A Right Royal Porphyria Fallacy

To the Editor:

We were intrigued by the cover image for the November 2011 issue of Clinical Chemistry, which showed a cover picture claiming that Mary Queen of Scots had a diagnosis of acute porphyria. The diagnosis of porphyria is usually assigned when a presenting patient is currently symptomatic; the range of indicative symptoms is diverse but relatively well defined. The 3 most common acute porphyrias are hereditary coproporphyria, variegate porphyria, and acute intermittent porphyria, all inherited as autosomal dominant disorders. Many individuals who inherit the enzyme abnormalities remain asymptomatic through out life. Abdominal pain is the most common symptom of an acute attack, although nausea, vomiting, constipation, neuropathies, and psychiatric symptoms may also accompany the abdominal pain. Hormonal changes, drugs, and nutritional factors may precipitate or aggravate the disorder. The definitive diagnosis relies on a combination of biochemical analyses to define the type of porphyria, followed by molecular analysis that enables screening of potentially affected family members. Occasionally, clinicians encounter patients with vague symptoms and an uncertain family history of porphyria, for which testing of the index case may not be possible. In this scenario of a potentially latent porphyria, results of all screening tests may well be normal and molecular testing is not justified. Many clinicians will adopt a “wait and see” approach, collecting samples at the time of an acute attack if one eventuates.

Owing to the complexity of the porphyrias, a retrospective diagnosis based on clinical observation alone is fraught with problems and would seldom be accepted. Recent research has thrown doubt on the assumption based on historical observation that the English Royals, notably King George III and Mary Queen of Scots, had one of the porphyrias. The array of symptoms attributed to King George III have been presented as meeting the WHO International Classification of Diseases, 10th Revision, criteria for type I bipolar affective disorder with mood-congruent delusions, consistent with previous reports of manic depressive psychosis and with subsequent Alzheimer-type dementia, rather than acute porphyria (1,2). Evidence generated by the computerized diagnostic aid SimulConsult has also shown that King James VI of Scotland and I of England (the son of Mary Queen of Scots) had features strongly suggestive of attenuated Lesch-Nyhan disease and an autistic spectrum disorder, possibly Asperger syndrome (3). Furthermore, a review in the Journal of Clinical Pathology (4) suggests that neither King George nor his relatives had porphyria, a conclusion based on rarity, penetrance, symptoms, and natural history.

Although all of these reports come from a single group, we believe that the evidence underpinning the assertion that Mary Queen of Scots had porphyria is not robust and potentially perpetuates a myth.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures or Potential Conflicts of Interest: No authors declared any potential conflicts of interest.

References
continue to limit translation of the holoTC concept, despite its theoretical attractiveness.

Validation efforts have typically compared holoTC against serum B₁₂ in general populations and have used imperfect tools with often-arbitrary cutpoints to define B₁₂ status. Such approaches and their reliance on methylmalonic acid (MMA), despite its questionable diagnostic specificity, have been critiqued (2). Valente et al. reuse this model with just 1 modification, the substitution of erythrocyte vitamin B₁₂ (RBC-B₁₂) for MMA as the arbiter of B₁₂ status. That innovation misfires, however, because RBC-B₁₂ is probably an even poorer standard than MMA. No convincing data anywhere, including the references cited by Valente and colleagues, justify the report’s assumption that RBC-B₁₂ represents tissue B₁₂ status. Very little is known about what RBC-B₁₂ concentrations really mean. The measurement of RBC-B₁₂ has been deemed insufficiently informative (3), has rarely been used diagnostically, and has generated remarkably limited published data [cited in (1, 3)].

Yet, even the sparse RBC-B₁₂ literature shows reduced RBC-B₁₂ values, not only in B₁₂ deficiency but also in untreated iron deficiency, untreated folate deficiency, pregnancy, polycythemia, and other conditions. Valente et al. cite a few such reports but largely ignore the confounding issues. They mistakenly downplay nonspecificity on the tautological grounds that holoTC predicted 44% of RBC-B₁₂ variation (high-probability confounders, such as iron status, were not tested). Although acknowledging the large contribution that B₁₂-rich reticulocytes make to RBC-B₁₂ values, Valente et al. do not consider the equal likelihood that RBC-B₁₂ probably diminishes if reticulocyte counts are low for any reason whatsoever. Even if incomplete and unexplained (and setting aside the disparities between previous RBC-B₁₂ values and the values of Valente et al.), the broad roster of potential confounders mandates skepticism about the reliability of RBC-B₁₂ as a diagnostic standard for B₁₂ deficiency.

Indeed, compromised iron status almost certainly explains the significantly lower mean corpuscular volume that accompanied low RBC-B₁₂ values in the study of Valente et al. and that puzzled the authors. Unrecognized iron depletion, not B₁₂ deficiency, probably accounted for many of the RBC-B₁₂ abnormalities.

Until B₁₂ deficiency and its diagnostic standards can be defined properly, a moratorium seems advisable on surveys comparing holoTC and B₁₂, which have produced consistently marginal differences (2) or flawed data. Fresh approaches should redirect attention to the crucial but insufficiently studied unknowns surrounding the interpretation of holoTC (4). Does holoTC measure B₁₂ metabolism, absorption, or both? What conditions unrelated to B₁₂ influence holoTC? (Many conditions have been suggested, but few have been studied.) Is an isolated holoTC abnormality truly the earliest marker of B₁₂ deficiency, or can it mislead? (Transiently diminished B₁₂ intake or medication-induced malabsorption, for example, might reduce holoTC without ever creating deficiency.) Do isolated holoTC abnormalities usually progress, or do they spontaneously reverse?

The thrust of most holoTC studies is mismatched with the diagnostic primacy that Valente et al. advocate. Their “first-line procedure” recommendation aims at clinicians and clinical laboratories, but validation research has confined itself to nonclinical populations in which B₁₂ deficiency was infrequent, vaguely defined, and usually nonprogressive and subclinical (2). Vitamin B₁₂ deficiency is frequently misperceived as a single, progressive continuum, a perception that blurs the many substantive differences between subclinical deficiency (isolated, minimal biochemical abnormalities of variable permanence in persons without signs or symptoms) and clinical deficiency (typically malabsorptive, progressive, and symptomatic, as in pernicious anemia) (2). Populationwide data often have little relevance to patients: few studies have identified clinically important subsets of individuals; provided useful hematologic, clinical, or prognostic information; assessed absorption status; or evaluated clinical response. Additionally, individual discrepancies (in either direction) between holoTC and B₁₂ results, astonishingly, have rarely been analyzed by investigators for performance insights.

Without answers to the many important, unaddressed questions, holoTC remains an inadequately explored enigma, and assigning its primacy in clinical diagnosis is premature. If better diagnostic yardsticks than MMA and RBC-B₁₂ will confirm that holoTC outperforms B₁₂ chiefly in very early subclinical B₁₂ deficiency, holoTC could become a valuable tool for needed research on early B₁₂ deficiency.

The diagnostic advantages of holoTC appear minor, however, in the clinical setting, where concerns revolve around illness and health, not subclinical deficiency. It bears reemphasis that current holoTC validation data apply only to subclinical B₁₂ deficiency. They do not apply to the tiny, ignored, but crucial subset with clinical deficiency, for which B₁₂ measurement, although an imperfect marker, offers a diagnostic sensitivity of 95%–97%, compared with only 38%–39% for subclinical deficiency (2). The earliest possible detection of...
subclinical deficiency is not a clinical imperative. Indeed, isolated biochemical changes progress only infrequently (5). The risk of overt-dx is presently an undesirable trade-off, because neither the health risks caused by subclinical deficiency nor the health benefits of treating it with B₁₂ have yet been proved (2).

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures or Potential Conflicts of Interest: No authors declared any potential conflicts of interest.

References

Ralph Carmel²,³

² Department of Medicine
New York Methodist Hospital
Brooklyn, NY
³ Department of Medicine
Weill Cornell Medical College
New York, NY

*Address correspondence to the author at:
Department of Medicine

In Reply

Requests for the determination of vitamin B₁₂ (B₁₂) status remain a clinical reality but current laboratory methods for this have limitations (1). In recent years, measurement of holotranscobalamin (holoTC), the fraction of B₁₂ that can enter cells, has been proposed as an improvement, and attempts to demonstrate this improvement have used increased serum methylmalonic acid (MMA) and/or total homocysteine (tHcy) as indicators of deficiency of B₁₂. Both MMA and tHcy may be compromised by renal function and other factors, particularly in an elderly population. The clinical utility and analytical aspects of holoTC have recently been reviewed (2) and current evidence merits continued study of the marker, both for diagnosis of B₁₂ deficiency and assessment of vitamin B₁₂ status.

We investigated indicators of B₁₂ status in an elderly population and used low red cell B₁₂ concentrations (RBC-B₁₂) as a biologically different standard of deficiency. Carmel makes several points regarding the shortcomings of B₁₂ status assays and we agree that these extend to RBC-B₁₂. We do not suggest RBC-B₁₂ has a routine role. However, we observed correlations between RBC-B₁₂ and the B₁₂ markers that were as biologically expected. Our primary analysis was based on ROC plot and stepwise multiple linear regression analyses, which do not depend on selecting cutoffs for the dependent markers. ROC plot analysis demonstrated a significant difference in the area under the curve for holoTC (0.9) compared to MMA (0.78) and serum total B₁₂ (0.8). The same significant hierarchy remained whether we selected RBC-B₁₂ concentrations at the lower or higher 95% CI limits of the reference value used. We also found that with the use of RBC-B₁₂ the positive predictive values of total B₁₂ for deficiency were similar to those reported by Matchar et al. (3), who used clinical diagnosis.

Carmel raised the question of iron status confounding the results. Our hemoglobin data indicated that the prevalence of anemia was high in both the RBC-B₁₂ deficient and nondeficient populations (58% and 42%, respectively), which indeed suggests that many individuals probably had iron deficiency. As Carmel notes, our stepwise multiple regression analysis indicated that 44% of the variation in RBC-B₁₂ concentrations was attributable to holoTC. In this analysis, hemoglobin was not a significant factor, suggesting that other causes of anemia, such as iron status, had much less influence than, for example, holoTC.

Concerning the use or misuse of such assays, Carmel (4) gives an excellent account of the diagnosis, treatment, and management of patients with suspected clinical B₁₂ deficiency. He is very cautious with respect to what to do with what he calls subclinical deficiency (5). He concludes that even in elderly patients, until well-designed clinical trials are completed (a goal that we think is problematic) supplementation has unknown value and specific intervention is not warranted (4). He suggests treating low B₁₂ accompanied by increased MMA or tHcy by injection, which complicates ruling in or out dietary deficiency. This approach would leave follow-up of such patients open.