Folate assays: serum or red cell?

ABSTRACT – Tests for folate and vitamin B_{12} deficiency are frequently requested by clinicians in many different specialties. An audit of folate assay methodology was undertaken to establish the number of tests and types of assay performed in different centres, and to analyse the indications for these investigations, with a view to advising on the most appropriate assay for use in the laboratory. A questionnaire was sent to 30 centres, 24 (80%) of which participated in the audit. The types of folate assay performed, number of requests, reference range and method of analysis differed between centres. The major specialty users of the service were general practitioners, general physicians and geriatricians. A detailed analysis of 1,259 consecutive requests for folate assays from a single representative laboratory showed a significant correlation between serum and red cell folate levels ($r = 0.49$, $p < 0.001$). However, in patients with low serum folate, there was no correlation with red cell folate in the absence of macrocytosis. The major indication for folate analysis was for haematological abnormalities but 36% of cases were for nonspecific indications. A haematologist with an interest in folate metabolism was invited to moderate the results at an audit meeting of haematologists. The consensus was that the most appropriate screening test for folate deficiency is the serum assay, which can be combined easily with vitamin B_{12} assay.

Folate and vitamin B_{12} assays constitute a major component of both district and teaching hospital haematology laboratory workloads. Whilst many of the assays are automated they are costly and time-consuming. Many tests are requested as part of a screening programme in the investigation of patients with a variety of medical conditions. It is important that both false positive and false negative results be minimised by an appropriate choice of investigations and to have adequate quality controls. Several authors have suggested that there is no advantage in assaying red cell folate (RCF) over serum folate (SF)[1–4]. In addition, haematologists in South Thames Region who regularly participate in quality assurance programmes agree that RCF assays as currently performed give more variable results than the SF assay, even though the same test reagents are used. We therefore reviewed folate assays in South Thames Region to determine which method to use in patients suspected of having folate deficiency. This was done as a prospective study of 1,259 requests for folate assays to a single laboratory site performing both SF and RCF assays over a 6-week period. No patients were excluded from the analysis.

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Methods

A questionnaire was designed by the audit coordinator in conjunction with a lead physician to seek information on folate assay techniques in South Thames Region. In addition, each centre was requested to supply details of its reference range, quality control procedures, and to identify user specialties as main, moderate, minor or insignificant. The questionnaire was posted to all senior Medical Laboratory Scientific Officers (MLSOs) in the 30 district or teaching hospitals in South Thames Region. Response was requested by a specific date and the MLSOs were telephoned if questionnaires were not returned. After coding, the data were analysed using a desktop digital computer running commercial statistical software. A representative centre (a large district general hospital) using both SF and RCF assays was identified from which detailed information was collected prospectively. Consecutive requests for either RCF or SF analysis received by this haematology laboratory were studied over a 6-week period. Information on the following variables was abstracted from the computerised laboratory system: sex, date of birth, clinical indication for test, haemoglobin (Hb), mean cell volume (MCV), RCF, SF and serum vitamin B_{12} level. Statistical analysis was by linear regression.

In light of the findings, one of us (AVH) was invited as moderator to discuss the results and to draw up guidelines at the South Thames Regional Haematology Specialist Committee audit meeting (January 1995).

Results

The questionnaire was completed by 24 (80%) of the 30 centres. Of the 24 laboratories 10 (42%) used only SF assay for evaluating folate status, 11 (45%) measured only RCF, and 3 (12.5%) used both assays. The 24 laboratories performed a total of 6,673 folate assays (mean 278, range 5–1,000) per month: 2,685 (40%) were SF assays (range <5–1,000 tests per month) and 3,988 (60%) were RCF assays (range

Table 1. Users and extent of usage of folate assay tests in the 24 centres.

	Main user	Moderate user	Minor user	Non or insignificant user	Not recorded
Dermatologists	1	2	6	12	3
ENT sugeons	–	2	2	17	3
Gastroenterologists	6	8	1	4	5
General physicians	10	11	–	–	3
General practitioners	13	7	2	–	2
General surgeons	1	3	14	3	3
Geriatricians	8	12	1	–	3
Haematologists	7	8	6	–	3
Neurologists	7	6	3	3	5
Obstetricians & gynaecologists	–	2	9	10	3
Ophthalmologists	–	–	8	12	4
Paediatricians	–	2	15	4	3
Psychiatrists	2	6	8	4	4
Urologists	–	–	4	15	5
Other*	–	4	1	–	–

*Biochemistry, dental/maxillofacial unit, oral surgeons, rheumatology.

10–820 per month). In addition they performed 5,862 B_{12} assays (60–1,000 per month), and 15 (63%) centres used a combined B_{12} and folate assay technique. The reference ranges varied greatly between laboratories. The lower limit for B_{12} ranged from 130–200 ng/l and the upper limit from 710–2,000 ng/l. The lower limit for SF varied from 1.5–4.2 µg/l and the upper limit from 7.6–20.6 µg/l and for RCF the lower limit varied from 95–180 µg/l and the upper limit from 500–860 µg/l depending on the technique used. Reference ranges were derived from manufacturer data, 'in house' normal samples or published population based data. All laboratories subscribed to a local manufacturer or an external quality assurance scheme. The main user groups of the service within the region are listed in Table 1.

The method of analysis used by the representative centre's laboratory for folate assay was the Becton Dickinson SNB radioimmuno isotope test which uses a 1 : 20 dilution with ascorbic acid to lyse red cells. Their normal reference ranges were: RCF 160–640 µg/l; SF 3.0–7.6 µg/l; MCV 80–100 fl; Hb 115–145 g/l (female), 125–150 g/l (male). A total of 1,259 consecutive requests for a folate assay at the representative district centre was prospectively analysed. The mean age of the 1,259 patients was 61 years (range 1–98); 428 were male and 827 female (ratio 1 : 2); the sex of four patients was unrecorded. Both SF and RCF were measured in 797 (63%) patients. There was a highly significant correlation between SF and RCF levels (r = 0.49, $p < 0.001$; Fig 1). Of the 797 patients, 159 (20%) had a low folate level; 79 (10%) of them had a SF <3 µg/l and 80 (10%) a RCF <160µg/l; only 20 (2.5%) samples were abnormal by both assays. In patients with a low SF, no correlation was found with RCF level (r = 0.16, p = 0.154) but in patients with a low RCF there was significant correlation with SF level (r = 0.26, p = 0.018). There was a significant correlation between SF and RCF in patients with macrocytosis as defined by MCV > 100 fl (n = 46, r = 0.48, p = 0.001; Fig 2), and it was even higher in patients with macrocytic anaemia (Hb < 110 g/l) (n = 205, r = 0.66, $p < 0.001$; Fig 3).

The 1,259 folate assays were requested for a wide variety of clinical indications. They fell into three main groups: haematological 415 (33%), neurological 126 (10%) and gastrointestinal 88 (7%). For 176 (14%) no reason was given for the request, but the largest group of requests (36%) was for non-specific indications ranging from atrial fibrillation, 'deterioration' and fever, to foot pain, polyuria, vaginal bleeding, routine screening and urticaria.

Discussion

Folate is an essential cofactor in DNA synthesis. Deficiency results in a megaloblastic anaemia indistinguishable from that produced by vitamin B_{12} deficiency, another cofactor in the conversion of N-methyltetrahydrofolate into tetrahydrofolate. In addition to the haematological abnormalities there may be other nonspecific clinical features such as stomatitis and glossitis and, more recently, neural tube defects have been described in children born to folate deficient mothers[5]. Folate levels can be measured in both serum and red cells. The audit identified a variation in practice amongst haematologists, with 10 (42%) of the 24 respondents performing SF assay alone and a further 3 (12.5%) performing both assays.

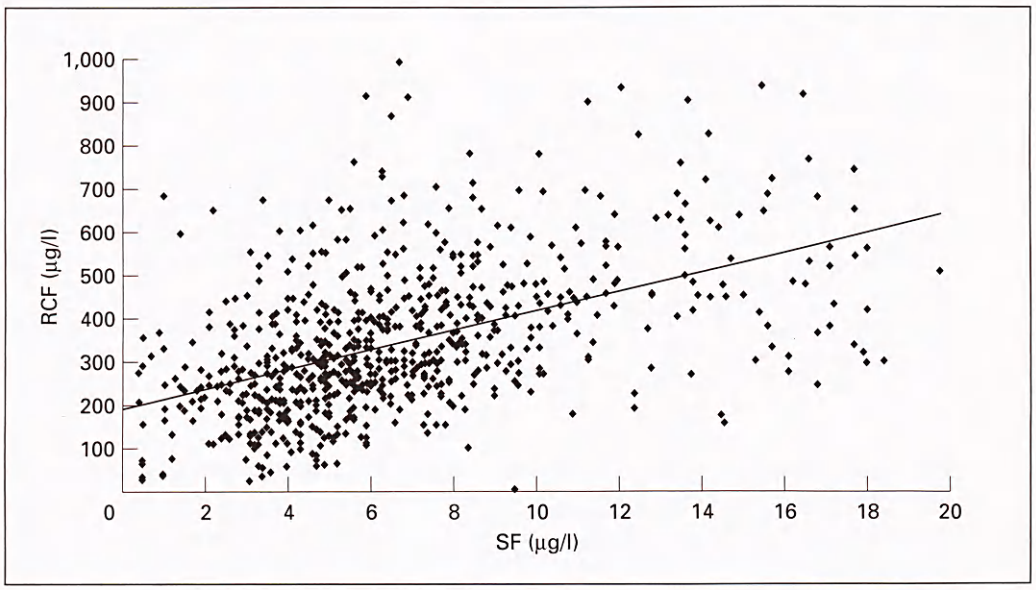

Fig 1. Correlation between serum folate and red cell folate levels in patients for whom both were assayed; the graph excludes outliers.

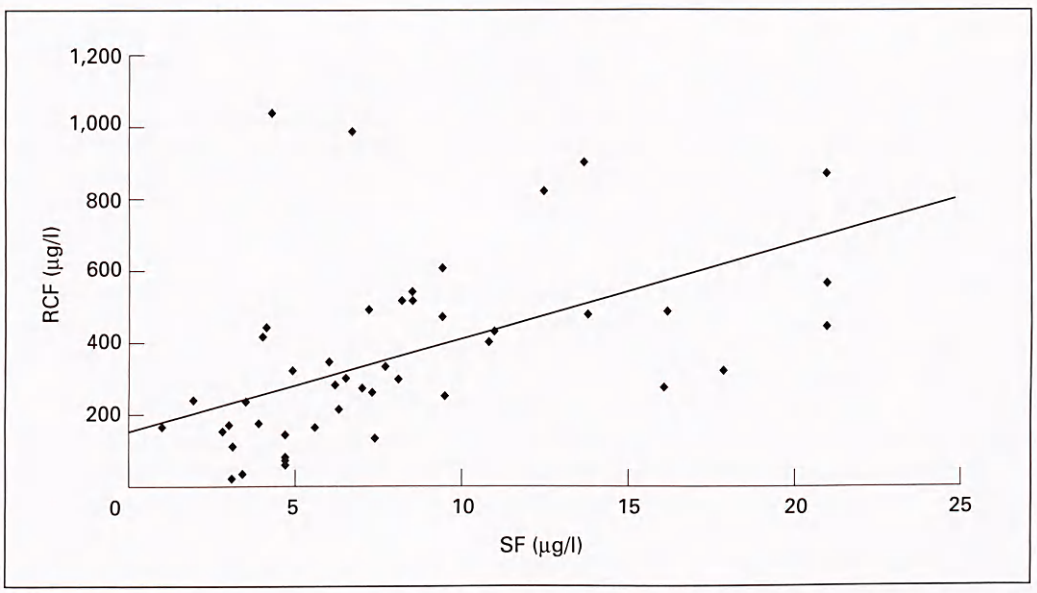

Fig 2. Correlation between serum folate and red cell folate levels in patients with macrocytosis (MCV > 100 fl).

In certain circumstances, both assays can give both false positive and negative results. In the presence of B_{12} deficiency, which not infrequently accompanies folate deficiency, RCF levels may be spuriously low, and SF assay may therefore be of more value than RCF assay for routine investigation of megaloblastic anaemia. On the other hand, RCF may be normal despite folate deficiency if the patient has recently been transfused because the transfused red cells retain their folate content throughout their lifespan. It has been argued that RCF levels give a better indication of tissue availability and are therefore a better test of folate status[6]. However, where it has been measured, folate level in hepatocytes correlates equally well with

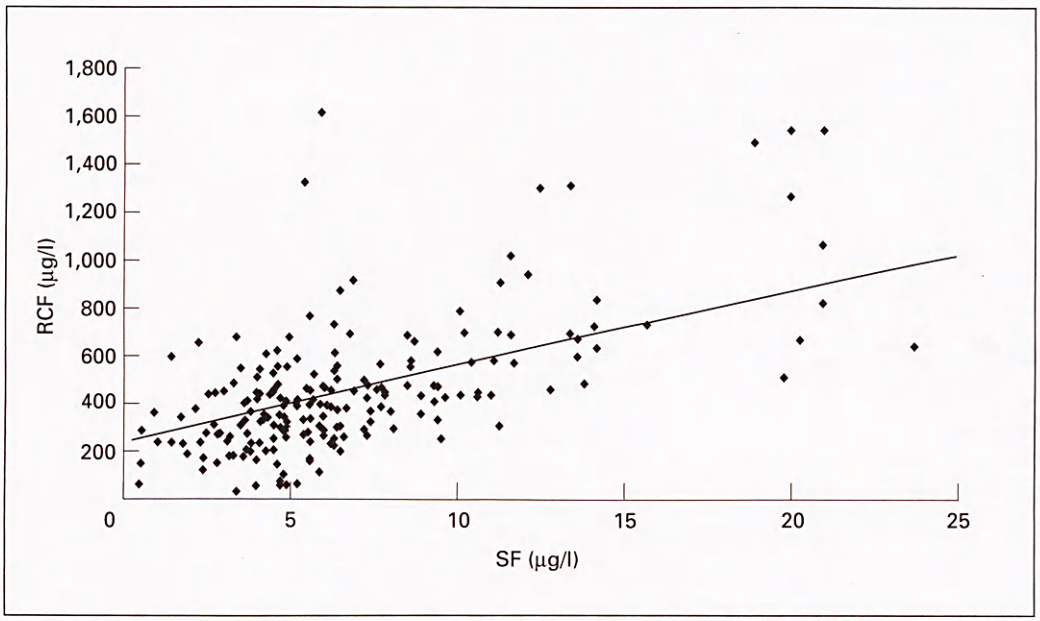

Fig 3. Correlation between serum folate and red cell folate levels in patients with macrocytic anaemia (Hb < 110 g/l); the graph excludes outliers.

serum and red cell folate[7], supporting the contention that SF assay is as useful an indicator of tissue folate availability as RCF assay.

Jaffe and Schilling[8], in a retrospective study over a 69-month period of all patients with a low RCF (n = 57) and a control group with normal RCF (n = 53), found no significant difference between the groups that could not have been equally well defined using SF values. They concluded that the measurement of RCF rarely gave additional useful information when the SF level was known. The present study also showed a highly significant correlation between the two folate assays (n = 797, r = 0.49, $p < 0.001$), as there was for patients with macrocytosis (r = 0.48, p = 0.001), and an even stronger correlation was found for patients with macrocytic anaemia (r = 0.66, $p < 0.001$). Furthermore, only 20 (2.5%) of the 797 investigations were abnormal by both assays.

This audit was not designed to evaluate the appropriateness of clinical requests; a further audit would be necessary to address this issue. However, a substantial proportion of requests for folate assay appear to have been ordered merely as part of non-specific screening tests, since 14% of requests supplied no clinical details at all and a further 36% were assessed as being for inappropriate clinical indications, eg vaginal bleeding. At the regional specialist audit meeting there was a consensus that it would be difficult to reduce at a laboratory level the number of tests being performed. The ease of the radioisotope dilution assay, the convenience of dual isotope kits for B_{12} and folate assays and the more reproducible results with SF compared with RCF assay lead us to advocate the use of the serum folate assay for screening requests. In line with the British Society of Haematologists guidelines[9] we also recommend that all patients being investigated for folate deficiency should, as a part of the protocol, have a full blood count and blood film examination. The morphological findings of macrocytosis and hypersegmented neutrophils in the peripheral blood are suggestive but not specific for B_{12}/folate deficiency, and in those patients who have a low SF in the absence of anaemia the RCF may be performed to define tissue status better. It is important to be aware of the various factors such as diet, that can affect the SF assay levels, and samples should be taken as soon as possible once folate deficiency is suspected. Furthermore, the wide variation in normal ranges in the different participating laboratories makes it important for physicians to be aware of their own laboratory's normal range when interpreting results and for laboratories to check their normal range.

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