Editorial

Clinical application of serum vitamin B₁₂ assay

The resolution of megaloblastic anaemias is haematological bread and butter. Since Cooper and Whitehead¹ documented their disconcerting findings with serum vitamin B₁₂ levels yielded by radioisotope dilution assay (RIDA) kits, many haematologists may have sensed a crisis of confidence, amplified by similar personal experiences. Though we aspire to work with sufficient circumspection not to be thrown off course by a single erroneous result, it is not so much the ability to identify a state of vitamin B₁₂ depletion that appears to have been lost as the grounds for its exclusion from clinical suspicion.

There is some debate about the incidence of false normal serum B₁₂ results by RIDA, but few critics would claim a figure as high as the 20% observed by Cooper and Whitehead. Nonetheless a disparity is noted, not only between proprietary kit systems and reference microbiological assay methods but between classical RIDA and microbiological assay methods too. These are not just trend differences, which can be accommodated for clinical purposes by modulation of the reference ranges, but sporadic disparities from otherwise reassuring co-ordinates.

Ubiquitous phenomena have been invoked to explain the disparities. Kolhouse et al.² submitted evidence that cobalamin analogues present in plasma, which do not support the growth of Euglena gracilis or Lactobacillus leichmannii, featured as true cobalamins in some RIDA in which non-intrinsic factor ‘R-binder’ constituted a significant proportion of the B₁₂ binding ingredient. However, from the evidence of their paper, cobalamin analogues are found in virtually all sera and account for about 40% of the total detectable cobalamin. Though disparities had been observed³ these were attributed to the serum extraction techniques.⁴

Misleading disparities are observed not only using RIDA kits. In our own experience using a classical RIDA method, we are aware of delayed diagnosis in a few subjects from whom further samples also yielded normal results. Dr Sourial’s study⁵ does not identify cobalamin analogues by Escherichia coli. Hence if they are present in serum, E. coli cannot exploit them for growth. Furthermore, Zacharakis et al.⁶ report concordance in their RIDAs, comparing intrinsic factor and ‘R-binders’. Thus a satisfactory explanation for the observations of Cooper and Whitehead (and for the somewhat less frequent diagnostic difficulties encountered by others) remains to be found.

Meanwhile, however, it is necessary to adopt a practical, clinically efficient, and realistic approach to the management of subjects with macrocytic features in the peripheral blood. Bone-marrow examination has been the Lynch-pin in the work-up of macrocytic anaemias. Is it an inescapable and immediate clinical obligation? Only if there is reason to believe that the sample will not be megaloblastic, namely, myelodysplastic or hypoplastic. If the informed assumption is that the marrow is megaloblastic, then the results of the assays should in due course identify the nutritional cause. If the results of the assays do not represent depletion then marrow aspiration may be the next step, unless excessive alcohol consumption can be confidently implicated. Such departures from more doctrinaire procedures will include upwards of 80% of macrocytic subjects. In the others, by virtue of severity either of symptoms and signs or of the haematological disorder, a bone-marrow examination follows the blood count without further ado.

The current convention seems a consequence of historical habit. Before the Coulter counter and similar instruments yielded such reliable values for the mean cell volume, it must be recalled that haematologists agonised over peripheral blood films in which gross anisocytosis did not offset the macrocytes: was it or was it not macrocytic? The suspicion lingered, and a marrow sample was taken in the hope of clinching the matter. The aspirate was required by virtue of the greater uncertainty in which conclusions were reached. Likewise with the marrow there remain conditions in which the most widely acclaimed afficionados still cannot make up their minds.

It is for just such circumstances that the deoxyuridine suppression test receives its strongest advocacy,⁷ reputedly providing a quantitative calibration of the megaloblastic defect and a functional index of B₁₂ or folate depletion. It remains, however, corroborative rather than a first-line diagnostic procedure, and its

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use has so far been fundamentally academic. Perhaps there are grounds for its wider application.

The modulation of diagnostic strategy places an even greater reliance upon the authenticity of assay results. Nonetheless the possibility that a result is incorrect or misleading, for whatever reason, is inescapable. With respect to B12 results, it would be remarkable if two such different assay systems as microbiological assay and RIDA were not responsible for occasional disparities by virtue of unrelated artefacts. Which estimation provides the true value? Is it always microbiological assay? Is microbiological assay always correct, for, if it is, there seems no good reason to use or to develop RIDA at all. When serum B12 levels have been measured blind by microbiological assay and by RIDA, in samples known to be depleted, without historical privilege or reference status accorded to microbiological assay, we shall see whether, on grounds of reliability alone, there is anything to choose.

Meanwhile the context provides the most exacting quality control, without which the test and the result are a ritual gesture rather than a medically purposeful enquiry.

References
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