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Immunoscintigraphy of Hodgkin's Disease: *In Vivo* Use of Radiolabelled Monoclonal Antibodies Derived from Hodgkin Cell Lines

Patrice Carde, Ligia Da Costa, Luc Manil, Michael Pfreundschuh, Jean-Denis Lumbroso, Jean-Claude Saccavini, Bernard Caillou, Marcel Ricard, Frédéric Boudet, Marcel Hayat, Volker Diehl and Claude Parmentier

The Hodgkin associated monoclonal antibody (Mab) HRS-1 reacts with Hodgkin and Reed–Sternberg cells (HR-S) in all HD subtypes. HRS-1 Mab was labelled with radioiodine and injected into 10 patients for immunoscintigraphy (IS). Seven patients were injected with HRS-1 Mab radiolabelled with ^{131}I and three patients were injected with HRS-1 Mab labelled with ^{123}I. A control anti-alpha-fetoprotein (anti-AFP) Mab was radiolabelled with another iodine isotope and was injected simultaneously in five cases. Six out of eight patients with proven HD had a true positive scan (nodal, splenic and bony involvement). Imaging was equivocal or failed in the two other patients. In the last two patients IS imaging was truly negative due to the absence of residual HD in one patient and to an erroneous histological diagnosis of HD in another patient. These results, although preliminary, demonstrate that IS with radioiodine-labelled HRS-1 Mab is feasible and may prove to be informative in the staging of HD.

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INTRODUCTION

THE PURPOSE of this study was to evaluate the use of radiolabelled monoclonal antibodies (Mabs) as a reliable non-invasive method for the staging of Hodgkin's disease (HD) and detection of involved sites. The advent of monoclonal antibodies raised against Hodgkin derived cell lines made possible immunoscintigraphy (IS) of human Hodgkin tumors grown in nude mice. In an attempt to image suspected HD, 10 patients underwent IS using one of the Hodgkin associated Mabs, HRS-1 [1].

MATERIALS AND METHODS

Monoclonal antibody (Mab)

The Mab HRS-1 demonstrates a restricted reactivity to Hodgkin and Reed–Sternberg cells and was produced by using the Hodgkin derived cell line L428 [2–4] as the immunogen. The subtype of HRS-1 is found to be a mouse IgG2a [5].

This antibody recognizes a 120 kd glycoprotein (CD30) which is expressed in HR-S cells of all histological subtypes [5] of Hodgkin's disease. In normal lymphoid tissue it only recognizes a minor subpopulation of cells.

The HRS-1 Mab was purified from hybridoma ascites of BALB/c mice by the combination of two chromatographic methods, protein A Sepharose 4B and fast performance liquid chromatography (FPLC), with mono Q columns. The purity was greater than 98% as determined by two-dimensional gel electrophoresis [6] and by high performance liquid chromatography (HPLC).

Immunoreactivity was determined by a cell binding assay using L428 cells and ^{131}I radiolabelled HRS-1 Mabs, and also by immunohistology using the alkaline phosphatase method (APAP) on Hodgkin involved lymph nodes.

Labelling HRS-1 Mabs

Using the iodogen method, approximately 1.7 mg of whole HRS-1 Mab was radiolabelled with 77 MBq (2.08 mCi) of ^{131}I (Table 1) and injected into seven patients. Fragments of HRS-1 Mab were radiolabelled with 152 MBq (4.1 mCi) ^{123}I, and injected into three patients. A labelling efficiency greater than 80% was achieved in all cases. After removal of free iodine, the Mab was sterilized by filtration through 0.22 μm Milex HA filters (Millipore). In addition, a control IgG2a, referred to as AF01 [6] and directed against alpha-fetoprotein (anti-AFP), was labelled with ^{123}I and injected with [^{131}I]HRS-1 Mab into five patients for sample tissue counting. All the Mabs used in the study were found to be apyrogenic and sterile.

Patients

Ten patients reported to have biopsy-proven Hodgkin's disease and no previous allergy were studied (Table 1).

Staging evaluation included biopsies of lymph nodes, bone marrow and other involved organs as well as peripheral blood evaluation. Studies were performed under an IGR-approved protocol and all patients gave their informed consent.

All patients were given 30 drops daily of Lugol's iodide solution for 5 consecutive days prior to and after Mab injection. Mab was administered by i.v. infusion (in 250 ml normal saline over 15–30 min). No clinical or biological reaction to Mab was observed.

Imaging

Scintillation camera images were recorded with a large field-of-view gamma camera within the first 6 h (day 1) until day 6 postinfusion. The [^{131}I]HRS-1 was imaged with a high energy collimator using a 20% window centered over the 364 keV photo-peak. Anterior and posterior whole-body images as well as spot views (10–15 min) were recorded as film or acquired as digital data on a data analyzer. No blood-pool or organ subtraction was performed. Serial images were analyzed with clinically and radiologically drawn regions of interest (ROI) of the anterior and posterior liver, spleen, positive lymph nodes, and bone marrow.

Positive tumor uptake was graded as weak (+) or strong (++). Values were expressed as cpm per pixel corrected for isotope decay and background. A specificity index [7] (SI) estimated the counting on biopsies of specific over control activity in a tumor node and of specific over control activity in normal tissue. SI was calculated in three patients who underwent a lymph node biopsy (with surrounding soft tissue sampling) after they were injected both the [^{131}I]HRS-1 Mab and the control [^{123}I]anti-AFP Mab. The radioactivity of nodal and soft tissue were both counted with a gamma counter in two energy windows for ^{131}I (peak 364 keV ± 20%) and for ^{123}I (peak 159 keV ± 20%). An immunohistological control of the HD material with the HRS-1 Mab by the indirect immunoperoxidase staining technique was performed in all cases.

RESULTS

An overview of the clinical and pathological data is given in Table 1. Eight out of 10 patients had actual sites of Hodgkin's disease involvement. These included: nodes in supra-diaphragmatic areas (patients 1, 3, 4, 5) and infra-diaphragmatic areas (patients 9, 10); spleen (patient 2); bone (patient 6); muscle (patient 10). Two patients (7, 8) were found to be free of HD: one patient with supradiaphragmatic HD whose staging laparotomy turned out to be negative (patient 7) and whose the only node initially involved had been removed for diagnostic biopsy; the other (patient 8) had been referred by another hospital, allegedly for relapsing HD; however, HD involvement was neither observed on sections of the initial biopsy nor at time of relapse leaving only eight patients suffering from HD out of the total of 10 in the series.

A summary of the results (Table 2) showed a true positive imaging in six of the eight patients with actual sites of HD involvement (patients 1, 2, 3, 4, 6, 10). In these six patients all sites evaluated as involved with HD on conventional work-up were actually imaged as positive by IS. The control anti-AFP MAb was injected into two of the six patients who had positive HRS-1 MAb imaging without any demonstrable uptake in HD sites (patients 3 and 4). Immunoperoxidase staining of the histopathology material using the HRS-1 MAb (or the closely related HRS-4 MAb) was positive in all six patients whose IS was also positive with the HRS-1 MAb.

True negative imaging occurred in the two patients who were clinically suspected of having HD and who therefore underwent IS but were found to be free of HD involvement: patient 7 presented as clinical stage I supradiaphragmatic HD but with a palpable tip of the spleen. Faint spleen uptake was indeed

Correspondence to P. Carde.
P Carde, L. Manil, J.-D. Lumbroso, B. Caillou, M. Ricard, F. Boudet, M. Hayat and C. Parmentier are at Institut Gustave-Roussy and U66 INSERM, Villejuif, France, L. Da Costa, M. Pfreundschuh, and V. Diehl are at Medizinische Universitätklinik I, Köln, F.R.G. and J.-C. Saccavini is at ORIS Industrie, Gif-sur-Yvette, France.

Table 1. Hodgkin's immunoscintigraphy: overview of clinical and pathological data in 10 patients; labelling of the HRS-1 and control monoclonal antibodies

Patient	Known site of involvement (R = relapse)	Histology	Injected activity HRS-1 Mab Labelling		Control Mab*	
1	Nodes: cervical, mediastinal	NS	^{131}I 0.5 mg 1.8 mCi			
2	Spleen: (needle biopsy)	MC	^{131}I 0.5 mg 1.9 mCi			
3	Nodes: mediastinal	NS	^{131}I 0.5 mg 1.3 mCi	^{123}I	0.5 mg	2.8 mCi
4	Nodes: cervical	MC	^{131}I 0.5 mg 1.8 mCi	^{123}I	0.5 mg	4.3 mCi
5	Nodes: mediastinal	NS	^{131}I 0.2 mg 1 mCi	^{123}I	2 mg	7 mCi
6	Bone: vertebra, humerus, iliac (R)	NS	^{123}I 2 mg† 6.8 mCi			
7	0: negative laparatomy	NS	^{123}I 2 mg† 4.8 mCi	^{131}I	1 mg†	0.38 mCi
			^{125}I 2 mg† 0.05 mCi			
8	0: abdominal, inguinal nodes	Not HD	^{123}I 0.5 mg† 0.8 mCi			
9	Nodes: abdominal (R)	NS	^{131}I 2 mg 1.8 mCi	^{125}I	2 mg	0.2 mCi
10	Psoas muscle/node (R)	MC	^{131}I 8 mg 5 mCi			

*Control Mab = anti-alpha-fetoprotein.
†$F(ab')_2$.
Abbreviations: NS, nodular sclerosis; MC, mixed cellularity; Mab, monoclonal antibody.

observed at day 1, but subsequently faded away. Splenectomy and staging laparotomy were negative, as was immunoperoxidase staining of the spleen with HRS-1 Mab. Immunoperoxidase staining with the HRS-1 Mab in patient 8 who was proven not to have HD (adenocarcinoma) was also negative.

No patients had false positive imaging in a site which was not involved with HD.

Table 2. Hodgkin's immunoscintigraphy: summary of results in 10 patients

Eight patients with active HD	True positive	Patients 6/8 (patients 1, 2, 3, 4, 6, 10) Sites imaged: 9/11 (82%) cervical/mediastinal nodes, spleen, bone, psoas muscle IS assessment with a control Mab: negative (patients 3, 4) HRS-1 immunohistology: positive (patients 1, 2,* 3, 4, 6, 10)
	Equivocal	1/8 (patient 9) Retroperitoneal relapse in irradiated area
	False negative	1/8 (patient 5) Superimposition with heart shadow: suboptimal HRS-1 quantity: 0.2 mg SPECT unavailable
Two patients with no HD	True negative	2/2 Negative staging laparotomy (patient 7) Retroperitoneal ilio-inguinal nodes of another origin (patient 8) HRS-1 immunohistology: negative (patient 7, 8)
	False positive	None

*Patient 2: immunohistology with the HRS-4 Mab, closely related to the HRS-1 Mab.

Patient 1 had right cervical and anterosuperior mediastinal involvement; there was positive uptake in both regions. One of the nodes imaged with IS was biopsied and showed positive immunoperoxidase staining. Patient 2 had a normal sized spleen (clinically and on CT scan) which showed a ++ positive image (Fig. 1). The spleen was macroscopically normal at laparotomy and normally homogeneous at CT scan examination. Only needle biopsy confirmed the IS findings by showing histological evidence of HD. In contrast to patient 7, whose initial vascular spleen image seen on day 1 vanished thereafter and who subsequently had a negative splenectomy, the splenic image in patient 2 showed increasing contrast with time. In patient 4, IS with 0.5 mg of the HRS-1 MAb labelled with 1.8 mCi ^{131}I showed + + positivity in the cervical nodes (Fig. 2); subsequent work-up showed these to be the only areas involved. Patient 6 relapsed in three bony sites: IS with 2 mg of $F(ab')_2$ fragments of the HRS-1 MAb labelled with ^{123}I (6.8 mCi) showed a ++ uptake in all three sites (Fig. 3). A periosteal paraspinal uptake was also observed at the level of L_3. Patient 10 relapsed exclusively in the right psoas and was imaged (++) by IS with 8 mg of HRS-1 MAb labelled with ^{131}I (5 mCi).

DISCUSSION

There was no definite correlation between the quality of the images and the techniques employed. However in patient 5, for whom clear imaging of mediastinal nodes was not obtained, there are two possible explanations: only 0.2 mg of HRS-1 were injected and the mediastinal nodes were small and superimposed on the cardiovascular image in IS. Conversely, better images were available for patients 6 and 10 who had received a higher activity of ^{131}I, even though tumoral tissue was not bulky. HRS-I $F(ab')_2$ fragments could only be used once (patient 6) and good results were obtained.

IS with HRS-1 MAb may be especially helpful in evaluating the spleen in Hodgkin's disease patients [8]. Infradiaphragmatic involvement is present in approximately 1/3 of HD patients presenting with apparently localized supradiaphragmatic disease. Progress in radiology (lymphangiography, CT scan) and ultrasonography has improved the effectiveness of staging [9]. However, spleen involvement, which is a major prognostic factor [10, 11] and determinant of treatment [12], is under-

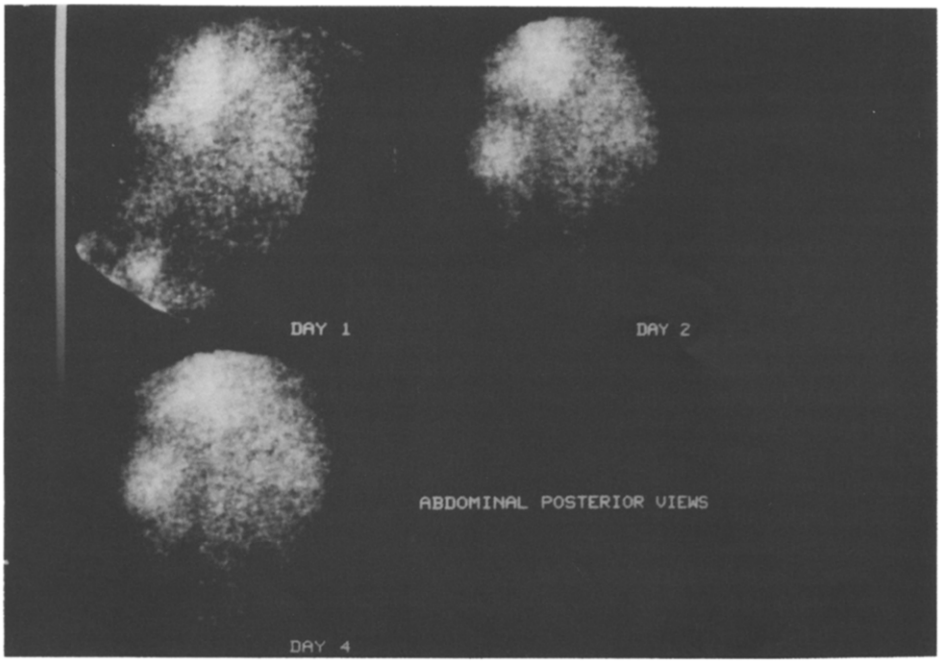

Fig. 1. Positive immunoperoxidase staining of spleen sample obtained in patient 2 by needle biopsy under laparoscopy (the HRS-4 used is a formaldehyde-resistant anti-Reed–Sternberg cell Mab close to the HRS-1 which is more efficient for paraffin-embedded section staining).

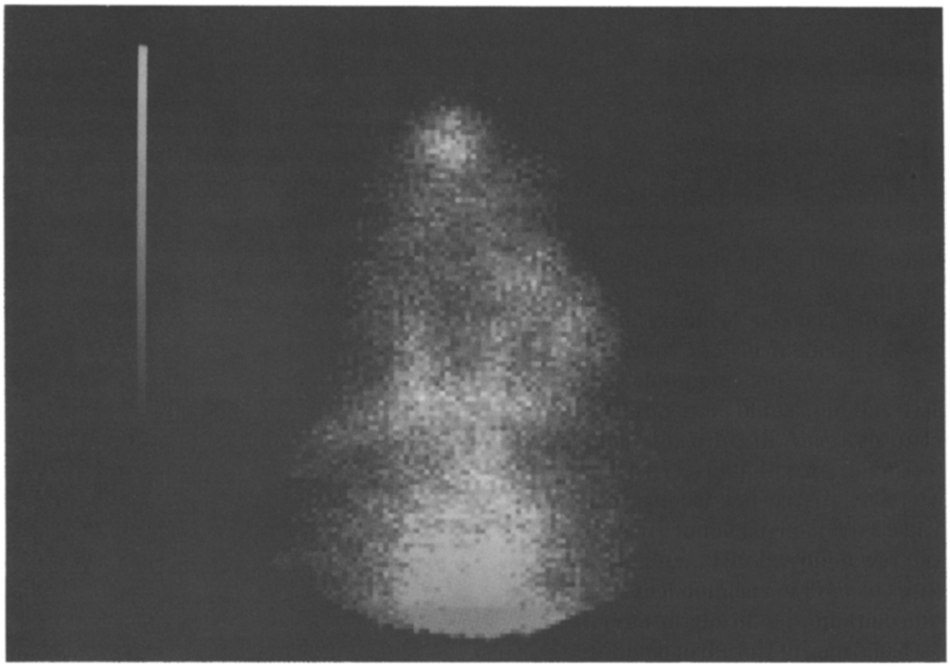

Fig. 2. Positive nodal immunoscintigraphy (++) in left supraclavical nodes in patient 4 after injection of 0.5 mg of the HRS-1 Mab (^{131}I 1.8 mCi). Anterior view on day 4. The ratio of nodal over thyroid uptake is correct. A control MAb labelled with ^{123}I was injected in parallel (on the node biopsied at day 5, the specificity index as 1.3 and a positive immunoperoxidase staining was observed with the HRS-1 Mab).

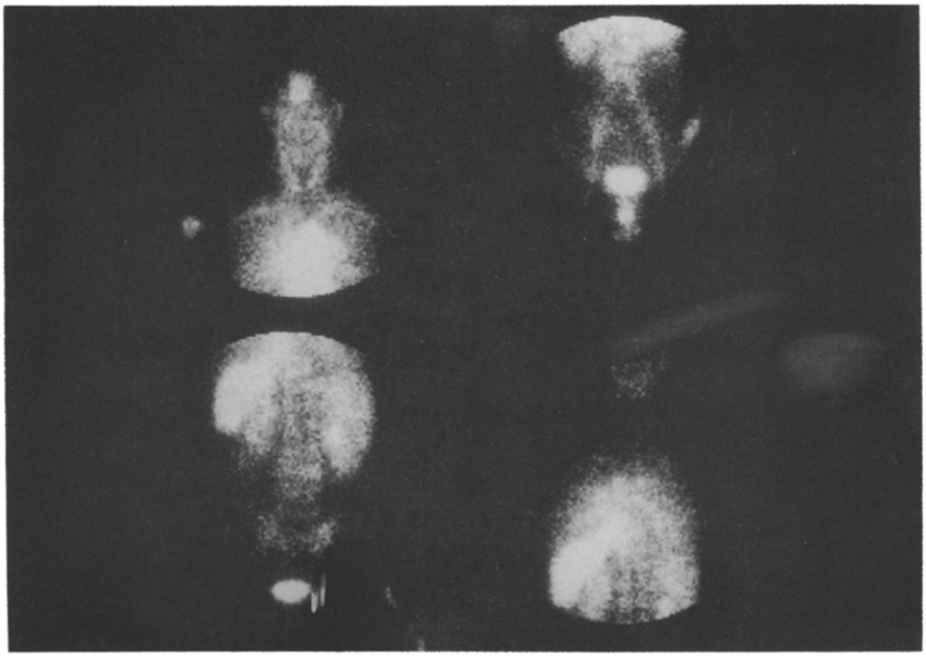

Fig. 3. Positive bone immunoscintigraphy (++) in patient 6. Anterior view. Confinement of the relapse to the three bony spots imaged by the IS was otherwise confirmed: for the L3 vertebra, pain, ^{99m}Tc scintigram, CT scan, NMR; for the left anterior iliac crest, pain, ^{99m}TC scintigram, biopsy; for right superior humerus, pain, ^{99m}Tc scintigraphy).

diagnosed. In supradiaphragmatic presentations, clinically normal spleens may be the only site of infradiaphragmatic involvement (5–10% of cases). Conversely, 1/3 of enlarged spleens are free of disease at pathological examination [13]. Ultrasonography and CT scan are not particularly helpful [9] in detecting HD in the spleen owing to their poor sensitivity (33%), specificity (76%) and accuracy (58%). Splenectomy itself has major drawbacks [14]. IS in the present study has shown encouraging results in the detection of foci of HD. In patient 2, the spleen size was normal, as was the lymphangiogram and spleen density and morphology on the CT scan. Nevertheless, distinct focal positivity was observed with IS and HD involvement was proven histologically. In contrast, in patient 7, the spleen was palpable and visible during the vascular phase at day 1 but the image vanished after 24 h and the spleen was found normal at subsequent staging laparotomy.

CT scan coupled with IS targeting may prove to be of value in some clinical situations. In patient 1, the unsuspected upper mediastinal nodes on chest X-ray were detected on IS and confirmed by CT scan. In patient 3, mediastinal involvement shown by CT scan was best recognized through the double labelling technique comparing the images obtained by the specific HaRS-1 MAb and by the control anti-AFP MAb.

In patient 6, HRS-1 IS demonstrated three bony sites of involvement corresponding to painful and increased ^{99m}Tc diphosphonate uptake areas whereas conventional X-rays were normal. The accessible (anterior iliac crest) involvement was confirmed by biopsy. Although both CT scan and IS were able to recognize a paraspinal mass related to a periosteal reaction at the level of L3, IS allowed a non-active vertebral lesion at the dorsal level (previously irradiated) to be distinguished from an active lesion at L3. Data from this patient provided information on the evolution of IS positivity in relation to treatment: irradiation to the dorsal region was performed 4 weeks prior to IS and the scan was negative at this level; single agent therapy had been given 10 days prior to IS, which was positive at L3. These observations suggest that IS may prove to be an early indicator of treatment response.

Adequate quantities of Mabs, high activity and tomographic reconstruction [15] as in patient 3, or single photon emission tomography (SPECT) may be required when a smaller target volume is superimposed on a vascular area (pericardium, lower mediastinal nodes). An example was the failure to image case 5. IS may be valuable in the appraisal of residual masses after treatment, particularly in the mediastinum. Patients in this category are at higher risk of relapse, but conventional post treatment evaluation usually fails to predict which patients are likely to relapse.

IS has also been attempted in HD using a polyclonal ^{131}I antiferritin IgG since ferritin is present in a variety of solid tumors and in HD in the form of a tumor-associated protein. SPECT imaging and ^{131}I antiferritin antibodies at therapeutic doses have been attempted [17]. Unlike other types of tumors, HD has not exhibited anti-antibodies. The need for 'recycling' with antibodies from different animal species in the event of iterative treatment is thus avoided [18].

The advantages of IS with the HRS-1 Mab in HD patients include the monoclonality of the HRS-1 Mab and because of the purification method its biological behavior is reproducible [6]. The iodogen radioiodination method adequately preserves antibody activity [19]. Along with current research in other tumors [19–23] different isotopes may be tailored to specific HD sites (e.g. ^{131}I for non-hepato-splenic localization).

SPECT is now technically available in many institutions for both ^{131}I and ^{125}I and allows a second control non-specific Mab to be used for both *in vivo* and *in vitro* control of specificity. The

use of HRS Mabs in a larger series of patients should permit correlations with ^{67}Ga scanning to be undertaken and the initiation of immunolymphoscintigraphy studies analogous to those achieved in mycosis fungoides with ^{111}In (T101) Mab [8].

The development of specific Mabs in HD and related disorders may provide new insights into diagnostic procedures and new approaches for *ex vitro* (marrow purging) and *in vivo* treatment.

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Growth Factor and Electrolyte Concentration in Human Breast Cyst Fluid

H. Hamed, D. Y. Wang, J. W. Moore, G. M. G. Clark and I. S. Fentiman

INTRODUCTION

GROSS CYSTIC DISEASE in women has been shown to be associated with a 2–3-fold increase in the risk of breast cancer [1]. Greater increases in risk have been found in a major subgroup where the benign breast lesions contained atypical hyperplastic changes of the epithelium [2, 3].

Correspondence to: D. Y. Wang.
H. Hamed and I. S. Fentiman are at the Clinical Oncology Unit, Guy's Hospital, St Thomas Street, London SE1 9RT and D. Y. Wang, J. W. Moore and G. M. G. Clark are at the Clinical Endocrinology Laboratory, P.O. Box 123, Lincoln's Inn Fields, London WC2A 3PX, U.K.

The concentrations of electrolytes in breast cyst fluid (BCF) have been shown to be correlated with the amount of dehydroepiandrosterone sulphate and the pathohistology of the breast [3, 4]. The suggestion is that the latter is related to subsequent breast cancer risk [3].

Recently it has been suggested that growth factors are important in the autocrine or paracrine control of breast carcinogenesis [5]. We have reported on the concentration of insulin-like growth factor-I (IGF-I) and epidermal growth factor (EGF) in BCF [6]. We now extend those results by reporting on their relationship to electrolyte levels in BCF.