Eur J Cancer Clin Oncol, Vol. 24, No. 8, pp. 1337–1343, 1988.
Printed in Great Britain

0277–5379/88$3.00 + 0.00

Characteristic Variations of Serum Alkaline DNase Activity in Relation to Response to Therapy and Tumor Prognosis in Human Lung Cancer

ANNA ECONOMIDOU-KARAOGLOU, MAGDA OPSOMER, GENEVIEVE PETIT, MARC LANS, HENRYK S. TAPER and MARCEL B. ROBERFROID

Unité de Biochimie Toxicologique et Cancérologique, Faculté de Médecine, Université de Louvain, UCL 7369, B-1200 Bruxelles, Belgium

Abstract—*The objective of the present study was to evaluate serum alkaline DNase activity (SADA) variations as a useful means of lung cancer monitoring therapy. SADA was measured in 40 patients with non-small cell and small cell carcinomas. Blood samples were collected before (Time 0), during the treatment and months after therapy.*

A decrease in SADA during the first treatment indicates a good clinical response, whereas an absence of decrease indicates a non-response to treatment. In patients who respond to therapy, three types of variations of SADA are observed during the clinical course. A progressive regaining of SADA up to a value largely exceeding the level of the initial SADA value (T0) correlates with a complete remission. An incomplete regaining of enzyme activity corresponds to a partial remission, whereas no regaining of SADA precedes a fatal evolution.

Such variations in SADA observed in the 40 patients with lung carcinomas support our previously published clinical results, confirming that the variations of SADA could be a reliable marker for the therapeutic monitoring of different human malignancies.

INTRODUCTION

RESEARCH continues to investigate the field of new circulating markers due to their utility in assessing response to therapy more rapidly and more accurately than by physical means [1, 2]. Numerous substances have been evaluated for their usefulness as biological markers in small cell (SCLC) and non-small cell (non-SCLC) lung carcinomas [3–6]. However, the need for simple biological tests as a predictor of response to therapy and thus of survival remains an essential problem for lung cancer patients.

Previously published histochemical observations [7–9] have indicated that alkaline DNase is implicated in the process of cancer, and once the tumor is established, its variations are correlated with the response of the tumor to treatment. It has been shown that alkaline DNase is also present in the serum of all mammals [10, 11]. Love *et al.* [12] have observed that there are at least five isoenzymic forms in human serum, and that the major isoenzymic form of human alkaline DNase may originate from the pancreas. However, as yet no definite physiological role has been assigned to alkaline DNase [13].

Our previous clinical studies on alkaline DNase variations concerned different kinds of malignancies [14–17]. These studies have shown that serum alkaline DNase activity (SADA) has characteristic variations once appropriate treatment is given. The good correlation of these variations with clinical and biological parameters suggest that SADA variations could be a useful biological marker to monitor response to treatment and to predict the long-term evolution of cancer patients. SADA also appears to be useful in predicting a recurrence of the disease before clinical signs of relapse in acute non-lymphoblastic leukemia patients [17].

Based on these observations, the present study analyzes the possibility of utilizing SADA as a clinical marker in patients with non-SCLC and SCLC. The measurements of SADA were done before, during and after therapy and are correlated to clinical and laboratory data.

MATERIALS AND METHODS

The study included 40 patients from three hospitals in Belgium; 29 patients were non-SCLC and 11 SCLC patients, at different stages of disease (Table 1A,B). They were classified as having extensive disease (ED) when dissemination outside the

Accepted 1 April 1988.
Address for reprints: A. Economidou-Karaoglou, M.D., Unité de Biochimie Toxicologique et Cancérologique, UCL 7369, B-1200 Bruxelles, Belgium.
This work has been supported by a grant from Sopar S.A. and Ministère des Technologies Nouvelles de la Région Wallonne, Belgique.

Table 1. Clinical data for patients with SCLC (A) and non-SCLC (B) examined for variations in SADA during therapeutic monitoring

Patient No.	Age	Sex	Stage*	Therapy Chemo	Radio	Response†	Duration of response‡ (months)
A. *SCLC patients*							
1	50	M	LD	+	−	CR	12+
2	68	M	LD	+	+	CR	10+
3	72	M	ED	+	−	PR	9
4	65	F	ED	+	−	PR	7
5	65	M	ED	+	+	PR	7
6	69	M	LD	+	−	MR	5
7	64	M	ED	+	+	MR	7
8	54	M	LD	+	+	MR	8
9	76	M	ED	−	+	MR	3
10	65	M	ED	+	+	NR	2
11	63	M	ED	+	+	NR	5
B. *Non-SCLC patients*							
1	59	M	LD	+	+	CR	13+
2	55	M	LD	+	+	PR	8+
3	53	M	LD	−	+	PR	7
4	56	M	ED	−	+	PR	5
5	60	M	LD	−	+	PR	9
6	67	F	LD	−	+	PR	10
7	62	M	ED	−	+	PR	5
8	65	F	ED	+	−	PR	5
9	55	M	ED	−	+	MR	3
10	59	M	LD	+	−	MR	3
11	60	M	ED	−	+	MR	2
12	62	M	ED	+	+	MR	2
13	75	M	LD	−	+	MR	5
14	59	F	LD	+	−	MR	9
15	76	M	LD	−	+	MR	3
16	64	M	ED	+	−	MR	5
17	63	M	ED	+	+	MR	3
18	60	F	ED	+	+	MR	5
19	62	F	ED	+	−	MR	4
20	65	M	ED	−	+	NR	1
21	55	M	ED	+	+	NR	4
22	48	M	ED	+	−	NR	4
23	69	F	ED	−	+	NR	1
24	66	M	ED	−	+	NR	5
25	67	M	ED	−	+	NR	2
26	60	M	ED	+	+	NR	3
27	50	M	ED	+	+	NR	8
28	64	M	ED	−	+	NR	1
29	69	M	ED	−	+	NR	1

*Stage of disease at diagnosis. ED: extensive disease; LD: limited disease.
†Response to therapy. CR: complete response; PR: partial response; MR: minor response with fatal evolution; NR: no response.
‡Duration of response in months after the beginning of therapy. + indicates patients still in remission.

affected hemithorax was proven. Tumor growth confined to the hemithorax was designated as limited disease (LD). The diagnoses were based upon histological as well as cytological examinations of the sputum or pleural effusions. The patients were treated either with chemotherapy and/or radiotherapy according to clinical findings and physicians' decision.

The present study consists of monitoring individual patients and of analyzing the values of SADA by comparing them to the initial activity (*T*0) determined for each case before any treatment.

Serum samples from each patient were collected before, during and after each therapy.

Five to ten milliliters of blood were collected in tubes without anticoagulants. After coagulation the blood was centrifuged and the serum obtained was stored at −20°C. Under these conditions, SADA is stable for more than 6 months.

For the biochemical detection of SADA the gener-

ally known spectrophotometric technique originally described by Loiselle and Carrier [18] was adapted [14]. The results are expressed in International kilo units/liter (kU/l). The intra- and inter-assay coefficients of variation were 3.6% and 8.5%, respectively.

Clinical information concerning diagnosis, treatment follow-up, criteria of tumor response such as physical and X-ray examinations were collected, analyzed and used to evaluate the efficiency of the treatment.

Complete remission (CR) was defined as the total disappearance of tumor at the end of therapy, partial remission (PR) was defined as a decrease of approx. 50% in tumor size after treatment. Initial, but minor response (MR), was defined when tumor regression was less than 50%, and no response (NR) was determined when no regression in tumor size was observed.

RESULTS

At the time of diagnosis, before any treatment (*T0*), the level of SADA varied from patient to patient among the 40 patients with lung carcinomas (Fig. 1A,B). However, several measurements done at different day intervals in sera taken from the same patient before therapy indicated that the individual *T0* levels remained stable (see Fig. 7A,B in Ref. [14]). Since differences in SADA *T0* values existed between the patients examined, each patient was individually analyzed and SADA variations were compared to the *T0* value determined before treatment.

After clinical examinations, 3–4 weeks from the onset of the first treatment, 28 patients had an initial response, whereas 12 patients showed no response.

A decrease in SADA was observed in all 28 initial responders within 1–2 weeks from the beginning of the first therapy (Fig. 1A). These patients presented at *T0* a mean SADA value of 18 ± 3 kU/l. An absence of decrease was seen in the 12 non-responders within the same period of time (Fig. 1B). All the 12 had very low mean values of SADA at *T0* (mean value: 4 ± 0.6 kU/l).

The 12 non-responders who had no decrease in SADA after the first treatment showed no variations of enzyme activity even after successive therapy. Moreover, SADA remained at very low levels all along the follow-up of these patients (data not shown). A lack of therapeutic response was clinically verified for these 12 cases. All the 12 died rapidly during the follow-up.

During the clinical course of follow-up, the 28 initial responders who had a decrease in SADA during their first treatment showed three different forms of clinical responses after successive therapy: (a) complete response; (b) partial response; (c) minor response, but fatal evolution.

Three patients showed a total disappearance of tumor. In these patients, a regaining of SADA exceeding largely, in each case, their initial *T0* value was found (Fig. 2A,B,C). All three are still alive up to date.

Ten patients showed a partial decrease in tumor size and a partial regaining of activity not exceeding

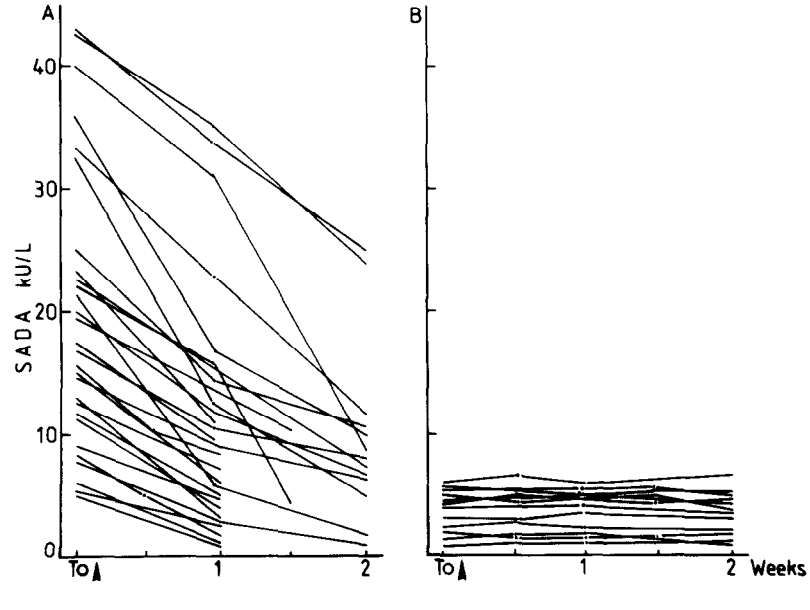

Fig. 1. SADA variations measured from T0 *to the lowest level reached during or after the first treatment in 40 patients with non-SCLC or SCLC. A: 28 patients showing an initial response; B: 12 patients showing no response. The arrow indicates the beginning of therapy.*

the initial T0 value as exemplified by four individual graphs, representing typical SADA variations for this group (Fig. 3A,B,C,D). Seven of these patients died during the clinical course.

Fifteen patients with a minor clinical response had a fatal evolution, and showed no regain of SADA, as exemplified by four typical individual graphs (Fig. 4A,B,C,D). All 15 patients died during the period of observation.

DISCUSSION

The variations of SADA in patients with lung carcinomas confirm our previous clinical results obtained on other malignancies [14–17]. They show that SADA varies characteristically after effective treatment of a tumor. The recapitulative Fig. 5 summarizes all the data of the 40 patients examined in correlation with their clinical response. Indeed, once appropriate treatment is given, a reduction of SADA (with a mean of 50%) within 1–2 weeks from the onset of the first therapy is observed. The observed decrease in SADA may be interpreted as an early sign of good clinical response which corresponds to therapeutically induced tumor necrosis. A lack of tumor response to treatment corresponds to an absence of decrease in SADA.

Although it appears from these results as well as from our previous ones [15–17] that responders have a higher T0 value than the non-responders, this observation requires further confirmation before it can be concluded that T0 activity may have a prognostic value in cancer.

An immediate decrease in SADA which corresponds to an initial response to therapy can be observed approx. 2 weeks ahead compared to the clinical signs of response. However, such an initial decrease in SADA after the first treatment does not necessarily mean that the course of the disease will always be favorable.

The initially responding patients who show a decrease in SADA during their first course of therapy demonstrate three types of further variations after intensive therapy: a progressive regaining of SADA exceeding the initial T0 value corresponds to a complete clinically confirmed tumor regression. An incomplete regaining of SADA corresponds to a partial tumor regression, and no regaining of the enzyme activity is found when a fatal evolution is observed, after a non-significant immediate therapeutic response. In the patients without any early SADA changes, even repeated therapy does not cause significant variations in the enzyme activity.

Since there are individually different T0 values in cancer bearing patients similarly as in healthy individuals (manuscript in preparation), it is worth pointing out again that only the individual follow-

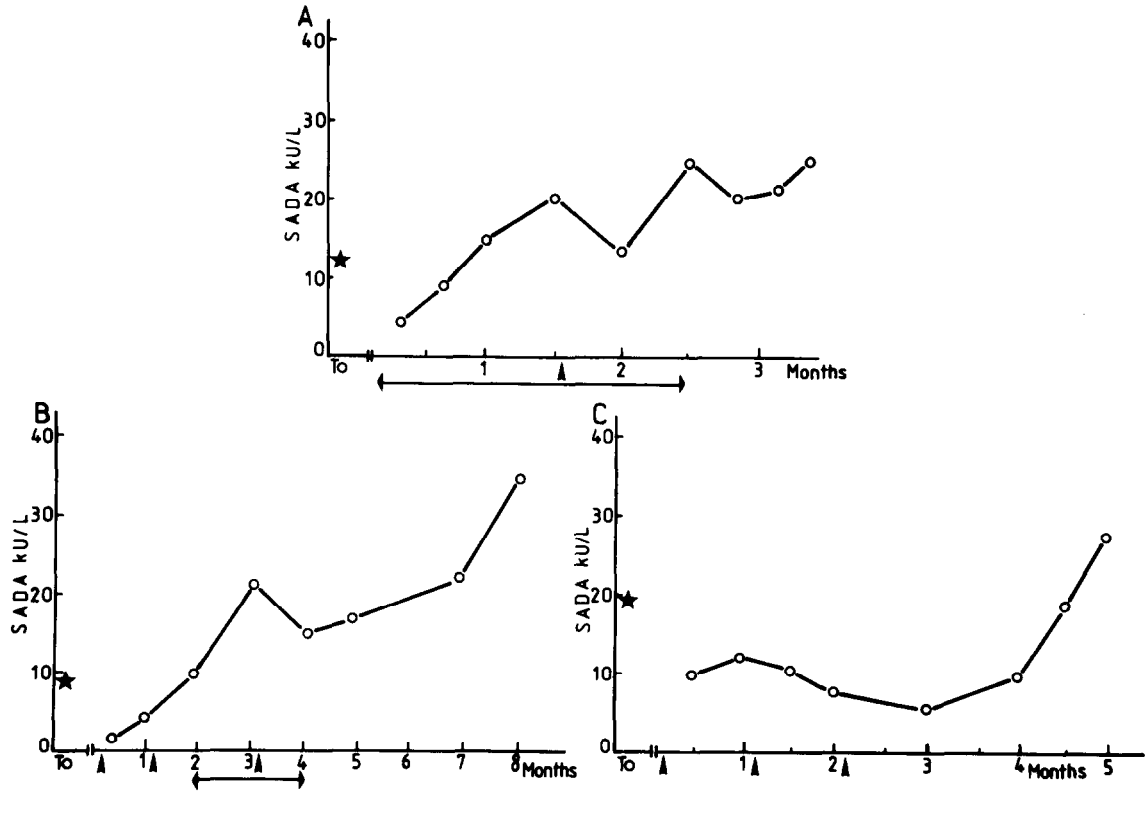

Fig. 2. SADA variations in patients with complete, therapeutically induced remission of malignancy. One patient with non-SCLC (A) and two patients with SCLC (B,C) are presented. The T0 *reference value for each patient measured before treatment is indicated* (*). ▲ *indicates chemotherapy,* (↔) *radiotherapy.*

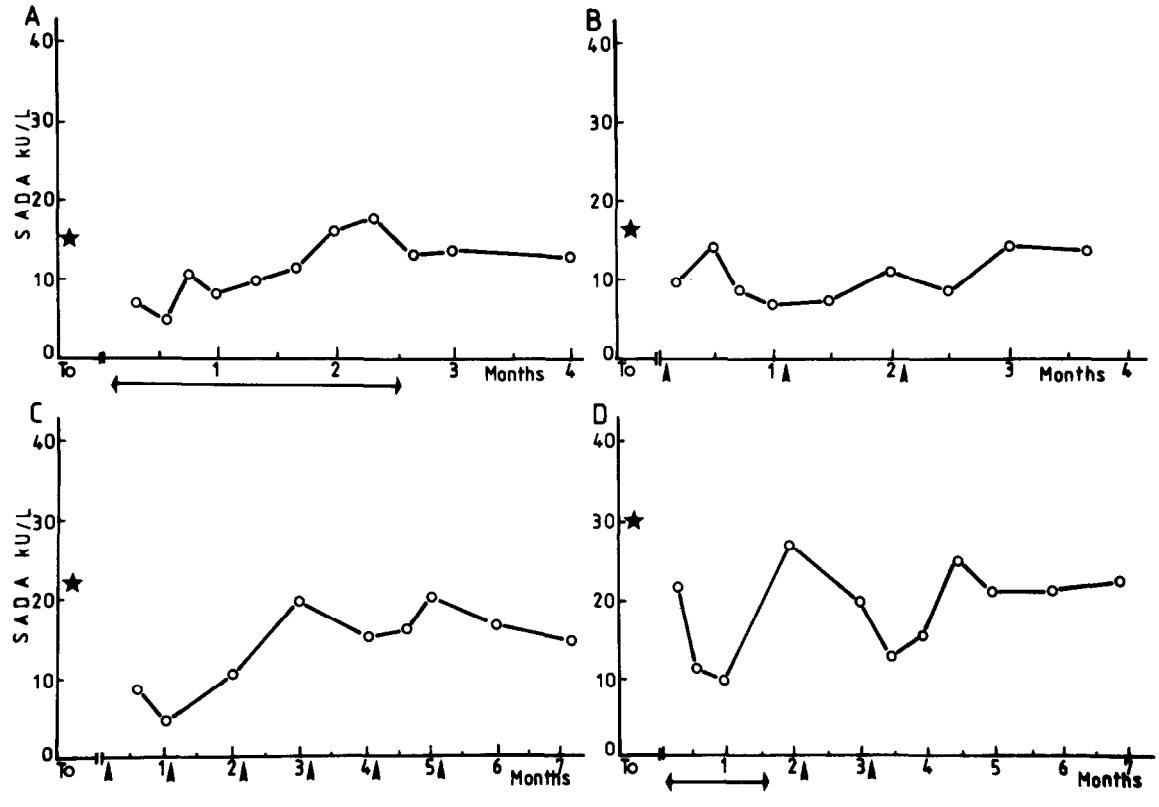

Fig. 3. Typical SADA variations in patients with partial, therapeutically induced tumor remission. Curves of patients with non-SCLC (A,B) and with SCLC (C,D) are presented. The T0 *reference value for each patient measured before treatment is indicated* (*). ▲ *indicates chemotherapy,* (↔) *radiotherapy.*

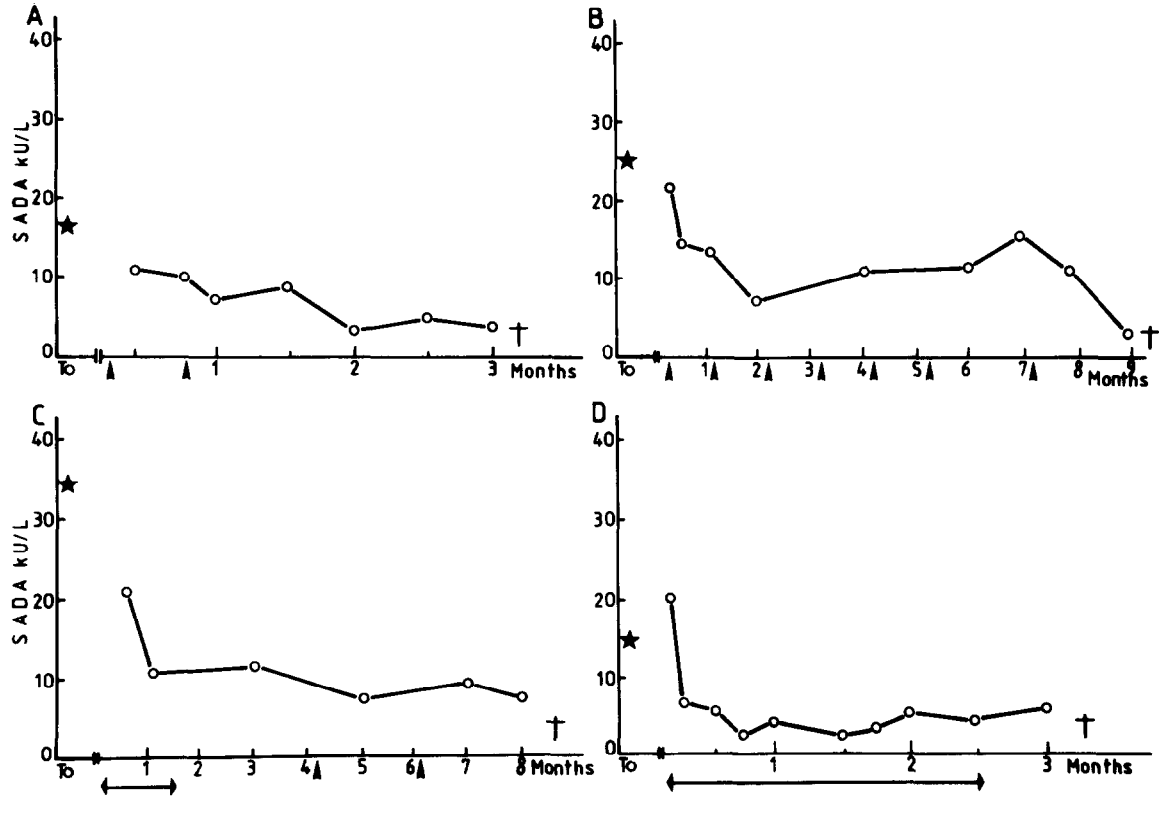

Fig. 4. Typical SADA variations in patients showing an initial response to the first therapy with a fatal evolution. Curves of patients with non-SCLC (A,B) and with SCLC (C,D) are presented. The T0 *reference value for each patient measured before treatment is indicated* (*). ▲ *indicates chemotherapy,* (↔) *radiotherapy.*

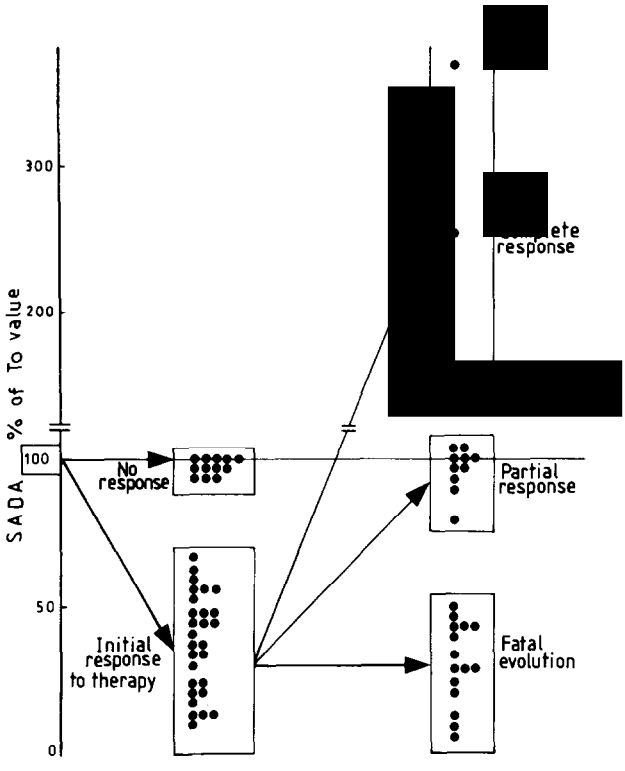

Fig. 5. Relative variations of SADA levels in all the 40 patients analyzed, according to their initial and final clinical response. The T0 value of each patient is considered as 100%.

up of patients and the interpretation of SADA variations during treatment, always compared to the initial *T0* value of each patient, furnish valuable information.

The above described SADA variations can at present be explained only hypothetically on the basis of other observations. It has been shown that viable [7, 19–21] and necrotic cells [9] of malignant tumors have characteristic changes of alkaline DNase activity, which may depend upon natural inhibitor(s), e.g. actin [22–25].

Two hypotheses may explain the direct decrease in SADA after effective treatment: either an inhibitor is released into circulation, or there might be an 'uptake process' of the serum enzyme by the tumor. As membrane alterations occur after effective therapy, this may facilitate the uptake or release processes. Obviously these processes may be mediated by other factors.

At present it is difficult to explain the fact that patients with an initial low SADA value typically do not respond to therapy. But the fact that after an initial response to therapy a rising SADA value correlates with a durable remission could be explained. This may imply a lack of enzyme uptake and/or a decrease in the quantity of the enzyme inhibitor(s), due to the total disappearance of the tumor. Following this hypothesis, we suppose that SADA value largely exceeding *T0* in patients with complete remission could be the level the patient had before he developed his tumor (this aspect is at present under investigation in animal models).

Results obtained throughout the therapeutic monitoring in patients with lung carcinomas support our previous observations [14–17]. They confirm that variations of SADA could be a simple, rapid and valid marker for the therapeutic monitoring of malignant tumors and thus a new means for the evaluation of therapeutic tumor prognosis and disease evolution.

The fundamental mechanisms of the variations of SADA are at present under investigation.

Acknowledgements—The authors thank Dr. C. Deckers, Cliniques St. Luc, Centre des tumeurs et de radiothérapie, Dr. Bauduin, Service d'Oncologie et de Médecine Nucléaire (Jolimont), Dr. E. Salamon Service de Radiothérapie (Clinique St. Elisabeth) and Dr. G. Delzenne for giving all the clinical facilities for this study and Ms. V. Allaeys, Ms. C. Heusdains and Ms. P.S. Dolatabadi for their excellent technical assistance and M.S. Suciu for his valuable comments.

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