Simple method of assessing splenic function in coeliac disease

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Summary

1. Erythrocytes with membrane abnormalities seen on interference contrast microscopy (‘pitted erythrocytes’) were counted in venous blood samples from patients with treated coeliac disease and dermatitis herpetiformis, and from control subjects and patients who had had an elective splenectomy.

2. The percentage of ‘pitted erythrocytes’ was compared with the 99mTc-labelled heat-damaged erythrocyte clearance, and with the splenic size computed from scintiscans.

3. There was a significant correlation between percentage ‘pitted erythrocytes’ and the above methods of measuring splenic function and size. Increase in the percentage of ‘pitted erythrocytes’ above the control range indicated splenic hypo-function.

4. The number of coeliac patients with percentage ‘pitted erythrocytes’ above the control range increased with increasing age at which they started a gluten-free diet.

5. Counting of ‘pitted erythrocytes’ is a simple and sensitive method of assessing splenic hypo-function in treated coeliac disease. It avoids radiation exposure, and is applicable to all ages and repeatable without risk.

6. The incidence of splenic hypo-function in coeliac patients may be related to the duration of untreated disease.

Key words: coeliac disease, spleen.

Introduction

It has been suggested recently that patients with impaired splenic function should receive prophylactic pneumococcal vaccine (Sullivan, Ochs, Schiffman, Hammerschlag, Miser, Vichinsky & Wedgwood, 1978; Lennard, 1979) to give protection against the serious infections which may occur in hyposplenic states (Bullen & Losowsky, 1979; Eichner, 1979). Apart from splenectomy, one of the commonest causes of splenic hypo-function is coeliac disease (Marsh & Stewart, 1970; Bullen, Hall, Gowland & Losowsky, 1978), in which abnormalities of the immune response have been related to reduced spleen size (Baker, Jones, Peacock & Read, 1975; Bullen, Hall, Cooke & Losowsky, 1977). Although there are several reports of overwhelming infection associated with splenic atrophy (Parr, Shipton & Holland, 1953; Whitaker, 1969; Bisno & Freeman, 1970; Grant, Horowitz, Lorian & Brodman, 1970; Bensinger, Keller, Merrell & O'Leary, 1971; Falter, Robinson, Kim, Go & Taubkin, 1973; Ryan, Smart, Holdsworth & Preston, 1978), these are not for patients with coeliac disease. The infection risk associated with splenic atrophy in coeliac disease is not known, and clinicians have no guidance as to whether prophylaxis should be given.

In order to obtain further information about the mechanism, incidence and complications of splenic atrophy in coeliac disease, accurate assessment of splenic function in a large number of patients is necessary, and this has been hampered by the lack of a simple quantitative non-invasive test of splenic function. Methods which have been previously applied are either insensitive and semi-quantitative, or are difficult to perform, time-consuming and, in addition, involve radiation exposure.

Counting of erythrocytes with membrane abnormalities, visible as ‘pits’ on direct-interference contrast microscopy (‘pitted erythrocytes’),
has been used in patients with haemoglobinopathies (Casper, Koethe, Rodey & Thatcher, 1976; Sills & Oski, 1979) and post-splenectomy hyposplenism (Pearson, Johnston, Smith & Touloukian, 1978) as a simple method of assessing splenic function. The method has not hitherto been applied in patients with coeliac disease or correlated with other quantitative tests of splenic function.

We have determined the percentage of 'pitted erythrocytes' in treated patients with coeliac disease and dermatitis herpetiformis, and, in order to assess the sensitivity of the method, we have compared the results with those of other tests of splenic function.

Patients and methods

'Pitted erythrocytes' were counted in 44 patients with coeliac disease, seven patients with dermatitis herpetiformis, 22 control subjects and six patients who had had an elective splenectomy at least 1 year previously. All patients with coeliac disease or dermatitis herpetiformis had been treated with a gluten-free diet for periods ranging from 4 months to 15 years, and the diagnosis was based on improved histology of the small bowel biopsy after gluten withdrawal with, in patients with dermatitis herpetiformis, typical skin lesions.

The controls comprised healthy volunteer subjects and patients with a variety of medical conditions, excluding haematological, hepatic or splenic disorders. The age range of the controls (12-80 years) was similar to that of the coeliac patients (7-72 years).

'Pitted erythrocytes' were counted by the method of Pearson et al. (1978). A drop of fresh venous blood was mixed with 0.5 ml of 3% buffered glutaraldehyde solution, pH 7-4, in a plastic tube. The fixed cells were examined in a wet preparation (magnification x650) with a direct-interference contrast microscope (Zeiss Universal Research Microscope equipped with Nomarski optics). Two thousand erythrocytes from each sample were counted by the same observer, who had no information about the subjects from whom the samples were obtained, and the percentage of cells with one or more 'pits' was calculated.

The percentage of 'pitted erythrocytes' was compared with heat-damaged erythrocyte clearance in 33 subjects, and with splenic volume computed from scintiscans in 42 subjects. The results of these methods of measuring splenic function in coeliac disease are reported elsewhere (Robinson, Bullen, Hall, Brown, Baxter & Losowsky, 1980). Briefly, autologous erythrocytes were labelled with 1-4 mCi of sodium [99Tc]-pertechnate (Dacie & Lewis, 1975) and damaged by heating for 20 min at 50°C. After re-injection of the cells, blood samples were taken at 3 and 20 min. The radioactivity remaining in the blood at 20 min, expressed as a percentage of the amount of radioactivity present at 3 min, was taken as a measure of heat-damaged erythrocyte clearance. The normal range for this method was found to be from 17.5 to 46.5%.

Splenic volume was computed from scintiscans obtained after injection of radio-labelled heat-damaged erythrocytes or 99mTc-labelled sulphur colloid. The normal range of computed splenic volume was found to be from 158 to 577 cm3.

All subjects gave informed consent and approval was obtained from the local Ethical Committee. Investigations using radio-labelled substances were not performed in children or young women.

Results

The results of counting 'pitted erythrocytes' in the various groups are shown in Fig. 1. Twenty out of 22 control subjects had less than 1% 'pitted erythrocytes' and the range was from 0.05 to 4%. The range in six splenectomized patients was from 32 to 49%. In coeliac patients the percentage of 'pitted erythrocytes' ranged from 0.05 to 47% and in dermatitis herpetiformis from 0.15 to 43%. Fourteen out of 44 coeliac patients (32%) had more than 4% 'pitted erythrocytes'. Three out of seven patients with dermatitis herpetiformis had more than 4% 'pitted erythrocytes'.

The percentage of 'pitted erythrocytes' plotted against the clearance of autologous heat-damaged erythrocytes is shown in Fig. 2. There is a significant correlation between these two methods of measuring splenic function ($r_s = 0.806, P < 0.001$; Spearman rank correlation). All of the 17 subjects judged to have normal splenic function had less than 4% 'pitted erythrocytes'. All but one of the 16 subjects judged to have splenic hypofunction by heat-damaged erythrocyte clearance had less than 4% 'pitted erythrocytes'.

Fig. 3 shows the percentage of 'pitted erythrocytes' plotted against computed splenic volume. There is a significant inverse correlation between these two measurements ($r_s = -0.820, P < 0.001$; Spearman rank correlation). All of the 17 subjects with computed splenic volume below 158 cm3 had more than 4% 'pitted erythrocytes'.
All but one of the 25 subjects with computed splenic volume in the normal range had less than 4% 'pitted erythrocytes'.

To assess the incidence of hyposplenism with respect to age at starting a gluten-free diet, the patients were grouped according to age at starting treatment and the percentage of patients in each group with more than 4% 'pitted erythrocytes' was calculated.

Table 1 shows that the percentage of patients with impaired splenic function increases with age at starting treatment.
Table 1. Incidence of splenic hypofunction in relation to the age at starting a gluten-free diet in patients with coeliac disease

<table>
<thead>
<tr>
<th>Age at starting gluten-free diet (years)</th>
<th>Mean duration of diet (years)</th>
<th>No. of patients</th>
<th>No. with &gt;4% 'pitted erythrocytes'</th>
<th>Percentage with &gt;4% 'pitted erythrocytes'</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under 30</td>
<td>7.8</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>30–39</td>
<td>4</td>
<td>12</td>
<td>4</td>
<td>33</td>
<td>0.033</td>
</tr>
<tr>
<td>40–49</td>
<td>3.8</td>
<td>9</td>
<td>4</td>
<td>44</td>
<td>0.14</td>
</tr>
<tr>
<td>50 or over</td>
<td>6.3</td>
<td>9</td>
<td>6</td>
<td>67</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* By Fisher's exact test: all comparisons with 'Under 30 years' patients.

Discussion

Although interference contrast microscopy suggests the presence of 'pits' in a proportion of erythrocytes, this appearance is probably due to vacuoles of low absorbance lying beneath the plasma membrane (Holroyde & Gardner, 1970) whereby, in the absence of a functioning spleen, the erythrocyte rid itself of solid material (Schnitzer, Rucknagel, Spencer & Aikawa, 1971). Normal erythrocytes acquire 'pits' after infusion into an asplenic recipient and the 'pits' in the erythrocytes of an asplenic donor disappear after infusion into a normal recipient (Holroyde & Gardner, 1970). One of the functions of the spleen, therefore, is to remove the 'pits' from the circulating erythrocytes.

Our results show a close correlation between the percentage of 'pitted erythrocytes' and both the heat-damaged erythrocyte clearance and computed splenic volume in treated coeliac patients, suggesting that counting of 'pitted erythrocytes' is a useful alternative to these methods of evaluation of splenic function. Counting of 'pitted erythrocytes' gives a quantitative result with a wide range of measurements. The method is simple to perform, requires only one drop of blood, is repeatable without risk and can be applied in children and young women, in whom radiation should be particularly avoided. In this study, the percentage of 'pitted erythrocytes' was calculated from a count of 2000 erythrocytes, as in that of Pearson et al. (1978). Other workers using the same technique have examined fewer erythrocytes: Holroyde & Gardner (1970) counted at least 500 erythrocytes, Casper et al. (1976) 500 erythrocytes, and Sills & Oski (1979) 250–500 erythrocytes. In our experience, examination of 2000 erythrocytes is preferable in order to obtain an accurate, reproducible result with little observer variation.
Splenic function in coeliac disease

Careful counting takes from 30 to 40 min, the necessary expertise is quickly acquired, and the method, although slightly tedious, gives an immediate estimate of splenic function, in contrast to methods using isotopes, which take longer to perform, demand greater expertise and do not give an immediate result.

The results show that splenic function in coeliac disease ranges from normal to negligible by comparison with normal subjects and electively splenectomized patients. The increased incidence of hyposplenism after treatment in those coeliac patients who start a gluten-free diet later in life suggests that splenic atrophy occurs after many years of untreated disease, which might cause prolonged lymphocyte depletion (Bullen & Losowsky, 1978) or reticuloendothelial blockade by prolonged immune complex uptake (Williams, Pussell, Lockwood & Cotton, 1979). Although spleen weight normally decreases with age, between 30 and 60 years (the age range of the majority of our patients) spleen weight remains relatively constant (DeLand, 1970), and none of our 11 controls aged from 50 to 80 years had more than 4% 'pitted erythrocytes'.

It must be noted that the good agreement of these tests of splenic function and size has been found in treated patients. This is not necessarily so in untreated coeliac disease (Palmer, Sherriff & Holdsworth, 1979; Robinson et al., 1980) or active inflammatory bowel disease in which there is a reversible component to splenic hypofunction not depending on changes in splenic size (Ryan et al., 1978; Smart, Ryan, Holdsworth & Preston, 1978). The finding of splenic atrophy in patients who have been treated with a gluten-free diet for a long time, with improvement of the mucosal histology, indicates that there is sometimes irreversible loss of splenic function and volume. This is clearly the more significant factor in relation to the long-term effect on the patient. We suggest, therefore, that counting of 'pitted erythrocytes' in treated coeliac patients may be of further use in investigation of disturbances of immunity in treated patients, in determining the necessity for an effectiveness of any form of prophylaxis, and in advising individual patients.

We suggest that at present patients with hyposplenism should be advised that infection should be promptly treated (Eichner, 1979) and that if they visit malarial zones prophylaxis should be assiduously taken (Shute, 1975).

References


