J Clin Pathol 1984;**37**:1090–1094

Demonstration of carcinoembryonic antigen in bone marrow from patients with carcinoma

A GRAY, R DOWNING, R HILL, RW PAYNE, CWO WINDSOR

From the Departments of Surgery and Haematology, Worcester Royal Infirmary, Ronkswood Branch, Worcester

SUMMARY Primary and secondary tumour and bone marrow trephine biopsies from 20 patients with carcinomas were stained for carcinoembryonic antigen by the three stage immunoperoxidase method. Six marrow biopsies contained tumour deposits, five of which were positive for carcinoembryonic antigen. A further five marrow biopsies contained single carcinoembryonic antigen positive cells of uncertain origin. Carcinoembryonic antigen staining may be a useful adjunct to conventional histology in the diagnosis of marrow metastases.

Few patients with carcinomas have demonstrable metastases in the bone marrow. In an examination of bone marrow specimens taken from 2191 such patients only 8·5% showed metastatic tumour using conventional stains.[1] Multiple biopsies, multiple sections, and the simultaneous use of aspirates, clot sections, and trephine biopsies have all been used in attempts to increase the detection rate. More recently, immunochemical stains have been introduced for specific tumour antigens. Metastatic adenocarcinoma expressing carcinoembryonic antigen (CEA) has been shown in regional lymph nodes from patients with colonic and breast carcinoma,[2 3] and Cal antibody has been used to distinguish malignant from non-malignant cells in serous fluids.[4] Immunochemical detection of epithelial membrane antigen in sections of bone marrow aspirate has yielded an increased number of positive samples and shown a greater extent of tumour infiltration.[5] In the present study, tumour tissue and bone marrow samples from patients with carcinomas of varying origin were stained for CEA using an immunoperoxidase method.

Patients and methods

PATIENTS

Twenty patients with carcinomas were studied. A Tc-99m radioisotope bone scan and chest *x* ray examination were performed and painful bony areas were also examined radiographically. Scans and *x* ray films were considered positive only if there was unequivocal evidence of metastatic disease.

PLASMA CARCINOEMBRYONIC ANTIGEN

Concentrations were measured using the double antibody radioimmunoassay method.[6] CEA values were estimated preoperatively in patients undergoing surgical removal of tumour. One patient (Table 1) had a primary tumour excised in 1953 and had a plasma CEA estimated at the time of first recurrence.

PRIMARY AND SECONDARY TUMOURS

Biopsy samples were fixed in 10% buffered formol-saline and embedded in paraffin wax; 5 μm sections were stained with haematoxylin and eosin and for CEA.

BONE MARROW

Bone marrow was obtained from the sternum or iliac crest using a Salah needle. Smears were fixed in 95% methanol and stained with May-Grünwald-Giemsa; similarly fixed smears were stained for CEA.

Bone marrow aspirate, collected directly into fixative of equal parts of 15% formalin and absolute isopropanol, was embedded in celloidin and paraffin wax using a modified Raman method.[7] Sections (5 μm) were dewaxed with xylene, and the celloidin was removed with acetone to prevent loss of sections during staining with haematoxylin and eosin and for CEA.

Trephine biopsies were obtained from the iliac crest using a Jamshidi needle, fixed in 10% buffered formol-saline, and decalcified in 10% edetic acid solution (pH 7·0) for a minimum of five days at 50°C before embedding in paraffin wax. Sections (5 μm) were stained with haematoxylin and eosin and for CEA.

CARCINOEMBRYONIC ANTIGEN ANTISERUM

CEA antiserum was a commercially available

Table 1 *Patients with marrow trephine biopsies containing tumour metastases (haematoxylin and eosin)*

Primary tumour		*Metastases*		*Marrow trephine biopsy*	*Plasma CEA (μg/l)*	*Bone scan*	*x ray film*
Site	*CEA stain*	*Site*	*CEA stain*	*CEA stain*			
Prostate	+	None		+	51	ND	Positive
Prostate	+	None		+	15·1	Positive	Positive
Prostate	−	None		+	581	Positive	Positive
Breast	−	None		++	85·6	Positive	Negative
Breast*	++	Skin	+++	+++	211	Positive	Positive
Prostate	−	None		−	54·4	Positive	Positive

CEA = carcinoembryonic antigen.
ND = not done.
*Primary tumour excised in 1953.
+ = few, ++ = moderate, +++ = many positive cells.

immunoglobulin fraction prepared in rabbits by immunisation with highly purified human CEA obtained from hepatic metastases of colonic adenocarcinoma (Dakopatt anti-CEA: Code A115). Rabbit CEA antiserum contained antibody to non-specific cross reacting antigen (NCA), which was absorbed with a human spleen extract treated with perchloric acid before application. The method described by Krupey *et al*[8] and modified by Smith[9] was used.

IMMUNOPEROXIDASE METHOD

Paraffin sections were dewaxed with xylene, taken to water, and stained for CEA by the indirect, or three stage, immunoperoxidase method.[3 10]

CONTROLS

Removal of anti-NCA activity from the CEA antiserum was assessed by applying the unabsorbed and absorbed antisera to blood smears prepared from a patient with chronic granulocytic leukaemia and to sections of normal spleen.[11] Sections of normal colon, stomach, breast, and prostate were negative controls, while sections of carcinomas of these tissues were positive controls for absorbed antiserum. Normal trephine biopsies from patients with non-malignant conditions were also included. Slides of all control specimens were included in each staining batch.

INTERPRETATION

Immunoperoxidase staining was graded according to the number of positive cells seen: + = few; ++ = moderate; +++ = many. Intensity of staining was not quantified.

Results

Interpretation of bone marrow findings has been based on trephine biopsies alone since loss of tissue during the process of immunoperoxidase staining often precluded the valid use of bone marrow smears and clot sections.

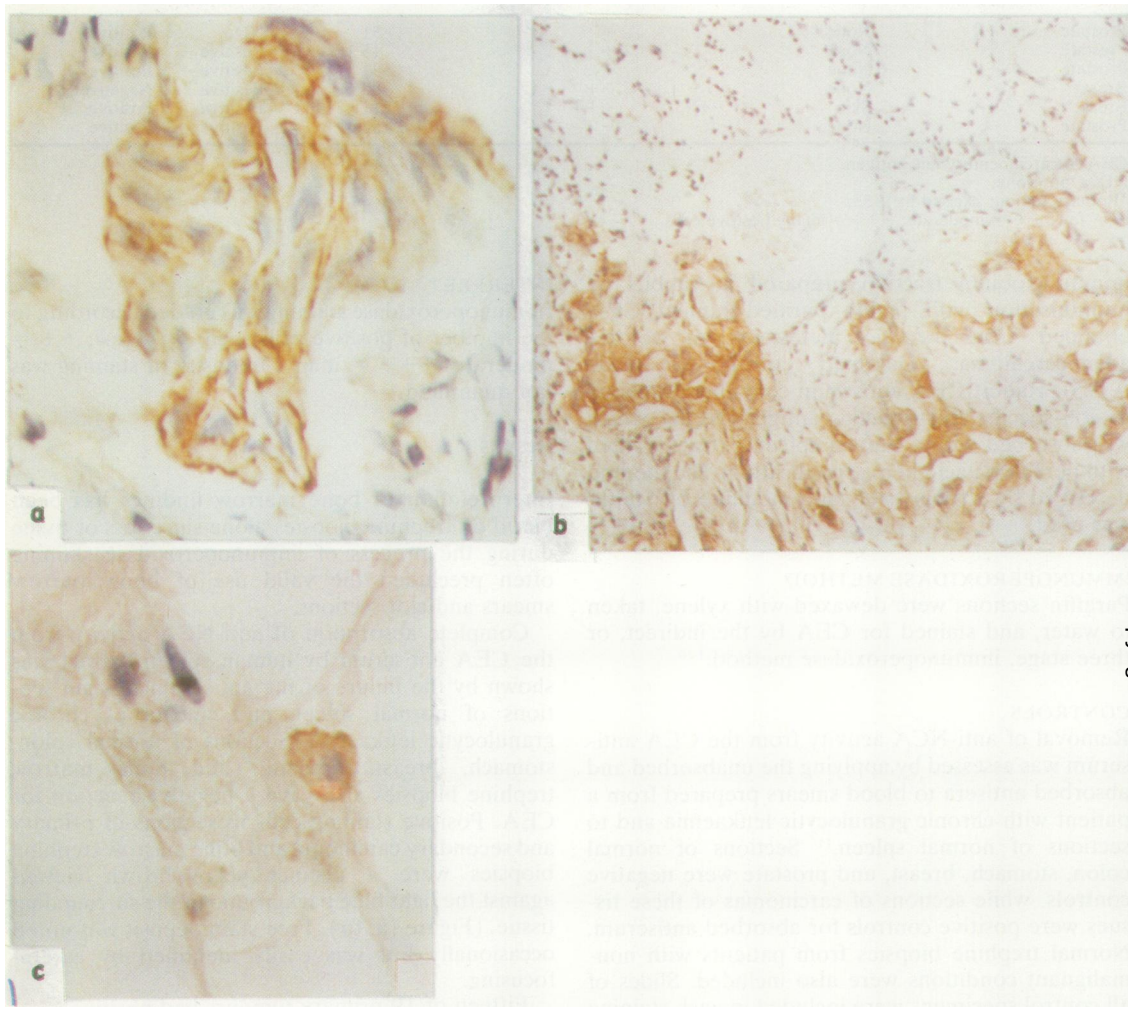
Complete absorption of anti-NCA activity from the CEA antiserum by human spleen extract was shown by the failure of the antiserum to stain sections of normal spleen and smears of chronic granulocytic leukaemia. Sections of normal colon, stomach, breast, prostate, and bone marrow trephine biopsies also gave a negative reaction for CEA. Positive staining cells in sections of primary and secondary carcinoma and bone marrow trephine biopsies were a distinct yellow-brown viewed against the light blue background of the surrounding tissue. (Figure (a, b)). Free stain deposit was noted occasionally but was easily identified by careful focusing.

Fifteen of 19 primary tumours had positive reactions for CEA; the least number of positive reac-

Table 2 *Patients with marrow trephine biopsies negative for tumour metastases (haematoxylin and eosin) but positive for carcinoembryonic antigen (CEA) stain*

Primary tumour		*Metastases*		*Marrow trephine biopsy*	*Plasma CEA (μg/l)*	*Bone scan*	*x ray film*
Site	*CEA stain*	*Site*	*CEA stain*	*CEA stain*			
Breast	−	Node	−	+	ND	Positive	Negative
Breast	+	Node	+	+	25·6	Negative	Negative
Breast	+	None		++	>1000	Negative	Positive
Stomach	+++	None		+	13·7	Negative	Negative
Colon	+++	Node	+++	+	16·4	Negative	Negative

ND = not done.
+ = few, ++ = moderate, +++ = many positive cells.



Carcinoembryonic antigen (CEA) positive bone marrow trephine biopsies. (a) Metastatic deposit of breast carcinoma. ×400. (b) Infiltrating metastatic carcinoma of prostate. ×100. (c)(d) CEA positive single cells in unaffected marrows (haematoxylin and eosin) of two patients with breast carcinoma. ×630.

Table 3 *Patients with marrow trephine biopsies negative for tumour metastases (haematoxylin and eosin) and carcinoembryonic antigen (CEA) stain*

Primary tumour		*Metastases*		*Plasma CEA (μg/l)*	*Bone scan*	*x ray film*
Site	*CEA stain*	*Site*	*CEA stain*			
Stomach	+++	None		ND	ND	Negative
Stomach	+++	Liver	+++	117	Negative	Negative
Caecum	++	None		18·2	ND	Negative
Colon	+++	Bladder	+++	164	Negative	Negative
Rectum	+++	None		78·5	Negative	ND
Rectum	+++	None		15·3	Positive	Negative
Breast	++	Node	−	24·9	Positive	Positive
Bronchus	−	Node	−	27·9	Negative	Positive
Unknown		Omentum	++	ND	ND	Negative

ND = not done.
+ = few, ++ = moderate, +++ = many positive cells.

tions was found in sections of prostatic carcinoma (Tables 1–3). Sections of one primary tumour were not available for study. Metastatic tumour was biopsied in nine cases, of which six had positive and two had negative primary tumours. Six of these metastatic tumours were CEA positive, of which four had positive primary tumours. Only one patient had a CEA positive primary tumour with negative metastases.

Patient data have been tabulated in three groups on the basis of bone marrow staining. Tumour deposits were identified by haematoxylin and eosin staining in six of 20 marrows (Table 1). Five of these had CEA positive cells in both the primary tumour and in the marrow, while the sixth patient had a primary carcinoma of the prostate which was CEA negative. The median plasma CEA concentration was 70·0 μg/l. Four patients had both radiological and radioisotopic evidence of bony metastases.

Table 2 gives details of five patients in whom bone marrow trephine biopsies showed no evidence of tumour metastases on haematoxylin and eosin staining but contained single CEA positive cells which were dispersed throughout the sample (Figure (c, d)). Morphologically, these cells were not obviously malignant; nor could they be identified on review of conventionally stained marrow sections. The median plasma CEA concentration was 21·0 μg/l; only two patients had evidence of bony metastases on either isotope bone scan or *x* ray film.

Nine patients had no tumour metastases or CEA positive cells within the marrow (Table 3). The median plasma CEA concentration was 27·9 μg/l; evidence of bony metastases was present in only three patients, who had positive isotope bone scans or *x* ray films.

Discussion

The incidence of histologically confirmed bone marrow metastases in patients with carcinoma is variable.[1 12 13] This may reflect a low frequency of marrow involvement, the disease stage when the marrow was examined, or sampling error. It may also arise from difficulty in identifying small amounts of tumour tissue, especially single cells, within the marrow. A tumour marker which reliably demonstrates these cells might profoundly alter both the management and prognosis in these patients.

The immunoperoxidase (indirect peroxidase-antiperoxidase) technique is a highly sensitive method of demonstrating CEA in paraffin embedded tissues.[14 15] Both the specificity of the antiserum and the use of proper controls are critical to the correct interpretation of results[3] and are especially important in the application of this staining method to bone marrow which contains appreciable amounts of NCA, a glycoprotein which shares some antigenic determinants with CEA. NCA has been found in maturing granulocytes[11] and macrophages and monocytes[16]; erythroblasts do not stain for NCA. CEA is minimal or absent in all blood and bone marrow cells.[11]

This study has clearly shown that CEA positive tumours maintain their capacity to express the antigen within the environment of the bone marrow having withstood the fixing and decalcifying process undergone by trephine biopsies. The extraordinary stability of the antigen can be gauged by its demonstration in a primary tumour (Table 1) which was excised 12 years before CEA was first described by Gold and Freedman.[17] This confirms the feasibility of retrospective studies.

The marrows of five patients which contained single, CEA positive cells not identified in conventionally stained sections are intriguing but must be interpreted with caution. A morphological resemblance of some of these cells to erythroblasts and other normal constituents of marrow was noted, raising the possibility of false positive reactivity. This was considered implausible for the following reasons:

1 Similar cells were not seen in either control marrows or the marrows from nine other patients with carcinoma (Table 3). Indeed the vast proportion of haemopoietic tissue in the marrows in which these cells were seen was CEA negative. As stated above, erythroblasts do not stain for either NCA or CEA in both normal and leukaemic subjects[11] and cross reactivity of the CEA antiserum with blood group antigens is eliminated during its purification by absorption with erythrocytes until agglutination is abolished.

2 Passive absorption of circulating or locally secreted CEA by bone marrow cell membranes cannot be excluded. It certainly occurs with normal colonic mucosal cells which lie adjacent to benign or malignant tumours.[2]

3 Likewise, macrophages concerned with processing antigen could account for these cells. It may be that small numbers of unidentified tumour cells in the marrow elaborate CEA in concentrations below the threshold sensitivity of the staining method and that cells of macrophage monocyte origin might then bring about sufficient concentration of the antigen on the cell surface or in the cytoplasm to allow its detection. If such a process is occurring in some patients but not in others this may reflect important differences in host response to the tumour. Whatever the origin of these cells, they serve to identify a subpopulation of patients with carcinoma which we feel deserves further investigation with regard to the pattern of metastases, response to treatment, and prognosis.

With regard to plasma CEA, bone scan, and *x* ray film findings, statistical evaluation was precluded by the small number of patients. It was noted, however, that the highest median plasma CEA concentration (70 μg/l) and the highest incidence of positive bone scans and *x* ray films occurred in the group of patients with histologically confirmed marrow metastases (Table 1). These investigations failed to distinguish between patients in Tables 2 and 3.

In conclusion, we have shown that the peroxidase-antiperoxidase method may be applied to bone marrow trephine sections which have been fixed, decalcified, and embedded in paraffin wax, although in our hands it was not suitable for use with bone marrow smears or clot secretions. The staining method is suitable for use in the histology laboratory of any general hospital and uses commercially available reagents. Since we have convincingly demonstrated the expression of CEA by carcinoma cells after they have metastasised to the marrow, the stain may prove useful in the interpretation of infiltrated marrows where the cell origin is difficult to identify—for example, in poorly differentiated lymphomas. In addition, we have identified a group of patients with small numbers of CEA positive cells, which may be indirectly or directly related to the presence of tumour in the marrow. The use of monoclonal antibodies may help to dispel any doubt over the specificity of the CEA antiserum, and follow up of patients should show any correlation between the presence of single, CEA positive cells within the marrow and prognosis, in particular, the subsequent development of overt marrow involvement.

We are indebted to Dr R Smith for his technical assistance and to the Department of Biochemistry, East Birmingham Hospital, for CEA estimations. We also thank Mrs L Whyley for typing the manuscript.

References

[1] Anner RM, Drewinko B. Frequency and significance of bone marrow involvement by metastatic solid tumours *Cancer* 1977;**39**:1337–44.

[2] Goldenberg DN, Sharkey RM, Primus FJ. Immunocytochemical detection of carcinoembryonic antigen in conventional histopathology specimens. *Cancer* 1978;**42**:1546–53.

[3] Walker RA. Demonstration of carcinoembryonic antigen in human breast carcinomas by the immunoperoxidase technique. *J Clin Pathol* 1980;**33**:356–60.

[4] Woods JC, Spriggs AI, Harris H, *et al*. A new marker for human cancer cells. 3. Immunocytochemical detection of malignant cells in serous fluids with the Cal antibody. *Lancet* 1982;ii:512–5.

[5] Sloane JP, Ormerod MG, Neville AM. Potential pathological application of immunocytochemical methods to the detection of micrometastases. *Cancer Res* 1980;**40**:3079–82.

[6] Booth SN, King JP, Leonard JC, *et al*. Serum carcinoembryonic antigen in clinical disorders. *Gut* 1973;**14**:794–9.

[7] Raman K. A method for sectioning aspirated bone marrow. *J Clin Pathol* 1955;**8**:265–6.

[8] Krupey J, Gold P, Freedman SO. Physicochemical studies of the carcinoembryonic antigens of the human digestive system. *J Exp Med* 1968;**128**:387–98.

[9] Smith R. Studies in metastatic human breast cancer. Birmingham; University of Birmingham, 1981. MD thesis.

[10] Curran RC, Jones EL. Immunoglobulin-containing cells in human tonsils as demonstrated by immunohistochemistry. *Clin Exp Immunol* 1977;**28**:103–15.

[11] Warren B, Gahrton G, Hammerstrom S. Nonspecific cross-reacting antigen in normal and leukaemic myeloid cells and serum of leukaemic patients. *Cancer Res* 1980;**40**:2039–44.

[12] Ingle JN, Tormey DC, Tan HK. The bone marrow examination in breast cancer. *Cancer* 1978;**41**:670–4.

[13] Stonier PF, Evans PV. Carcinoma cells in bone marrow aspirates. *Am J Clin Pathol* 1966;**45**:722–7.

[14] Wagener C, Csaszar H, Totovic V, *et al*. A highly sensitive method for the demonstration of carcinoembryonic antigen in normal and neoplastic colonic tissue. *Histochemistry* 1978;**58**:1–11.

[15] Heidl G. Demonstration of carcinoembryonic antigen by means of the immunoperoxidase technique in paraffin-embedded specimens of tumour tissues. *Exp Pathol* 1982;**21**:79–88.

[16] Burtin P, Quan PC, Sabine MC. Non-specific cross-reacting antigens as a marker for human polymorphs, macrophages and monocytes. *Nature* 1975;**255**:714–6.

[17] Gold P, Freedman SO. Demonstration of tumour specific antigen in human colonic carcinomata by immunological tolerance and absorption techniques. *J Exp Med* 1965;**121**:439–62.

Requests for reprints to: Mr R Downing, MD, FRCS, University Department of Surgery, Queen Elizabeth Hospital, Edgbaston, Birmingham B15 2TH, England.