Monocyte esterase deficiency in gastrointestinal cancer

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Abstract

***Aims*—To substantiate the high incidence of monocyte esterase deficiency (MED) in gastrointestinal carcinoma already reported in a small group of patients; to compare the clinical findings in esterase deficient and esterase positive patients.**

***Methods*—Peripheral blood smears (n = 22) or cytocentrifuge preparations (n = 52) of mononuclear cells from the peripheral blood of patients with gastrointestinal carcinoma were stained by the non-specific esterase stain (pH 5·8) using a batch technique. Samples containing ⩾85% esterase negative monocytes were identified at light microscopic examination.**

***Results*—Seven of 74 patients were identified as having MED. This correlated exactly with the proportion (five of 46) found before, using an automated method, and was significantly higher than the 0·8% incidence in normal blood donors shown in that study. Comparison of the clinical details of the 12 MED patients with those of 105 esterase positive patients showed a significantly longer disease free survival in the MED cohort and increased occurrence of benign neoplasms—largely colorectal polyps—in this group also. Three patients had a borderline degree of deficiency and were excluded from comparisons, although they showed the same clinical tendencies as the MED group.**

***Conclusions*—There is a strong degree of association between monocyte esterase deficiency and gastrointestinal carcinoma. Further evidence must be sought to prove that the deficiency precedes the disease and therefore may predispose to it, or at least may identify subjects with such a predisposition. This could lead to early diagnosis and effective treatment of gastrointestinal carcinoma in a sizeable proportion of patients.**

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Screening procedures for early detection of large bowel cancer in the families of subjects with familial adenomatous polyposis reduce early mortality from this cancer. The prevalence of the APC gene is variously estimated at 1 in 5000 to 1 in 20 000. Monocyte esterase deficiency (MED) occurs in 0·8%[1] "or 'less than 1%' of normal individuals" (Technicon Manual, Technicon Instruments Corp, Tarrytown, New York). In 46 patients with gastrointestinal carcinoma the deficiency was shown in five.[1] It has been suggested that MED, whether constitutional[1,2] or acquired,[3] results in, or is linked to, a predisposition to lymphoproliferative neoplasia. A similar link with gastrointestinal cancer could indicate a putative relative risk of 14·3 (95% confidence limits 3, 67)[4] for gastrointestinal cancer in MED subjects. In that case it would be useful to identify esterase deficient subjects to monitor the development of gastrointestinal malignancy with a view to early resection.

The original association of MED with gastrointestinal carcinoma was found in a small group of 456 patients with various carcinomata. The purpose of the present study was to examine a greater number of patients with gastrointestinal carcinoma for MED to ensure that the original finding was not a false positive result due to a type I error from examination of multiple subgroups. A second reason was to identify a larger number of such patients with and without esterase deficiency so that their clinical details could be compared.

Methods

Blood samples were obtained from 74 patients with gastrointestinal cancer confirmed by histological examination. The patients were sampled at random depending on pressure of work on the surgeon concerned and the haematology department, as well as the consent of the patient. The project obtained local ethical committee approval. Peripheral blood smears were used to screen 22 patients for the deficiency while 10 ml of anticoagulated blood was taken from the other 52. Patients' clinical records provided clinical details such as a history of other malignant or benign neoplasms, Dukes' staging of colorectal cancers, the presence of benign polyps in resected bowel, the occupations of MED patients, and family history of neoplasia. It was considered undesirable to make any additional enquiries about family history of neoplasia, or to investigate relatives of patients identified as MED for the deficiency.

Peripheral blood mononuclear cells were stored at −20°C on cytocentrifuge slides and stained in batches for non-specific esterase at pH 5·8 according to the method of Li *et al.*[5] Cytospin slides of esterase positive monocytes

were included in each batch and dot positive lymphocytes provided on-slide controls. For peripheral blood smear screening, duplicate blood smears were stained by Wright's Romanowsky stain or the non-specific esterase stain.

Several hundreds of monocytes were visualised on cytospins, a minimum of 100 counted, and samples with $\geqslant 85\%$ esterase negative monocytes considered to be MED. For the blood smear screening method, differential white cell counts (500 cells) were performed on the Wright stained peripheral blood films and the monocyte percentage obtained. This was compared with the proportion of esterase positive monocytes present on the esterase stained slide and the percentage of esterase negative monocytes deduced. If $\geqslant 85\%$ monocytes were esterase negative an anticoagulated blood sample was obtained for analysis by the cytospin method.

Seven patients had anticoagulated blood samples taken at intervals varying from three weeks to 17 months (median five months) after the initial identification and these samples were processed and examined as above.

We took the incidence of MED shown in 474 blood donors in a previous study using an automated cytochemistry analyser, the Technicon Hemalog D 90, for comparison with the incidence shown in patients with gastrointestinal carcinoma in the present study. The Hemalog D 90 provided a monocyte count (M) on an esterase stained aliquot of each blood sample and a monocyte count on another aliquot stained for peroxidase activity. Any excess of peroxidase positive over esterase positive monocytes was identified as a positive remainder value (R), which represents esterase negative monocytes. The fraction R/R + M (R/M) represented the proportion of esterase negative monocytes in a sample. The average R/M value of multiple Hemalog D 90 analyses of a blood sample from a patient repeatedly shown during a six year period to have $\geqslant 85\%$ esterase negative monocytes by the manual cytospin method located the R/M value. This identified similarly deficient samples and established the incidence in blood donors.[1] This patient continued to have $\geqslant 85\%$ esterase negative monocytes by the cytospin method.

Fisher's exact two tailed test was used to compare the incidence of MED in the gastrointestinal series with that in normal blood donors and the incidence of other malignant and benign neoplasms in MED patients with that in esterase positive patients. The Mann-Whitney two tail test was used to compare the disease free survival, from resection to esterase analysis, of patients with and without MED, and the age and white cell counts at time of analysis of both cohorts. Confidence intervals were calculated for proportions and medians. Relative risk was calculated through the odds ratio.[4]

Results

Seven of the 74 patients analysed by manual methods were identified as MED (10%, CI 4–19%); the single patient identified by the peripheral blood method was confirmed as MED by the cytospin method (table 1). Five of 46 patients had already been identified as MED by the automated method (11%, CI 4 and 24%).[1] The incidence of MED in the 120 patients was 10% (CI 5–17%). These incidences were significantly increased when compared with the 0·8% (CI 0·2–2·1%) incidence of the deficiency already shown in 474 blood donors ($p < 0{\cdot}001$ in each case; Fisher's exact test).[1] There was no material change in the degree of MED noted in the repeat samples obtained from seven MED patients.

Table 1 Proportion of blood samples from patients with gastrointestinal carcinoma showing MED by automated or manual methods

Method	No of patients examined	Number (%) with MED
Peripheral blood smear	22	1* (4·5)
Cytospin preparations	52	6 (11·5)
Hemalog D (previous survey)	46	5 (10·8)
Total	120	12 (10)

*Subsequently confirmed by cytospin method.

Two patients examined by the cytospin method were difficult to categorise even after several stains not consistently quite fulfilling the $\geqslant 85\%$ negative criterion. These patients were segregated into an intermediate group with a third patient previously identified as having 80% esterase negative monocytes by the Haemalog D 90. This group has been kept separate and is not included in comparisons of MED patients with esterase positive patients.

In table 2 the occurrence of MED according to primary site of neoplasm is shown together with some variables of MED and esterase positive cohorts. There was no significant difference between the two cohorts for any of these.

MED occurred as often in patients who were apparently free of tumour at the time of analysis as in patients in whom tumour was present (table 3). Of the 73 esterase positive patients with tumours, esterase analysis was performed preoperatively in 20; 37 were analysed postoperatively but their disease had

Table 2 Monocyte esterase status according to primary site of neoplasm

	Esterase positive	Intermediate	Esterase deficient
Oesophagel, gastric, small intestine	25	2	1
Large bowel	74	1	9
(right side/left side)	(21, 53)	(0/1)	(2/7)
Liver and pancreas	5	0	2
Uncertain primary site (stomach/colon)	1	0	0
Total	105	3	12
Sex (M/F)	47/58	2/1	9/3
Age at analysis (median, quartiles)	69 (62, 78)	67 (64,69)	72 (66, 74)
Age at presentation of neoplasia	69 (64, 75)	66 (64, 68)	68 (58, 77)
White cell count at analysis × 10^9/l	8·7 (8, 10)	9·3 (7, 10)	9·6 (8, 15)

Table 3 Tumour status at time of esterase analysis

	Tumour present	*Tumour free*	*Recent resection*
MED patients	6	5	1
Esterase positive patients	73	17	15
Intermediate esterase status	2	0	1

been inoperable or incompletely resected, and 16 had recurrent or terminal disease. Patients who had had analysis within two months of apparently curative resections were considered to be unknown quantities with regard to tumour status and were kept in a separate group.

All patients free of cancer at the time of esterase analysis and with a disease free survival of more than two months had had colorectal cancer. The disease free survival of MED and esterase positive patients is detailed in table 4 as is the proportion of patients with Dukes' stage A, B, or C disease at presentation. The longer survival of MED patients achieved significance at the 5% level ($p = 0{\cdot}0309$; Mann Whitney-U test).

The incidence of second malignancies and benign neoplasms in both groups is shown in table 5. Excluding the intermediate group from analysis, the incidence of second primary cancers in MED patients was not significantly different from that of the esterase positive group, but benign tumours—largely colorectal polyps—were more common in the MED group ($p = 0{\cdot}0164$).

The three female patients were old age pensioners when esterase analysis was performed; no previous employment was recorded for any of them. Three male patients were employed as a lorry driver, (1) a quality control manager (1), and a fitter (1). One was unemployed (long-term). Five were old age pensioners; recorded previous employments for three were as an insulator, doing farming and building work, and a builder.

Thirteen of the esterase positive group had documentation in their hospital notes of a close relative who had had cancer. One of the MED group (a patient with two synchronous carcinomata of colon) thought his father had died (aged 76) of "bowel trouble".

Table 4 Disease free survival in MED and esterase positive patients, cancer free at analysis: disease stage at presentation

	MED patients	*Esterase positive patients*
Months free of disease after resection		
Median	60	22·5
Confidence intervals	11, 84	15, 34
Quartiles	33, 72	6, 36
Range	11, 84	2, 69
Number of patients	5	17
Stage at diagnosis		
A	1	1
B	2	10
C	2	5 (1 untraced)

Table 5 Incidence of second cancers and benign tumours according to esterase status

Patients	*Second cancers*	*Benign tumours*	
Med (n = 12)	2 (Both gastrointestinal)	Colonic polyps 4	Multiple 1 Double 1 Single 2
		Pituitary adenoma 1	
Esterase positive (n = 105)	11 (2 gastrointestinal)	Colonic polyps 9	Several 1 Double 2 Single 6
		Warthin's tumour 1	
Intermediate esterase status	1 (Gastrointestinal)	Colonic polyps 1	Several

Discussion

This study showed that the original finding of an association between MED and gastrointestinal carcinoma was not a type I error. There is a substantially increased incidence of MED in such patients. Although manual methods were used in the present study, the degree of control incorporated in it and the reference to the same standard as that used in the original one—that is, the patient with repeated demonstration of $\geqslant 85\%$ esterase negative monocytes—validated the comparison of results from the two studies.

We considered as MED only samples showing $\geqslant 85\%$ esterase negative monocytes by the cytospin method or the comparable R/M of the Haemalog D-90, because such samples have been shown to lack the monocyte specific isoenzyme band on isoelectric focusing.[2] Study of the intermediate group, however, identified in the present study, suggests that monocyte esterase deficiency of a lesser degree may be important.

The occurrence of the anomaly was not related to the age of the patient, tumour status at time of esterase analysis, resection, stage of disease at presentation, or primary site of neoplasm in the gastrointestinal tract. That exposure of blood to organophosphates in vitro inhibits monocyte esterase is well documented. In vivo inhibition has only been documented in two studies to our knowledge. Oehmichen *et al* described its occurrence in a patient who swallowed organophosphate insecticide,[6] and Mandel *et al* demonstrated a 22% reduction of monocyte esterase activity in workers exposed to organophosphates,[7] while workers elsewhere in the plant did not show a decrease. Levine *et al* described a 72% reduction in monocyte esterase activity in a group of production workers at a plastics manufacturing company but did not positively identify organophosphate as the causative agent, although this seemed likely.[8] Again, other workers at the plant did not show a reduction. The occupations of our patients at the time of esterase analysis show that such a degree of exposure to organophosphates is unlikely to have occurred in our MED patients. Moreover, the stability of the MED, as evidenced by its presence in repeat samples many weeks after the first sample, further indicates that such exposure cannot be implicated as the cause of the deficiency in our series. The patient described by

Oehmichen *et al* regained monocyte esterase activity 15 days after the poisoning episode, indicating that newly produced monocytes retained the capacity to form monocyte esterase, although the enzyme itself is irreversibly inhibited by organophosphates. In MED patients with lymphoproliferative or autoimmune diseases we have shown a high familial incidence of the deficiency.[1 3 10] The lack of a demonstrable acquired cause for the deficiency in the present study raises the possibility that it could be constitutional in this instance as well.

We have already suggested that MED may result in, or be linked to, a predisposition to lymphoproliferative disease.[3 10] This was based on the demonstration of a defect in natural cytotoxicity of esterase negative[10] or esterase inhibited[11] monocytes, and on the demonstration of an increased incidence of familial MED in lymphoproliferative neoplasia. If MED in patients with gastrointestinal carcinoma were constitutional then screening for the deficiency should identify a large cohort at risk for this cancer. Moreover, MED subjects may have diminished ability to detoxify organophosphates and possibly, therefore may be at risk from the mutagenic effect of the latter and so could be advised to avoid exposure to these substances.

As only 12 MED patients were identified, no firm conclusions can be drawn from the comparisons of MED and esterase positive patients. On the one hand, lack of association of the deficiency with earlier age at presentation of disease and lack of association with family history of neoplasia could challenge the hypothesis of a predisposition to neoplasia. On the other hand, the tentatively increased incidence of benign tumours, known to be linked to colonic carcinoma, and the tendency to longer survival (analogous with that of familial colonic carcinoma) of the MED cohort could corroborate it. We suggest that a comprehensive study, incorporating screening for MED both in patients and their close relatives, together with detailed occupational histories and full investigation into the occurrence of cancer within families, should be undertaken to test the hypothesis.

The lack of a simple screening procedure for monocyte esterase deficiency poses a considerable difficulty. The method used for this study is too cumbersome for screening large numbers of patients; the Haemalog D-90 automated cytochemistry analyser has been replaced by improved instruments which, however, lack the esterase channel, and, because inactive isoenzyme seems to be present in MED subjects (personal communication, Dr K Ennis, Department of Medical Genetics, Queens University Belfast), antibody screening methods will not detect the deficiency state unless an antibody to the active site is produced. The semi-automated method of Ross *et al*[12] should permit identification of the deficiency state, but it is clear that a fully automated screening procedure would greatly simplify the proposed investigation.

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