Megaloblastic anaemia in Chinese patients: a review of 52 cases

JCW Chan, HSY Liu, BCS Kho, RW Chu, ESK Ma, KM Ma, PT Choi

A prospective study of Chinese patients with megaloblastic anaemia was conducted at the Pamela Youde Nethersole Eastern Hospital from 1 May 1994 to 31 August 1997. Megaloblastic anaemia was diagnosed in 57 patients, 52 of whom were eligible for further evaluation. The median age of these 52 patients was 73.5 years and the male to female ratio was 1.08:1. The serum cobalamin level (median, 56 ng/L) was low in 46 (88.5%) patients. In five (9.6%) patients, both serum cobalamin and red blood cell folate concentrations were low. Isolated low red blood cell folate level was demonstrated in one (1.9%) patient. Serum antibodies against intrinsic factor and gastric parietal cells were detected in 32 (61.5%) and 26 (50.0%) patients, respectively; 19 (36.5%) patients had both types of antibody. The aetiology of megaloblastic anaemia included pernicious anaemia in 39 (75.0%) patients, postgastrectomy vitamin B_{12} deficiency in five (9.6%) patients, and nutritional deficiency in two (3.8%) patients; the cause was undetermined in six (11.5%) patients.

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Introduction

The majority of cases of megaloblastic anaemia result from the deficiency of either cobalamin (vitamin B_{12}) or folate. Among Caucasians, cobalamin deficiency is the main cause and is usually the result of pernicious anaemia. Indeed, pernicious anaemia occurs in nearly 1% of those aged more than 60 years who have a northern European origin.[1,2] In other populations, there has been a tendency to assume that folate deficiency underlies most cases of megaloblastic anaemia. A study from Zimbabwe, however, showed that pernicious anaemia is the chief cause of megaloblastic anaemia in adults from southern Africa.[2] In addition, Indians of East African origin have exactly the same incidence of pernicious anaemia as the indigenous population of the United Kingdom.[3,4]

Pamela Youde Nethersole Eastern Hospital, 3 Lok Man Road, Chai Wan, Hong Kong:
Department of Medicine
JCW Chan, FRCP
HSY Liu, MRCP
BCS Kho, MRCP
Department of Pathology
RW Chu, MRCPath
Department of Nuclear Medicine
KM Ma, MSc, FRCP
PT Choi, MRCP
Department of Pathology, Haematology Section, Queen Mary Hospital, Pokfulam, Hong Kong
ESK Ma, MRCP, MRCPath

Correspondence to: Dr JCW Chan

In this study, we attempted to determine the relative role of cobalamin and folate deficiency in causing megaloblastic anaemia among patients from a regional hospital in Hong Kong that serves a population of half a million.

Methods

Patient entry

We started collecting data from patients with megaloblastic anaemia at the Pamela Youde Nethersole Eastern Hospital in May 1994. Patients with macrocytic anaemia (mean corpuscular volume [MCV] ≥100 fL) and low serum vitamin B_{12} or red blood cell (RBC) folate were recruited for the study. All patients were evaluated with a full medical history and physical examination. Particular attention was paid to their dietary habits, alcohol ingestion, previous gastric operation, past or concomitant autoimmune disease, and any symptoms or signs of anaemia, infection, bleeding, or of disorders affecting the neurological and gastrointestinal systems.

Laboratory investigations

Diagnosis of megaloblastic anaemia

Full blood counts were performed and the MCV was measured with a Coulter Haematology Analyser (STKS Coulter Electronics Inc., Miami, USA). Peripheral blood smears were reviewed by a haematologist.

Hypersegmentation of neutrophils was defined as the presence of 5% or more five-lobed neutrophils, or one or more six-lobed neutrophils.[5] Bone marrow examination was performed with the patients' consent and megaloblastic haemopoiesis was diagnosed.

Determination of the cause of megaloblastic anaemia

Serum cobalamin, serum folate, and RBC folate levels were measured by using the fluorimetric method with an Abbot IMX analyser (Abbot Laboratory, Chicago, USA). The normal range of serum cobalamin was 220 to 1100 ng/L and that of RBC folate was 150 to 650 ng/L.

Diagnosis of pernicious anaemia

Serum antibodies against intrinsic factor (IF) and gastric parietal cells (GPCs) were detected by the titerplane technique, which was developed by Euroimmun (Laboratorium für Experimentelle Immunologie GmbH, Krefeld, Germany). Endoscopic examination of the stomach was performed for patients who consented to the procedure. The Schilling test used the combined 'Dicopac' method in which free cyanocobalamin Co 58 was given simultaneously with cyanocobalamin Co 57 attached to human intrinsic factor.[6] Patients were referred for the test after their cobalamin deficiency had been corrected. Thyroid function test was performed for all patients to determine levels of thyroid-stimulating hormone and free thyroxine.

Replacement therapy

Cyanocobalamin (vitamin B_{12}) 1 mg/d was given intramuscularly for 5 to 7 days, followed by 1 mg weekly, 1 mg monthly, and finally 1 mg 3-monthly for patients with cobalamin deficiency. Oral folate 5 mg was administered for those with isolated folate deficiency and for those with suspected combined vitamin deficiency (in which case, folate was given after the correction of the cobalamin deficiency).

Response to therapy

The response to therapy was defined as complete if the cell counts and haemoglobin level had returned to normal and macrocytosis had disappeared after adequate treatment. Response was described as partial if any one of the above parameters failed to return to normal.

Results

Diagnosis of megaloblastic anaemia

Between 1 May 1994 and 31 August 1997, 57 patients were diagnosed to have megaloblastic anaemia. Five patients were excluded from the study: three because of inadequate evaluation and two for not being Chinese (one from Britain and one from Pakistan). Of the remaining 52 Chinese patients, the male to female ratio

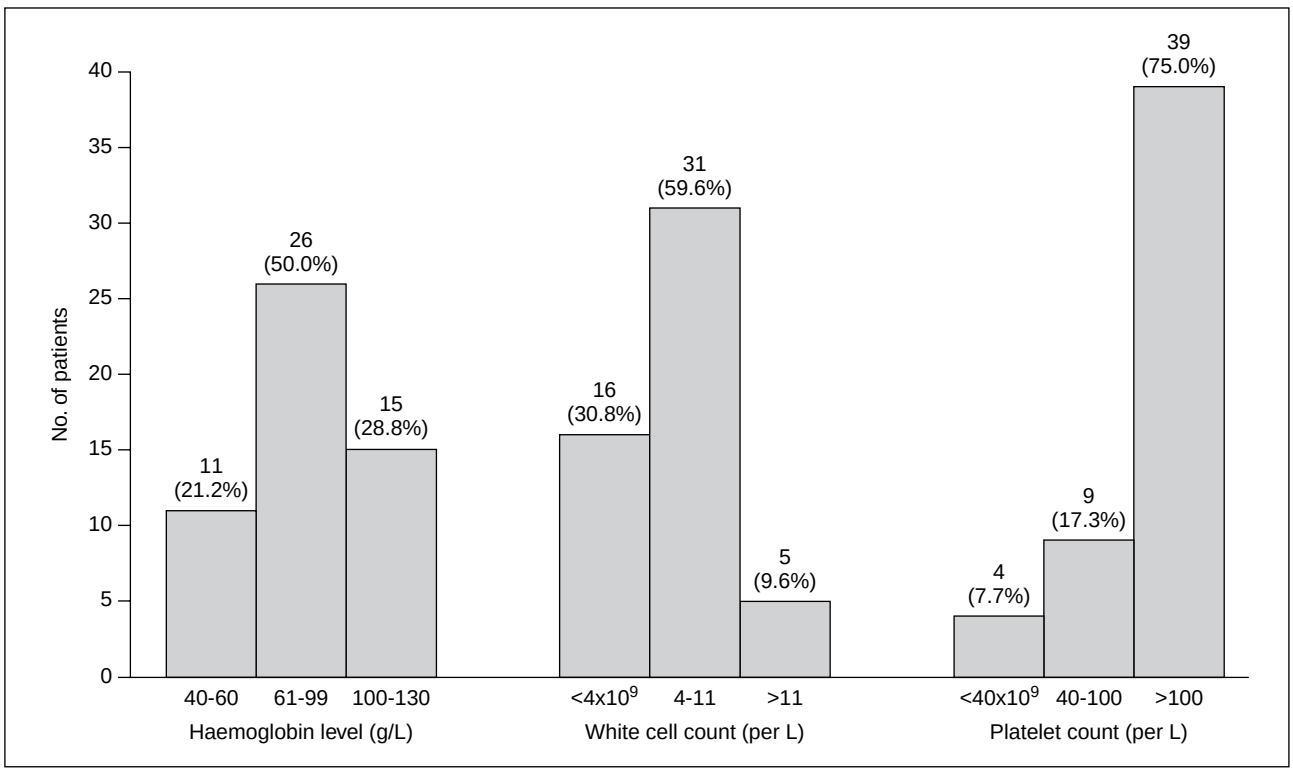

Fig 1. Blood investigations of Chinese patients with megaloblastic anaemia
The number of patients (%) in each category is shown above each bar

Table 1. Levels of serum vitamin B_{12} and red blood cell folate in Chinese patients with megaloblastic anaemia

Level of serum vitamin B_{12}/RBC* folate	Patients, n=52 No. (%)	Type of vitamin deficiency
Low vitamin B_{12} Normal folate	46 (88.5)	Vitamin B_{12}
Low vitamin B_{12} Low folate	5 (9.6)	Vitamin B_{12} and folate
Normal vitamin B_{12} Low folate	1 (1.9)	Folate

* RBC red blood cell

was 27:25 (or 1.08:1). The median age was 73.5 years (range, 43.0-90.0 years).

For 39 (75.0%) of the 52 patients, bone marrow examination confirmed megaloblastic haemopoiesis. Marrow aspiration was not performed for the remaining patients, but other features of megaloblastic anaemia were noted. These included neutrophil hypersegmentation and macro-ovalocytosis that resolved on treatment with vitamin B_{12} and/or folate. The median haemoglobin was 81 g/L (range, 47-130 g/L), the median white cell count was $5x10^9$/L (range, $1.3x10^9$-$16.7x10^9$/L), and the median platelet count was $157x10^9$/L (range, 15-280 /L) [Fig 1]. Twelve (23.1%) patients had pancytopenia. All 52 patients exhibited macrocytosis (MCV ≥100 fL) and 25 (48.1%) patients had MCV ≥120 fL. Hypersegmented neutrophils were noted in 43 (82.7%) patients.

Chief presenting features included anaemia in 32 (61.5%) patients, a weight loss of more than 5 kg in 15 (28.8%) patients, infection in 10 (19.2%) patients, glossitis in eight (15.4%), and peripheral neuropathy in one (1.9%). Dementia was the accompanying feature in six (11.5%) patients, while in eight (15.4%) asymptomatic patients, anaemia was identified by routine blood tests. No patient had any bleeding or thrombotic events. Two (3.8%) patients had concomitant Graves' disease (thyrotoxicosis), and the presence of antithyroid antibodies at the time of diagnosis of megaloblastic anaemia. Another two (3.8%) patients were positive for antithyroid antibodies but had normal thyroid function. One (1.9%) patient had a thymoma removed 2 years previously. Non–insulin-dependent diabetes mellitus was detected in 11 (21.2%) patients. Dietary history revealed that there were four alcoholics and one strict vegetarian. Five (9.6%) patients had undergone total gastrectomy to treat carcinoma of the stomach 5 to 6 years previously.

Diagnosis of types of vitamin deficiency

The levels of serum vitamin B_{12} and RBC folate in the 52 patients are shown in Table 1. The majority (88.5%) of patients had vitamin B_{12} deficiency. The median serum vitamin B_{12} level of these 46 patients was 56 ng/L (range, 0-159 ng/L). In five (9.6%) patients, both serum vitamin B_{12} and RBC folate levels were low. Their serum vitamin B_{12} concentration ranged from 42 to 156 ng/L. One (1.9%) patient showed isolated low concentration of RBC folate (140 ng/L): the concomitant serum vitamin B_{12} level (269 ng/L) was at the lower limit of the normal range.

Causes of vitamin deficiency and megaloblastic anaemia

Antibodies against IF were detected in 32 (61.5%) patients, and anti-GPC antibodies in 26 (50.0%) patients (Table 2); 19 (36.5%) patients possessed both types of antibody.

Table 2. Antibody levels of Chinese patients with megaloblastic anaemia

Level of serum vitamin B_{12}/RBC* folate	Diagnosis and presence of antibodies				
	Definite PA†		Probable PA		
	+IF‡/+GPC§	+IF/-GPC	-IF/+GPC	-IF/-GPC	Total
Low vitamin B_{12} Normal folate	18	13	7	8	46
Low vitamin B_{12} Low folate	1	0	0	4	5
Normal vitamin B_{12} Low folate	0	0	0	1	1
Total	19	13	7	13	52

* RBC red blood cell
† PA pernicious anaemia
‡ IF antibodies against instrinsic factor
§ GPC antibodies against gastric parietal cells

Among the 39 patients who had anti-IF and/or anti-GPC antibodies, the Schilling test was performed for 11—all had vitamin B_{12} malabsorption, which was corrected by the administration of IF. Endoscopy of the stomach was performed for 35 patients; this detected atrophic gastric mucosa in 11 patients, multiple benign polyposis in one patient, and intramucosal carcinoma in another. These 39 patients were assumed to have either definite pernicious anaemia (anti-IF antibodies present) or probable pernicious anaemia (anti-GPC antibodies present).

The remaining 13 patients had undetectable serum anti-IF and anti-GPC antibodies, and the possible aetiology of megaloblastic anaemia was deduced from clinical history (Table 3). Five patients had post-gastrectomy vitamin B_{12} deficiency and two had nutritional deficiency. The aetiology remained unclear for six patients.

Response to therapy

Thirty-eight of the 39 patients with definite/probable pernicious anaemia had a complete haematological response to vitamin B_{12}. The patient who did not have a complete response had undergone endoscopic polypectomy to treat intramucosal carcinoma and had remained anaemic despite receiving additional iron supplementation. There was, however, resolution of macrocytosis, leucopenia, and thrombocytopenia.

All five patients who had had a gastrectomy and who presented with macrocytic anaemia alone remained mildly anaemic (haemoglobin 110-120 g/L) despite adequate vitamin B_{12} and iron supplementation. The anaemia, however, became normocytic.

Two nutritionally deficient patients had resolution of pancytopenia and macrocytosis while receiving vitamin supplementation—one patient was given only folate the other was given both folate and vitamin B_{12}. Nevertheless, they had persistent normocytic anaemia, remained demented and bedridden, and had recurrent chest infections.

Three of the six patients who had megaloblastic anaemia of undetermined aetiology achieved a complete response to vitamin B_{12} supplementation. The other three patients were alcoholics. One of them had a complete response to vitamin B_{12} and folate replacement after having successfully abstained from alcohol and the remaining two patients continued alcohol ingestion and had persistent macrocytosis despite the normalisation of haemoglobin level and cell counts.

Discussion

We have reported on 52 Chinese patients who had classic features of megaloblastic anaemia, which resulted from florid deficiency of either cobalamin or folate. The clinical diagnosis proceeded in three sequential steps: the recognition of macrocytic anaemia, the confirmation of cobalamin/folate deficiency by vitamin assays, and the determination of the underlying cause of such deficiencies. This traditional diagnostic approach has some limitations. Firstly, macrocytosis can sometimes be masked by coexisting microcytic processes such as thalassaemia trait, iron deficiency, or anaemia from a chronic disorder.[7,8] An inclusion criterion of having an MCV ≥100 fL in the present study failed to identify macrocytic anaemia in patients who initially had a low MCV. What is more important for any individual patient is perhaps the change in MCV[7,9] and the appearance of the peripheral smear. Secondly, over the past decade, patients with cobalamin deficiency have frequently been reported to have mild or no anaemia but neurological features of anaemia.[10-14] If macrocytic anaemia were used as the sole criterion for pursuing a diagnosis of cobalamin deficiency, as in the present study, a significant proportion of patients with cobalamin deficiency would

Table 3. Postulated aetiology of megaloblastic anaemia in patients without antibodies against intrinsic factor or gastric parietal cells

Level of serum vitamin B_{12}/ RBC* folate	Postgastrectomy (for stomach carcinoma)	Nutritional deficiency	Undetermined aetiology (possible PA†)	Total
Low vitamin B_{12} Normal folate	4	0	4	8
Low vitamin B_{12} Low folate	1	1	2	4
Normal vitamin B_{12} Low folate	0	1	0	1
Total	5	2	6	13

* RBC red blood cell
† PA pernicious anaemia

escape detection. Thirdly, there is mounting evidence that serum vitamin assays lack sensitivity and specificity although they are still regarded as standard, first-line methods of screening.[11,12,15,16] Without more sensitive and reliable tests such as metabolite assays and the in vitro deoxyuridine suppression test, the detection of early and subtle stages of nutritional deficiency and the documentation of the exact type of vitamin deficiency is not possible.

Despite these shortcomings, the present study revealed some important information. Firstly, it demonstrated cobalamin deficiency as the major cause of megaloblastic anaemia in a cohort of Chinese patients at a regional hospital in Hong Kong. Judging by levels of serum cobalamin and RBC folate only, 88.5% of the patients were identified as having cobalamin deficiency, 1.9% as having folate deficiency, and 9.6% as having combined deficiencies. According to the 'methylfolate trap' or the 'tetrahydrofolate starvation' hypotheses,[8,11]—that the interaction of folate and cobalamin metabolism can bring about a reduction in the RBC folate level when there is cobalamin deficiency—the 9.6% of patients with both low serum cobalamin and RBC folate levels may have either combined deficiencies or cobalamin deficiency alone. Thus, in the absence of other supplementary tests, 98% of the patients can be estimated to have cobalamin deficiency, either alone or with concomitant folate deficiency. Folate deficiency was estimated to occur in 2% to 12% of the patients. The paucity of folate deficiency could be related to the fact that we did not screen obstetric, paediatric, or psychiatric patients in our study. In addition, tropical sprue/coeliac disease is rare among the Chinese population, and the nutritional status of most people in Hong Kong is satisfactory.

The second important finding of the study was that pernicious anaemia was a major cause of cobalamin deficiency among the patients. The diagnosis of pernicious anaemia in the present study was tentatively made on the demonstration of (1) megaloblastic haemopoiesis, either in the blood or in the marrow, (2) cobalamin deficiency by serum vitamin assay, (3) the presence of serum antibodies against IF and/or GPCs, (4) atrophic gastritis, and (5) a positive result from the Schilling test.[1,17] No attempt was made to obtain direct evidence of IF deficiency. At the time of analysis, only the first three criteria had been fulfilled completely. If we assume the presence of anti-IF antibodies to be a specific indicator of pernicious anaemia, and that of anti-GPC antibodies to be sensitive but less specific,[1,8] then the 32 (61.5%) patients who had anti-IF antibodies would have definite pernicious anaemia, and the seven (13.5%) who had only anti-GPC antibodies would at least have probable pernicious anaemia. The low frequency of endoscopic diagnoses of atrophic gastritis can be partly explained by the fact that biopsy and histological examination of the gastric mucosa were not performed for each patient. Likewise, the Schilling test was not performed for each patient, as the test was established in the hospital only in early 1996. This imperfect fulfillment of diagnostic criteria for pernicious anaemia, however, may not significantly affect our ultimate conclusion that 39 (75.0%) of the 52 patients had pernicious anaemia. If we consider the six patients in the category of 'megaloblastic anaemia of undetermined aetiology' as having pernicious anaemia with the absence of anti-IF and anti-GPC antibodies, the figure would rise to 86.5%. This is likely to be the case because anti-IF antibodies are present in only 55% to 60% cases of pernicious anaemia.[8]

The third important point raised by the study concerned patients who had undergone gastrectomy—9.6% of cases of megaloblastic anaemia were related to total gastrectomy that removed most or all IF-secreting cells. Megaloblastic anaemia has been recorded as early as 2 years and as late as 10 years after total gastrectomy, with the peak being about 5 years.[1,5] The period for the cobalamin deficiency to manifest largely relates to the size of the cobalamin stores. A course of prophylactic cobalamin might have to be started after total gastrectomy and continued for life. While anaemia after partial gastrectomy is common and is usually the result of iron deficiency, megaloblastic anaemia due to cobalamin deficiency has been reported to occur in 4% of such patients at least 5 years after the operation.[1,18]

Finally, dietary habit and nutritional deficiency may either directly cause cobalamin/folate deficiency or may aggravate existing deficiency states. For example, one of the patients in this series had been a strict vegetarian for 30 years and showed all the evidence of pernicious anaemia, including the associated Graves' disease. Another patient—a chronic alcoholic—was also shown to have definite pernicious anaemia. Hence, it may be worthwhile to look for pernicious anaemia even in patients with a recognised risk factor.

This report has described the haematological features, underlying aetiology, and response to treatment in a group of Chinese patients with megaloblastic anaemia who presented to a regional hospital in Hong Kong. Megaloblastic anaemia—in particular, pernicious anaemia—among Chinese patients has not been reported in great detail in any other series.

Although the exact frequency of pernicious anaemia in the Chinese population is unclear, this disorder must be regarded as one of the major causes of megaloblastic anaemia in Chinese patients.

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