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Breast Cancer and Serum Folate Binding Capacity*

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Abstract—*Total and unsaturated folate binding capacity (TFBC; UFBC) of serum have been measured in 44 normal volunteers, in 48 patients suffering from breast cancer (B.C.) and in 7 patients with cystic mastopathy (C.M.). The values were correlated with the presence of hormone receptors for estrogen and progesterone on the cancer tissue and also with the entity of cancer invasion.*

The mean normal values of TFBC and UFBC were 154 and 27 pg/ml, respectively. Both figures were statistically raised in patients with B.C. An even higher level of TFBC and UFBC was observed when B.C. was associated with C.M., condition which per se determines a raised concentration of TFBC. The amount of TFBC and UFBC was not correlated with the presence of hormone receptors, whereas a significant high concentration of TFBC was noted in patients with metastases.

INTRODUCTION

A SPECIFIC folic acid binding protein (FABP) has been found in many human tissues [1–2] and in serum of a variety of diseases [3–4]. The biological significance of this protein is still debated but some interesting findings recently published raise the possibility that it may play a strategic role in the synthesis of DNA [5]. This hypothesis is based on the high affinity of the binder for 5,10-methylentetrahydrofolate, the active folate essential for thymidilate biosynthesis. The *de novo* synthesis of DNA in chronic leukaemic cells with a high level of the binder is lower than in cells with normal or reduced amount of the protein [6]. FABP may be raised also in some solid tumors [2], probably released from cell into serum [7]. A high concentration of FABP has been detected in serum of women who are pregnant or taking oral contraceptives suggesting a possible role of sexual hormones in the bio-synthesis of the protein [8]. Methods are now available to measure the total folate binding capacity (TFBC) and unsaturated folate binding capacity (UFBC) of serum [3–4].

The aim of the present study was to measure the concentration of TFBC and UFBC in serum of patients with breast cancer (B.C.) and to correlate the results with the stage of tumor and presence of hormone receptors on cancer tissue.

MATERIALS AND METHODS

Radioactive folic acid (37 Ci/mmole) was supplied by Amersham Centre (Great Britain). Activated charcoal and bovine albumin were purchased from Sigma.

Patients

Serum was obtained from 44 normal volunteers aged 20–68 yr (20 males and 24 females, none of them taking oral contraceptives) and 48 patients with breast cancer (B.C.) staged following the TNM criteria suggested by WHO. The age of patients varied from 31 to 76 yr. In 14 patients the B.C. was associated with cystic mastopathy (C.M.): the concentration of TFBC and UFBC in this subgroup was compared with that of seven women with C.M. only. Of the remaining 34 patients, 13 were in pre- and 21 in postmenopausa. Metastases were present in eight patients:

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none of these patients had documented hepatic localisation. The hormone receptors (for estrogen and progesterone) were determined in 16 patients to study their possible relationship with the concentration of TFBC and/or UFBC. The study was performed before any therapy except in three women who previously underwent ovariectomy. The serum was obtained approximately 2 hr after breakfast by venipuncture, removed from clotted blood within 2 hr and stored at −20°C for not more than 15 days.

Determination of TFBC and UFBC

Performed using a recently described radioisotopic method based on the possibility to displace endogenous folate from the binder at low pH [4].

Determination of hormone receptors

The detection of hormone receptors on the cancer tissue was performed following the charcoal-dextran method [9].

Determination of serum folate

The serum folate was measured using a radioisotopic method (Schwarz Mann Folate Radioassay kit ^{125}I).

Statistical analysis

The results were statistically analysed using the *t*-test method.

RESULTS

Normals

The mean TFBC in normal serum was 154 ±53 pg/ml (range 89–257 pg/ml). No difference was found between males and females. The mean UFBC was 27, ranging from 66.2 pg/ml to unmeasurable traces. The mean normal saturation was therefore 127/154 =82%. No correlation was observed between the TFBC, UFBC and serum folate.

B.C. Patients

The mean concentration of serum of B.C. patients was 241 ±62 pg/ml, significantly higher than normal ($P<0.001$). The mean UFBC was 60.5 pg/ml and the saturation was therefore 74% (Table 1 and 2). In 14 patients with B.C. and C.M., the mean TFBC was 289 pg/ml, statistically higher than in both the normal group ($P<0.005$) and in B.C. patients without C.M. ($P<0.02$) (Table 1). The saturation of serum in these patients was also significantly reduced, the mean UFBC being 104 pg/ml (Table 2).

No significant difference was noted when the serum concentration of TFBC of women in premenopausa was compared with that of women in postmenopausa (221 ±86 pg/ml against 203 ±67 pg/ml). The mean UFBC was also similar in pre- and postmenopausa (41.8 ±21 and 49.0 ±24 pg/ml, respectively).

Table 3 shows the amount of TFBC and UFBC in serum of patients with different invasion of cancer: the concentration of TFBC in patients with metastates (M+) was higher than in patients without (M−) ($P<0.01$). As mentioned in Methods, in 16 patients the concentrations of TFBC and UFBC were compared with the presence of hormone receptors: in patients positive for receptors the mean TFBC (227 pg/ml) and UFBC (71 pg/ml) were similar to those found in patients negative for receptors (TFBC 246 and UFBC 59 pg/ml, respectively) (Table 4). In serum of three B.C. patients undergone ovariectomy before our study, the TFBC were 141, 102 and 127 pg/ml and the UFBC 34, 22 and 19 pg/ml, respectively.

Patients with C.M.

In seven patients with C.M. without cancer the mean serum TFBC (278 pg/ml) was higher than normal ($P<0.01$) but not different from that of B.C. patients with C.M. (Table 1). The highest value was 493 pg/ml and the lowest 91 pg/ml. The mean saturation of serum was remarkably lower than normal (43%) ($P<0.005$), even lower than saturation of the patients with B.C. only ($P<0.05$) (Table 2).

DISCUSSION

The method used for determination of TFBC and UFBC is rapid and reproducible. The serum can be stored at −20°C for 15 days without deterioration. The use of albumin-coated charcoal instead of chromatographic method allows to better distinguish between specific and non specific folate binding protein [2]. The serum concentration of TFBC did not differ between males and females. As recently demonstrated, neither TFBC nor UFBC are correlated with the level of serum folate [3, 4]. The saturation of binder in normal serum was 82%: the reason why nanograms of endogenous folate do not saturate the binder may be explained

Table 1. *Mean value of serum TFBC of patients studied, and statistical analysis*

Patients	Number	TFBC (pg/ml ± S.D.)	P_1	P_2	P_3	P_4
Normal	44	154 ± 53	—	—	—	—
Breast cancer	48	241 ± 62	<0.001	—	—	—
B.C. + C.M.	14	289 ± 180	<0.005	<0.01	—	—
B.C. − C.M.	34	198 ± 55	<0.002	<0.005	<0.02	—
C.M.	7	278 ± 177	<0.01	n.s.	n.s.	<0.05

Table 2. *Mean value of serum UFBC and statistical analysis*

Patients	Number	UFBC (pg/ml ± S.D.)	P_1	P_2	P_3	P_4
Normal	44	27 ± 23	—	—	—	—
Breast cancer	48	60 ± 51	<0.02	—	—	—
B.C. + C.M.	14	104 ± 89	<0.02	n.s.	—	—
B.C. − C.M.	34	44 ± 39	n.s.	n.s.	n.s.	—
C.M.	7	121 ± 96	<0.005	<0.05	n.s.	<0.05

Table 3. *Mean value of TFBC and UFBC in B.C. patients with (M+) and without (M−) metastases*

Patients	Number	TFBC (pg/ml ± S.D.)	P_1	UFBC (pg/ml)	P_2
B.C. M+	8	219 ± 61	—	79	—
B.C. M−	20	177 ± 56	<0.01	63	n.s.

Table 4. *No correlation was noted between the TFBC or UFBC and the presence of hormone receptors*

Patients	Number	TFBC (pg/ml ± S.D.)	P_1	P_2	UFBC (pg/ml)	P_3	P_4
Breast cancer	48	241 ± 62	—	—	60	—	—
B.C. Receptors +	7	227 ± 81	n.s.	—	71	n.s.	—
B.C. Receptors −	9	265 ± 83	n.s.	n.s.	54	n.s.	n.s.

with the lower affinity for the binder of methyltetrahydrofolate, the active form of folate in serum [1]. For the same reason the function of the binder should not be the transport of serum folate. It has been documented that FABP may be released from cells [7]; whether that occurred also in our patients remains to be ascertained although this hypothesis fits well with our findings that patients with B.C. had a higher level of serum TFBC than normals, especially when metastases are present.

When the cancer was associated with C.M., condition which *per se* determines a high level of binder, the TFBC was particularly high (Table 1). It has been suggested that the

synthesis of the binder is under hormone control [8]. The concentrations of TFBC and UFBC were similar in patients positive or negative for hormone receptors (Table 4), but in three patients undergone ovariectomy the TFBC was normal or low. However, the high concentration of TFBC found in patients with breast cancer may not be explained on hormonal basis since patients in premenopausa had similar TFBC level to patients in postmenopausa. A high level of binder was found in a variety of diseases, including hepatic disorders [3, 4]. However, since no hepatic localisations of cancer were present in our patients, the possibility has to be ruled out that the liver is the source of binder in our patients.

What is the possible role of binder inside the cell? No definitive explanation is so far available, but some interesting results raise the hypothesis that it may interfere with the pool of intracellular thymidilate. In fact, in presence of FABP, the three molecules (deoxyuridilate, 5,10-methylentetrahydrofolate and thymidine synthetase) essential for the *de novo* formation of thymidilate can not assemble because the high affinity of folate coenzyme for the binder [5]. This finding supports previous observations that the *de novo* synthesis of DNA by leukaemic cell with a raised concentration of FABP is lower than with normal or low concentration [6]. If this involvement of FABP in the regulation of thymidilate pool will be confirmed, FABP may represent a regulatory control mechansim of DNA synthesis. Further studies are necessary to correlate the level of FABP and the proliferation of normal and tumor cells.

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