Vitamin B₁₂ absorption after allogeneic bone marrow transplantation

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SUMMARY The B₁₂ absorption test (Schilling test) with intrinsic factor was used to examine ileal B₁₂ absorption in 26 patients after allogeneic transplantation. The test was well tolerated and showed a profound fall in B₁₂ absorption, which was maximal at two weeks after transplantation and recovered by eight weeks. The predominant influence on absorption at this stage was probably the conditioning schedule, and the presence of acute graft versus host disease (GVHD) was not associated with a further impairment of absorption. Six patients with chronic GVHD were studied. When compared with nine patients without GVHD there was a significant ($p < 0.005$) reduction of B₁₂ absorption. These findings suggest that the B₁₂ absorption test may be a useful non-invasive method of studying bowel function after bone marrow transplantation.

Bone marrow transplantation is associated with numerous influences on the gastrointestinal tract. The ablative conditioning regimen (usually cyclophosphamide and total body irradiation) damages the epithelial lining of the gut, resulting in nuclear atypia, abnormal surface epithelium, flattened crypts and crypt cell degeneration.¹ These changes usually resolve in three weeks. The bowel mucosa can further be damaged by graft versus host disease (GVHD). Acute GVHD is usually characterised by a combination of diarrhoea, skin rash, and jaundice.² Rectal biopsy specimens show crypt cell degeneration and crypt abscesses.³ Some patients may go on to develop chronic gut GVHD which may mimic systemic sclerosis⁴; rectal biopsy specimens from these patients are often normal.³ In addition to these causes of diarrhoea, after bone marrow transplant patients are also subject to colitis due to viral and other infections.⁵

The investigation of transplant patients, especially in the period immediately after transplantation, is difficult. They are often nursed in a protective environment and are neutropenic and thrombocytopenic. Rectal biopsy, even when the platelet count exceeds $20 \times 10^9/l$, can be hazardous³ and contrast radiology and gastroscopy are difficult to justify except in specific circumstances. We studied small bowel absorption to establish whether abnormalities could be correlated with the presence of acute or

chronic GVHD and to monitor the functional abnormalities of the gut resulting from bone marrow conditioning. The B₁₂ absorption test was chosen as a test of ileal absorption because it was relatively easy for the patients to undertake. Earlier experience taught us that within four weeks of transplantation patients were extremely intolerant of oral mixtures of sugar solutions used to determine small bowel absorption and permeability. The B₁₂ absorption test only required that patients swallowed capsules of vitamin B₁₂ and intrinsic factor and that urine was collected for 24 hours.

Patients and methods

Twenty six patients were studied, 16 both before and after transplant and 10 after transplant alone. The patients received allogeneic marrow from haploidentical sibling donors for acute myeloid leukaemia ($n = 10$), acute lymphoblastic leukaemia ($n = 8$), and chronic granulocytic leukaemia ($n = 8$). The age range was 11-43 (mean 23 years).

The bone marrow conditioning regimen was changed midway through the study. Sixteen patients received total body irradiation of 10.0 Gy midline dose in three fractions at 6 cGy/minute, together with cyclophosphamide 120 mg/kg, and cyclosporin A to prevent acute GVHD. A further 10 patients received the same dose of cyclophosphamide but the radiotherapy was delivered as 7.5 Gy in a single fraction at a fast dose rate (16 cGy/minute). In this group

GVHD was controlled by in vitro T cell depletion of the donor marrow using the monoclonal antibody Campath-I; cyclosporin A was not used.

During the patients' stay on the transplant unit vitamin B₁₂ absorption was assessed by the urinary excretion of a 1 µg oral dose of ⁵⁷Co B₁₂ after a loading dose of cyanocobalamin. The labelled B₁₂ was given with intrinsic factor to eradicate gastric influences. In the group of patients examined after discharge from the unit the B₁₂ absorption was assessed in most by the Dicopac method (Amersham International, Amersham, United Kingdom). This is the preferred method of most of the hospitals in the region, and for convenience the follow up of many of the patients was at their local hospital.

Vitamin B₁₂ excretion was measured before transplantation and at weekly intervals for four weeks after bone marrow transplantation and again at eight weeks, by which time the patients had been discharged. Six patients developed chronic GVHD at intervals of 108–1800 (mean 530) days after transplantation and these were compared with nine patients without evidence of chronic GVHD 547–1460 (mean 700) days after bone marrow transplantation. The difference between these groups was measured by the Mann-Whitney U test.

Results

The Schilling test proved acceptable to the patients even shortly after conditioning when nausea and diarrhoea were common. The intensive nursing care of the unit allowed accurate collections of urine to be made. In the group of 16 patients followed up prospectively through transplantation the test was not completed in 12.5% of instances. This was either because of early death, failure to administer the B₁₂ capsules at the correct time, or contamination of the collected urine with faeces. Before transplantation the mean absorption of B₁₂ was normal (15.4%), although four values were marginally below the normal range of >10%. After conditioning there was a sharp reduction in the ability to absorb vitamin B₁₂ (fig 1). This fell to its lowest value (mean 4.3%, range 0.1–11%) after two weeks but had returned to normal at eight weeks. Four of the six patients receiving marrow without T cell depletion had grade I–II acute skin and gut GVHD but none of the patients given marrow treated with Campath-I had any evidence of acute GVHD. Although the numbers are small, the presence of GVHD did not seem to be associated with a further impairment of B₁₂ absorption.

Vitamin B₁₂ absorption was abnormal in all the patients with chronic GVHD (mean 4.5%, range 1.4–7.6%) and differed significantly ($p < 0.005$) from the group of nine patients without chronic GVHD

Fig 1 Vitamin B₁₂ absorption before and after bone marrow transplantation in 16 patients (normal range > 10%).

Fig 2 Urinary B₁₂ excretion in two groups of patients with and without chronic GVHD.

Table Sites of chronic GVHD

Case No	Age (years)	Months after bone marrow transplantation	Site(s) of GVHD	B ₁₂ excretion percentage (normal > 10)
1	43	12	Liver	5.9
2	22	18	Liver, joints	4.0
3	33	60	Lung, vagina	5.0
4	35	4	Skin, gut*	3.9
5	12	12	Skin, joints	7.5
6	12	6	Skin, gut*	1.4

*Findings of rectal biopsy specimen compatible with GVHD.

(fig 2). The site(s) of chronic GVHD are shown in the table. Two of the patients had mild diarrhoea and rectal biopsy specimens compatible with chronic GVHD but the remaining patients denied gastrointestinal symptoms and had normal rectal histological results.

Discussion

Investigation of abnormalities of the gastrointestinal tract immediately after bone marrow transplantation is difficult. Although information has been available from rectal biopsy specimens,^{1,6} this procedure may not be desirable in neutropenic patients and may be associated with an unacceptable risk of bleeding.³ Contrast radiology is not practical for repeated studies of the bowel in these patients and is probably not justified as a research tool. We had initially wanted to study bowel permeability⁷ and bowel absorption using mixtures of disaccharides and polyethylene glycol. This proved impossible because the patients, who were generally anorectic, either refused the mixture or vomited. As a compromise the B₁₂ absorption test was introduced. This was well tolerated, having only a requirement to swallow two capsules and to collect urine for 24 hours. Despite this, 12.5% of the tests could not be analysed, usually because the urine sample had become contaminated with faeces in female patients with diarrhoea.

Notwithstanding these limitations, there was a clear and profound abnormality of B₁₂ absorption following the conditioning. It was not possible to establish whether the presence of acute GVHD was associated with a further reduction of bowel absorption in the small numbers studied. In view of the variability of the results obtained it seems unlikely that abnormalities of the Schilling test will provide any better information on the confirmation of the diagnosis of acute gut GVHD than the established methods.²

The findings in patients with chronic GVHD were interesting. Of the six patients studied, only two were suspected clinically of having gut GVHD. Both had moderate diarrhoea and a rectal biopsy specimen compatible with chronic GVHD. The remaining four patients had no evidence of gastrointestinal symptoms, although one patient had lost weight. Although the numbers are small, the results suggest that major changes of bowel function may occur in chronic GVHD without obvious clinical symptoms. The presence of normal rectal histology is well recognised in chronic bowel GVHD,³ and radiographically the disease can be patchy often affecting the terminal ileum.⁸

In conclusion, the B₁₂ absorption test was a simple and well tolerated test of ileal absorption, both during the transplant period and afterwards. In view of the simplicity of the test we believe that its use should be evaluated in a larger group of patients.

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