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Treatment of Advanced Malignant Melanoma by a Pyrogenic Bacterial Lysate. A Pilot Study

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Summary and Key Words

Fifteen patients (8 female, 7 male) with advanced melanoma received intravenous injections of a pyrogenic bacterial lysate consisting of streptococci and serratia marcescens. In three cases with skin metastases, this kind of treatment resulted in a total and long lasting remission. In another case with inguinal lymphnode metastases, a five month period of stability was achieved. The remaining eleven patients showed further progression of their disease. Side-effects of therapy included fever, nausea, headache, back pain, and – occasionally – herpes labialis. Laboratory analysis revealed a marked increase in the blood counts of monocytes 24 and 48 hours after injection of the bacterial lysate, while other hemopoietic cells did not show significant alterations. During peak temperatures four to six hours after injection, TNF-alpha serum levels were elevated correlating with the increase in body temperature. The increase in concentration of complexes of thrombin-antithrombin III, prothrombin fragments and soluble fibrin during peak temperatures indicate an activation of the coagulation system without evidence, however, of consumption coagulopathy.

Metastatic melanoma · Bacterial lysate · Fever

Zusammenfassung und Schlüsselwörter

15 Patienten mit metastasierendem Melanom wurden mit wöchentlichen i.v.-Injektionen eines fiebererzeugenden Bakterienlysats, bestehend aus Streptokokken und Serratia marcescens, behandelt. Dabei konnten drei vollständige und lang anhaltende Remissionen bei Hautmetastasierung sowie ein fünf Monate anhaltender, stabiler Zustand bei Befall der inguinalen Lymphknoten, im übrigen Progressionen beobachtet werden. Neben Fieber betrafen die Nebenwirkungen Übelkeit, Kopf- und Rückenschmerzen, vereinzelt auch Herpes labialis. Unter den Laboruntersuchungen fiel ein Anstieg der Monozytenkonzentration 24 und 48 h nach Bakterienlysatgabe auf, die lymphozytären Zellpopulationen verhielten sich uneinheitlich. Im Temperaturmaximum, d.h. ca. 4 – 6 h nach Injektion, waren erhöhte TNF-alpha-Spiegel im Serum nachweisbar, deren Höhe mit der Temperatur korrelierte. Weiter konnte im Temperaturmaximum anhand erhöhter Konzentrationen von Thrombin-Antithrombin III-Komplexen, Prothrombin-Fragmenten und löslichem Fibrin eine Aktivierung des Gerinnungssystems nachgewiesen werden, jedoch war laborchemisch kein Verbrauch des plasmatischen Hämostase-Potentials im Sinne einer Verbrauchskoagulopathie nachzuweisen.

Metastasierendes Melanom · Bakterienlysat · Fieber

Introduction

Apart from isolated hyperthermic limb perfusion with cytostatic drugs, no relevant progress in the treatment of metastatic melanoma have been achieved in the last few years [1]. Also, the use of cytostatic drugs and diverse cytokines either as single agent or in combination did not provide a relevant advance [2–5]. Alternatives to these therapies should be taken into consideration.

In 1869, Busch observed an involution of a sarcoma during an erysipelas infection [6]. Numerous reports of tumor regression induced by fever therapy with bacterial derivates followed [7, 8]. Nauts states that body temperatures of 40°C and higher are more often accompanied with tumor regression than lower temperatures. In most of these therapy studies, lysates containing gramnegative (serratia marcescens) and grampositive (streptococci) microorganisms were used as suggested by Coley [7].

Recent observations have indicated a stimulation of the immune response after intravenous application of endotoxin: At peak temperatures, increased IL-1, IL-2, IFN-γ, and TNF secretion were demonstrated. 48 hours after injection of endotoxin, the count of monocytes, NK-cells, B-, and T-lymphocytes increased in the peripheral blood, the ratio of T-helper-cells (CD4+) to suppressor cells (CD8+) was also enhanced [9–13]. These facts justify a phase-I study with a pyrogenic bacterial lysate, in which data concerning the dosage, side effects, and effects on the tumor should be collected.

Material and Methods

The study followed the guidelines of the declaration of Helsinki in the revised version of 1983. The patients were selected under the following criteria: Clinical, histological, photographic, by X-ray, or otherwise diagnosed metastasizing melanoma; age under 70 years; Karnofsky index of at least 80%. Criteria for exclusion were cardiac insufficiency, creatinin level above 2 mg%, brain metastases, seizures. Concomitant therapies such as cytostatics, immuno-suppressive agents including corticosteroids, prostaglandine antagonists or mistletoe extract were not allowed. Other therapy was allowed. In this trial all patients had no prior systemic therapy of their metastasizing disease, with the exception of one female (No. 1 in table 2) who underwent two isolated limb perfusions.

A commercially available autolysate containing streptococci and serratia marcescens (Vaccineurin®, Südmedica, Munich, FRG) was used for fever induction. The product contained the bacteria in stepwise increasing concentrations (1–10 Mill./ml). The endotoxin amount has been verified as between 0.2 ng/ml and 14 ng/ml. Depending on the tolerance of the first injection, the intravenous application was con-

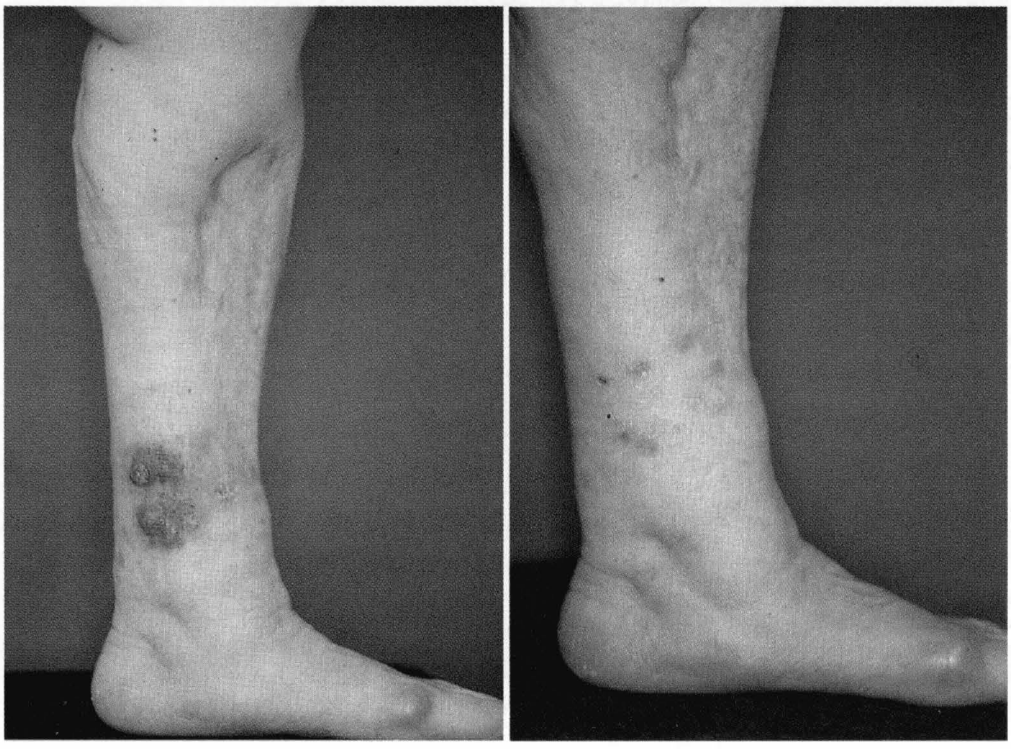


Fig. 1, 2. Regression of a larger metastases area with ulcerous nodules above the outer right ankle after 24 bacterial lysate injections in patient 1. The completely remittant tumors of the inner left calf are not shown.

tinued weekly up to 12 injections. In case of progression a breaking off of the therapy was planned after the sixth fever induction; in case of remission the treatment could be continued even longer than 12 weeks until complete remission was observed.

The manufacturers instructions for a stepwise increasing dose were followed with the aim of maintaining body temperatures of 39°C and more. Patients not achieving this temperature received higher doses with the next injection. No antipyretic medication was given.

Before therapy a complete health examination including tumor staging and routine laboratory analysis was conducted. In cases of striking results, the cardiovascular system was thoroughly examined. The intravenous injection of the bacterial lysate was given at 8 a.m. Patients were sober and had to rest in bed. Blood pressure, pulse, and temperature were measured in half hour intervals. At the temperature maximum reached between noon and 3 p.m., patients were allowed to drink and in the evening to take a light meal. Dismissal followed the day after injection or later in cases of extreme exhaustion. Metoclopramid was given against nausea, Tramadol-HCL against pain. A detailed examination similar to the one taken before therapy was carried out after the sixth and twelfth injection.

Supplementary laboratory tests:

Leukocyte and lymphocyte concentrations, B-cells, T-cells, CD4+/CD8+-quotients, NK-cells, and monocytes were determined through flowcytometrical analysis. The blood was taken at 8 a.m. – with the injection of the bacterial lysate –, at peak temperature, 24 and 48 hours following injection, respectively.

To determine the TNF-alpha (Quantikine-ELISA, British Technology, Ltd.), blood was taken only at the peak temperature, since in five preceding therapies elevated levels could not be measured before nor after therapy [10].

To determine the influence of the coagulation system, in 15 injections, blood was taken before, five, and 24 hours after injection. The thromboplastin time according to Quick (TPT), partial thromboplastin time (PTT), and antithrombin III-activity (ATIII) were determined through routine diagnostic tests. The determination of prothrombin fragments (PTF) and thrombin-antithrombin III-complexes (TAT) were done with ELISA-kits from the Behringwerke AG (FRG); soluble fibrin (SF) was measured using reagents provided by Boehringer-Mannheim, FRG (reagents are not commercially available) [14, 15].

Results

In order to reach a body temperature of at least 39°C, the plan of dosage enhancement given by the manufacturer proved to be helpful. All but two patients (table 2, Patients 8 and 14) reached the desired body temperature after each injection. The side effects are summarized in table 1. Each of the 172 bacterial lysate injections was separately registered. The intensity of complaints such as headaches, back pain, nausea varied intra- and interindividually, i.e. little pain after the first injection and increased pain after the later injections or vice versa. In cases of heavy side effects after the first injection, Tramadol-HCL and/or Metoclopramid were given with the next bacterial lysate injection. Side effects over a prolonged period were not observed. No therapy was interrupted due to side effects.

The effects on the tumor are shown in table 2. We observed three complete regressions of skin metastases and one stable condition, which progressed after five months. The complete regressions were achieved after six, twelve and 24 injections, respectively. All regressions were long-lasting, i.e. until today (32, 21, 15 months). An impressive finding is shown in figures 1 and 2. A 66-year old female, having undergone two isolated hyperthermic cytostatic perfusions without effect, achieved a regression of large ulcerous nodules in the right calf after 24 injections.

The variability of the leukocyte, lymphocyte, B-lymphocyte, and monocyte concentrations are shown in figures 3 to 6. All other parameters were not uniform or showed only little change. At peak temperature, a high TNF-alpha level was seen (fig. 7). This variability correlated with body temperature. After injection of the bacterial lysate the coagulation

Table 1. Side effects. Frequency refered to the total number (n=172) of injections. Intensity graduated according to WHO

Symptoms	Level 0	Level 1	Level 2	Level 3	Level 4
General Health*	0	0	0	0	172
Appetite	42	128	2	0	0
Nausea/vomiting*	25	139	8	0	0
Diarrhea*	165	7	0	0	0
Pain*	41	116	15	0	0
(Fever)	0	4	135	33	0
Herpes labialis	165	7	0	0	0
Hypotonia**	61	108	3	0	0

* only day of injection
** Level 1: RRsyst. < 100 mmHG
Level 2: RRsyst. < 80 mmHG

Table 2. Clinical patient data (CR = complete regression, NC = stable condition, P = progression)

	Sex	Age	Metastases	TNM (UICC 1987)	Inject.	Temp.max (median °C)	Effect on tumor	Survival time (months)
1	f	66	skin (left calf)	N2c	24	39.7	CR	alive
2	m	34	skin (right leg)	N2b	12	39.1	P	10
3	f	62	lung	M1b	12	39.1	P	6
4	m	51	inguinal LN	N	12	39.7	NC (5 months)	32
5	f	69	lung skin (left leg)	M1b	12	39.2	P	16
6	f	48	skin (right thigh)	N2b	12	39.0	P	1h
7	m	43	mediastinum inguinal LN	M1b	9	39.3	P	5
8	f	54	lung	M1b	8	38.8	P	alive
9	f	58	skin (right thigh)	N2b	12	39.2	CR	alive
10	f	50	intra-abdominal inter-vertebral	M1b	6	39.4	P	0,5
11	m	50	skin (trunk, extrem.)	M1a	16	39.2	P	11
12	m	22	skin	N2b	12	39.4	P	alive
13	m	68	liver, paraaort. Inguinal LN	M1b	7	39.1	P	1
14	m	59	ax., inguin. LN skin (trunk)	M1a	6	38.8	P	0
15	m	57	skin left (thigh)	N2b	12	39.4	CR	alive

system was activated. No substantial consumption occurred, however. At the fever peak, approx. five hours after the injection, the concentrations of prothrombin fragments (PTF), thrombin-antithrombin III-complexes and soluble fibrin (SF) increased (in mean) 3.3 to 5.5-fold up to pathological values (table 3). Then it normalized, and, 24 hours later, TAT und PTF concentrations were only slightly higher and the SF level had doubled in comparison to initial values. The TAT correlated with PTF and SF concentrations (n=45)

Table 3. Changes in blood coagulation after 15 injections of the bacterial lysate

	Standard value	Time after injection		
		0 h	5 h	24 h
TPT (s)	100	107.50	98.60	100.80
PTT (s)	35–45	41.10	37.10	44.60
AT III (%)	> 80	104.72	102.17	99.62
PTF (ng/ml)	0.5–1.1	1.31	4.30	1.74
TAT (ng/ml)	< 4.0	5.35	27.90	5.79
SF (µg/ml)	ca. 2.0	2.60	13.40	5.80

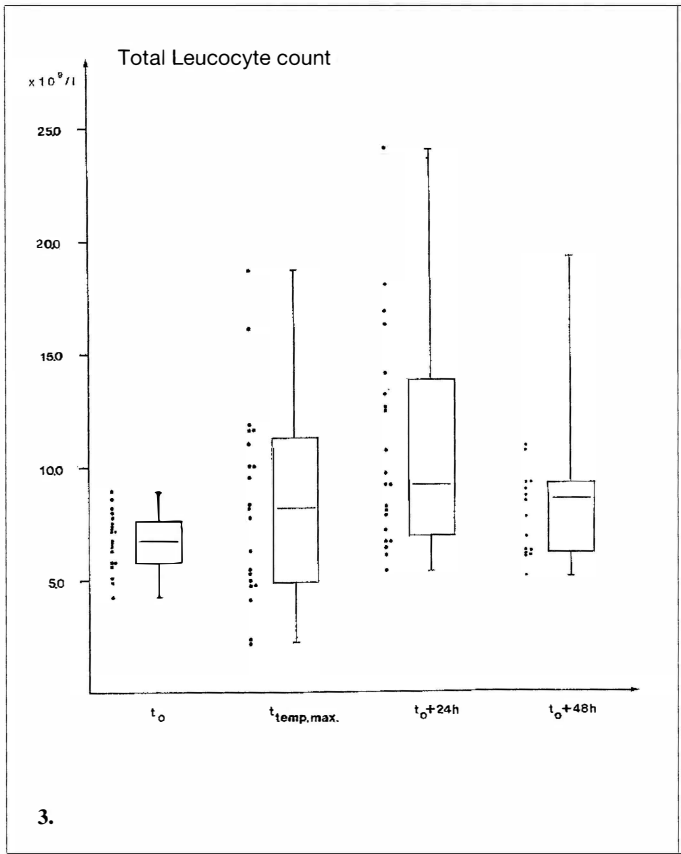
with r = 0.85 and 0.83, respectively. Compared with the distinct increase of thrombin generation and action, the coagulation potential shows only decent consumption: Thromboplastin time and partial thromboplastin time dropped only about 8%, antithrombin III about 5%. Slightly shortened PTT values during the fever peak might indicate a hypercoagulable state.

Discussion

Three complete regressions and one stable condition of 15 cases were achieved by weekly injections of a fever-inducing bacterial lysate of streptococci and serratia marcescens in increasing doses. To our knowledge, studies with bacterial mixtures like the so-called Coley toxin have not been reported in the therapy of metastasizing melanoma. Prior to this study one of the authors (E.G.) had reached a complete regression of lung and liver melanoma metastases using this therapy. Therefore it can be presumed, that this treatment is not only effective for stage III (UICC, 1987).

According to Abel, bacterial derivates are suitable in cancer therapy which as living infectious agents induce a strong reaction with high fever, succesfully defending the injection [8]. In former literature, 'spontaneous' tumor remissions are often reported after erysipelas [16, 17]. The induction of an infection or an infection-like state in cancer therapy was first tried using streptococci and its derivates. Coley added a gramnegative, endotoxin containing agent. In agreement with these reports, some aspects argue for the therapy with autolysed or lyophilized bacteria. This probably explains the observation of Hawkins et al., that a polyribonucleotide derived from streptococci induced a fever reaction, but did not affect the metastasizing melanoma [18]. OK 432 (Picibanil®), a substance produced from the weakly virulent Su-family of Streptococci pyogenes (Group A), has been admitted for tumor therapy by the Japanese Ministry of Health since 1975 [19]. Chiba et al. used this agent in combination with cytostatics in metastasizing melanoma. In a treatment of 15 patients, two achieved a complete and one a partial regression [20]. In an adjuvant setting, Hayasaka combined this substance with DTIC (Dacarbacin) and compared it to a group treated only with DTIC. After 36 months, the survival rate of the 84 patients treated with the combined preparation was 36%, in the 41 patients treated solely with DTIC 20% [21].

The monocytes showed high cell counts 24 and 48 hours after injection of the bacterial lysate. Changes of lymphocyte



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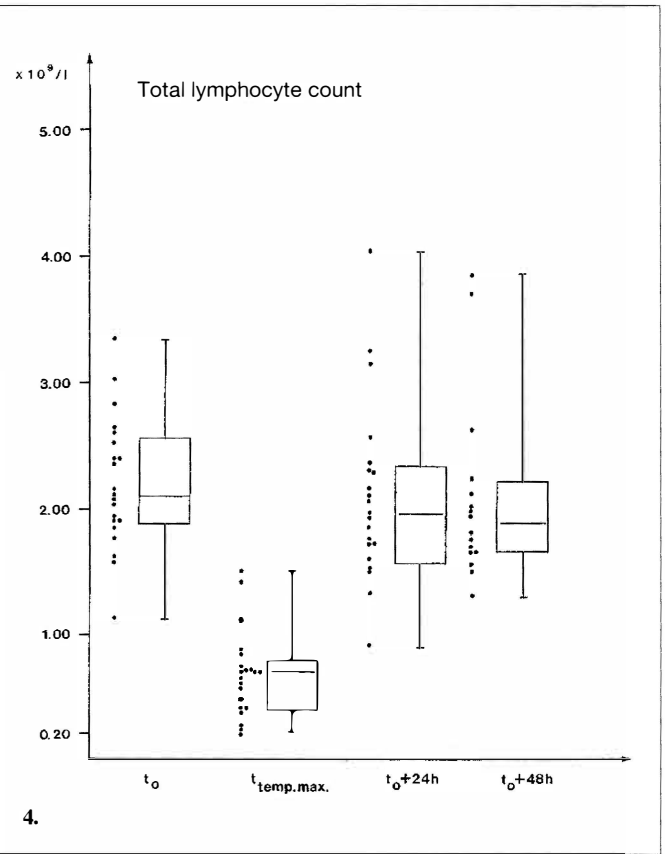

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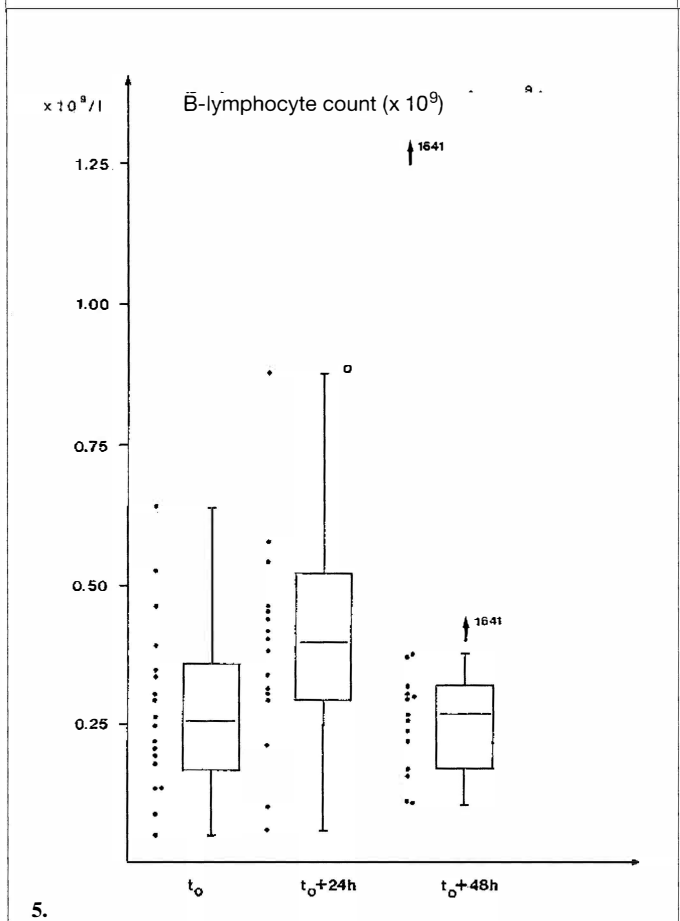

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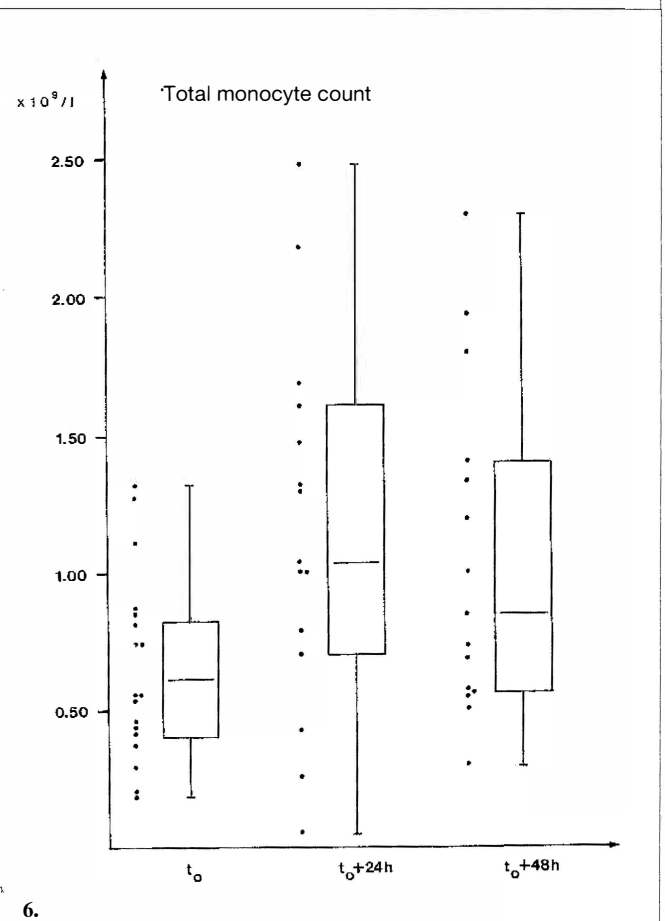

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Fig. 3–6. White blood cell counts (median, quartiles) at different times after bacterial lysate injection (t.). **3.** Total leukocytes. **4.** Total lymphocytes. **5.** B-Lymphocytes. **6.** Total monocytes.

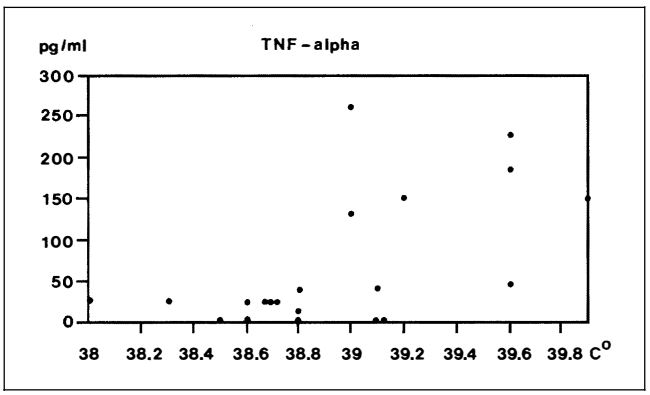

Fig. 7. Serum TNF-alpha level at peak temperature in dependence on the individually observed temperature maximum (21 patient data, 10 patients).

subpopulations differed intra- and interindividually. With respect to these findings it should be stated that the constellation of lymphocytes in blood flow promoting an immunological tumor defense is unknown up to now. In this trial the responders did not differ from the other patients.

At peak temperature, the TNF-alpha level was higher in most cases, depending upon the body temperature. When high TNF-levels are related with the antitumor effect, high body temperatures are necessary for this therapy. Since after administration both of endotoxin and streptococcal derivates high TNF-levels are observed, it remains open, wich agent was responsible for this effect in our trial [13, 22].

The bacterial lysate induced activation of the coagulation system corresponds to the known effect of endotoxin, in which the endothelial cells and tissue thromboplastin play an important role [23]. The activity is so low, that a clinically revelant disturbance such as a consumption coagulopathy seems not to occur. In order to avoid any thromboembolic complication during this therapy, a normal AT III and protein C activity as well as lacking thromboembolic events in history should be demanded.

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