retrospective study of the results of the serum vitamin B₁₂ levels from 2523 patients from six different hospitals provided a number of insights into the apparent value of this measurement in diagnosing vitamin B₁₂ deficiency states. First, regardless of whether the measurement was performed on all patients under evaluation for a hematologic abnormality (Group A) or specifically because of a physician’s suspicion of vitamin B₁₂ deficiency (Group B), the incidence of deficient values was extremely low. Only 11 of 2523 individuals (an incidence of 0.4%) were noted to have values below 100 μg/ml, the value considered diagnostic for vitamin B₁₂ deficiency, while an additional 57 patients had values between 100 and 200 μg/ml (maximum frequency of values < 200 μg/ml was 3.5% in Group B). This frequency is very much lower than that found in a study of a South Wales population, where 33 of 673 individuals (an incidence of 4.9%) were found to have levels below 100 μg/ml, and the incidence of 9.8% reported by Gaffney et al. in a study of 528 individuals, aged 12–94 yr. Furthermore, when the medical records of the 68 patients with vitamin B₁₂ levels below 200 μg/ml were carefully reviewed, only 17 patients (25%) demonstrated a hematologic abnormality directly related to the B₁₂ deficiency state. Thirteen of these patients had classical pernicious anemia, while four individuals had a small bowel defect causing B₁₂ malabsorption. This low incidence of pernicious anemia was a surprise, especially in the face of the large Scandinavian population of the greater Seattle area (5.6% as compared to a national average of 0.86%). It may reflect several characteristics of patient care in Seattle, including the tendency of the Scandinavian population to use other hospitals and variations in physician performance.

It was also of interest that the 13 patients demonstrating intrinsic factor deficiency (pernicious anemia) showed vitamin B₁₂ levels ranging from 65 to 200 μg/ml. This distribution of levels is considerably higher than that previously reported by a number of investigators who measured vitamin B₁₂ levels in patients with pernicious anemia with Euglena gracilis or Lactobacillus leichmannii (Table 5). This difference may reflect innate differences in assay systems; normal values for the radioassay system have been defined as 200–900 μg/ml, and those below 100 μg/ml clearly diagnostic of B₁₂ deficiency.

The ultimate value of the vitamin B₁₂ level in identifying B₁₂ deficiency is largely determined by the diagnostic approach of the physicians. A tendency for
physicians not to rely upon the B₁₂ measurement as the sole indicator of clinically significant B₁₂ deficiency was apparent. Physicians used other measurements of vitamin B₁₂ malabsorption to diagnose a deficiency state. This was especially true for those patients demonstrating a hematologic abnormality characteristic of pernicious anemia (macrocytic anemia in an elderly patient of Scandinavian descent); virtually all such individuals were evaluated in detail with marrow studies, B₁₂ malabsorption studies, and a therapeutic trial with the vitamin prior to the return of the vitamin B₁₂ level. At the same time, little attention was paid to the return of an unexpectedly low vitamin B₁₂ level in patients not demonstrating a typical macrocytic anemia. In the 51 patients who were not immediately suspected of having pernicious anemia, studies of vitamin B₁₂ malabsorption were only infrequently performed (Table 4). This was true despite evidence of gastric or small bowel defects in 15 and advanced age in another 14 patients. It would imply a judgment on the part of physicians to discount the vitamin B₁₂ laboratory value in lieu of their clinical appraisal. This fact tended to further diminish the value of the B₁₂ level as a detector of vitamin B₁₂ deficiency.

It may be concluded that merely making vitamin B₁₂ levels available on a routine basis will not necessarily result in the most effective use of the test in the diagnosis of B₁₂ deficiency. Certainly, as a mandatory screening test or routine test in all patients with a hematologic abnormality, the incidence of deficient values may be far too low to make it economically feasible. Even when ordered by trained internists because of suspected B₁₂ deficiency, the rate of success can be very low. The value of the method tends to be further reduced by the performance characteristics of the physicians. Not only were detailed studies of B₁₂ malabsorption and vitamin B₁₂ therapy initiated prior to the receipt of the serum vitamin B₁₂ level in most patients suspected of having classic pernicious anemia, but also unexpected low values were largely ignored in terms of stimulating further evaluation or therapy.

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