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Original article

Serum cytokine levels correlate with clinical parameters in Hodgkin's disease

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Summary

Background: It has been suggested that cytokines are involved in the pathogenesis of Hodgkin's disease. Enhanced expression of various cytokines has been demonstrated in cell lines and biopsy specimens from patients with Hodgkin's disease (HD).

Patients and methods: In this investigation 14 cytokines were analysed by ELISA in sera of a large panel of patients with HD and compared with clinical and serological parameters.

Results: Increased levels of soluble IL-2 receptors (sIL-2R), IL-6, IL-7, IL-8 and G-CSF, were found in many patients with HD as opposed to healthy individuals. In contrast, IL-1α, IL-1β, IL-2, IL-3, IL-4, TNFα, TNFβ and GM-CSF were rarely detectable. Serum concentrations of sIL-2R, IL-6 and IL-7 were significantly correlated with advanced stage of HD and, together with G-CSF levels, with the presence of B-symptoms. In addition, elevated cytokines correlated with several laboratory parameters. In the majority of patients the serum levels of cytokines decreased after therapy. However, elevated cytokine levels persisted in some patients in complete remission. Patients with normal IL-6 levels had better event-free survivals than patients with elevated IL-6 levels but this difference has not reached significance.

Conclusion: Our results indicate that enhanced levels of sIL-2R, IL-6, IL-7, IL-8 and G-CSF, are correlated with disease activity and clinical symptoms in HD.

Key words: cytokines, serum, Hodgkin's disease, stages, B-symptoms

Introduction

Cytokines are pleiotropic molecules and can act on a variety of normal and malignant cells. A number of reports have described expression of various cytokines (IL-1α, IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, TNFα, TNFβ, GM-CSF, M-CSF, TGFβ, IFNγ) and cytokine receptors (IL-2R, IL-6R, TNF-R) in cell lines and primary specimens from Hodgkin's disease (HD) [1–17]. It has been suggested that these molecules may be involved in the interaction between the tumor cells and reactive bystander cells [18–20] and in the pathogenesis of systemic symptoms such as fever, night sweats and weight loss which are frequently observed in HD patients. Recent studies demonstrated increased levels of sIL-2R, sCD-30, IL-6, GM-CSF, sTNF-R and ICAM-1 in sera of patients with HD [21–30]. We determined the concentrations of 14 cytokines in the sera of a large panel of patients with HD and control donors by ELISA and correlated the expression of these molecules with prognosis and clinical and serological parameters.

Patients and methods

Patients

Cytokine levels were measured in the sera of 210 patients (93 females, 117 males) with newly diagnosed Hodgkin's disease who had been registered in the multicenter trials of the German Hodgkin's Study Group since 1989 (Table 1). Serum samples were collected before treatment and stored at −70 °C until use. The diagnosis was confirmed by histological examination of tumor tissue. Staging was performed according to the Ann Arbor classification system. Patients who suffered from unexplained fever (>38 °C), night sweats or weight loss (>10% of body weight in 6 months) were considered to have B-symptoms. Their ages ranged from 15 to 71 years (mean: 35). All patients were treated according to protocols of the German Hodgkin's Study Group [31].

In addition, sera were taken from 9 patients with newly diagnosed relapse of HD and from 52 patients in complete remission after chemo- or radiotherapy. In 38 cases serum samples were collected and tested before and after therapy. The sera of 95 age and sex matched healthy persons were used as controls.

Cytokine assays

Cytokine levels were determined with commercially available sandwich-ELISAs in accordance with the manufacturers' instructions: QUANTIKINE, R&D Systems, Mineapolis (IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, TNFα, TNFβ, G-CSF, GM-CSF) and CELLFREE, T-Cell-Sciences, Cambridge, MA (sIL-2R and sCD23). Samples were assayed in duplicate and the mean values calculated. The following detection limits (upper limits of the linear range of the standard curve) were found: IL-1α 25 pg/ml; IL-1β 16 pg/ml; IL-2 60 pg/ml; IL-3 16 pg/ml; IL-4 15 pg/ml; IL-6 1 pg/ml; IL-7 6 pg/ml; IL-8 100 pg/ml; TNFα 16 pg/ml; TNFβ 80 pg/ml; G-CSF 16 pg/ml; GM-CSF 4 pg/ml; sIL-2R 50 U/ml; sCD-23 15 U/ml.

Statistics

The Mann-Whitney U-Test and the Kruskal-Wallis H-Test were used to compare cytokine levels in different subgroups of patients. Cor-

relations between cytokine levels and laboratory parameters were assessed by Spearman's rank correlation coefficient. p-values <0.05 were considered as significant. Freedom-from-treatment-failure (FFTF) plots were generated by the Kaplan-Meier method. FFTF was defined as the time from the beginning of treatment to disease progression, failure to achieve complete remission (CR) after therapy, relapse or death. The upper limit of the normal range for each cytokine was defined as the value which exceeds the 95% *percentile* of the healthy persons.

Results

The serum levels of sIL-2R, IL-6, IL-7, IL-8 and G-CSF were significantly higher than those observed in healthy individuals (Table 2 and Figs. 1–4). In contrast, only a few patients had detectable serum concentrations of IL-1α (1 of 38 patients tested), IL-1β (2/39), IL-2 (0/37), IL-3 (5/39), IL-4 (0/38), TNFα (5/39), TNFβ (2/38) and GM-CSF (1/38). Serum levels of sCD-23 were detectable in all 39 patients analysed, but not elevated above those of normal controls (U-Test: p = 0.57/Median: Patients: 129 U/ml; Controls: 163 U/ml). *The median IL-8-values of both patients and control persons were below the detection limit (100 pg/ml). However, U-test revealed significant differences since 46% of patients but only 5% of control persons had values above the detection limit.*

Patients with advanced stages (stages III + IV) had significantly higher serum levels of sIL-2R, IL-6 and IL-7 than did patients with lower stages (*Table 2* and Figs. 1–4). There was no significant correlation between stage and G-CSF or IL-8 level. Furthermore, patients with B-symptoms had significantly higher concentrations of sIL-2R and IL-6 (p < 0.0001). The levels of IL-7 and G-CSF were correlated with B-symptoms to a lesser extent (p = 0.0044; p = 0.0158). Patients with the NS-subtype had higher levels of G-CSF than those with other subtypes (p = 0.0057). Serum levels of cytokines were not correlated with any of the following clinical parameters: bulky disease, massive mediastinal mass, massive splenic involvement, bone marrow- or liver involvement, or age (<30 years vs. 30–50 vs. >50).

There was a significant correlation between IL-6 and IL-8 levels (r = 0.458; p < 0.001), between IL-6 and sIL-2R levels (r = 0.404; p < 0.001) and, to a lesser extent, between IL-7 and G-CSF-levels (r = 0.343; p = 0.02). A number of laboratory parameters were correlated moderately but significantly with sIL-2R, IL-6, IL-7 and G-CSF-levels (Table 3). In contrast, no significant correlations were found between IL-8 and other laboratory parameters.

Cytokine levels were not significantly correlated with response to therapy (complete remission [CR] vs. no CR) or the freedom from treatment failure (FFTF)

Table 1. Characteristics of HD patients.

		n
Stage	I	26
	II	87
	III	64
	IV	33
B-symptoms	A	113
	B	97
Histol. subtype	LP	18
	NS	129
	MC	50
	LD	5
	EC	3
	n.e.	5
Therapy response	CR	158
	no CR	16
	n.e.	36

LP = lymphocyte predominant; NS = nodular sclerosis; MC = mixed cellularity; LD = lymphocyte depletion; EC = epitheloid cell type. NE = not evaluable; CR = complete remission; no CR = partial remission, no change, progressive disease or death before end of therapy.

Table 2. Cytokines in serum and correlation with clinical parameters in patients with HD.

Cytokine	Group	n	% of values above normal range[a]	Median U/ml pg/ml	Signif. U-test p =
sIL-2R	Controls	57	3,5	480	0.0001
	Patients	207	76,8	1950	
	A	111	69,4	1613	0.0001
	B	96	85,4	2698	
	St I + II	111	68,4	1697	0.0001
	St III + IV	96	86,5	2480	
G-CSF	Controls	60	5,0	<16	0.0001
	Patients	161	38,5	28	
	A	89	33,7	22	0.0158
	B	72	44,4	42	
	St I + II	89	37,1	26	n.s.
	St III + IV	72	40,3	32	
IL-6	Controls	47	2,1	<1	0.0001
	Patients	177	72,3	6,7	
	A	95	58,9	2,9	0.0001
	B	82	87,7	12,4	
	St I + II	98	65,3	4,7	0.0036
	St III + IV	79	79,7	12,4	
IL-7	Controls	29	3,4	<6	0.0001
	Patients	87	39,1	12	
	A	50	30,0	7	0.0044
	B	37	51,4	18	
	St I + II	45	31,1	6	0.0049
	St III + IV	42	47,6	17	
IL-8	Controls	43	4,7	<100	0.0001
	Patients	154	46,1	<100	
	A	84	50,0	<100	n.s.
	B	70	41,4	<100	
	St I + II	89	48,3	<100	n.s.
	St III + IV	65	43,1	<100	

n.s. = not significant.

[a] The upper limits of the normal ranges were: sIL-2R = 1100 U/ml; G-CSF = 46 pg/ml; IL-6 = 2,4 pg/ml; IL-7 = 18 pg/ml; IL-8 = 100 pg/ml.

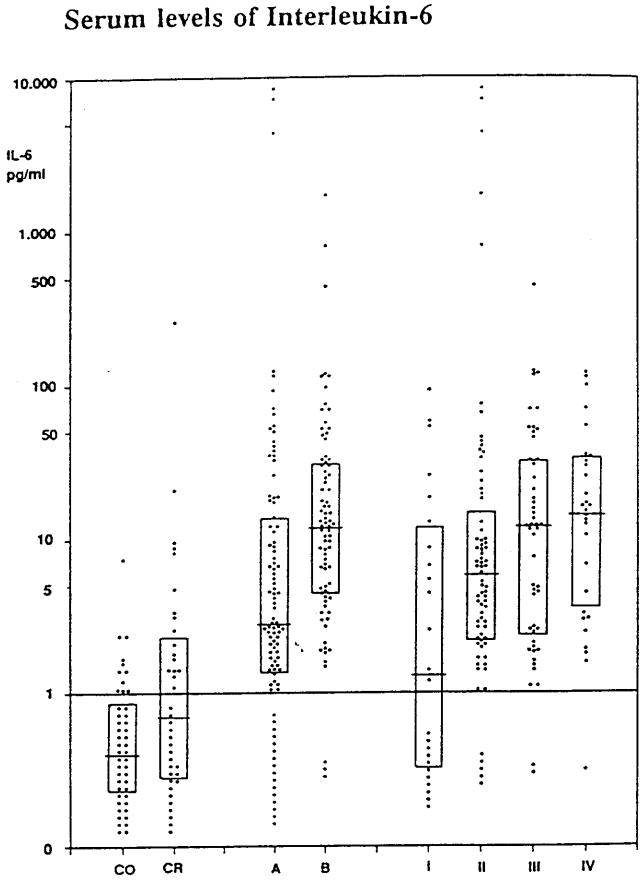

Fig. 1. Serum levels of IL-6 in patients with HD and in control sera (= CO). CR = patients in complete remission; A = pt. without B-symptoms; B = pt. with B-symptoms; I/II/III/IV = pt. in stages I, II, III or IV; horizontal lines represent the median values; boxes mark the 25% and 75%-quartils. Detection limit = 1 pg/ml.

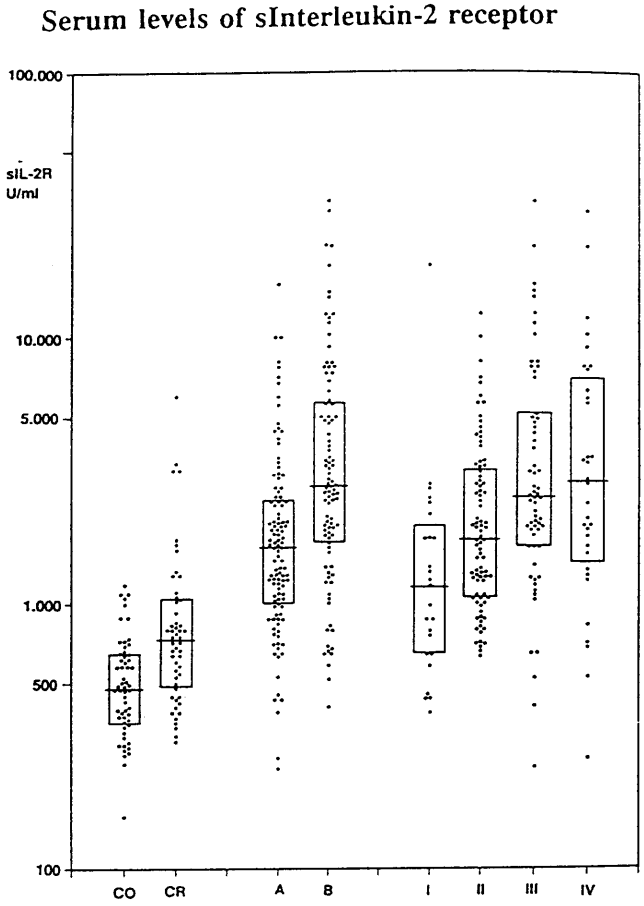

Fig. 2. Serum levels of sIL-2R in patients with HD and in control sera (= CO). CR = patients in complete remission; A = pat. without B-symptoms; B = pat. with B-symptoms; I/II/III/IV = pat. in stages I, II, III or IV; horizontal lines represent the median values; boxes mark the 25% and 75% quartiles.

rate. However, there was a trend toward a better FFTF in patients with normal IL-6 levels as compared to those with elevated levels ($p = 0.053$). The serum concentrations of sIL-2R, IL-6, IL-8 and G-CSF in patients in complete remission were lower than in untreated patients (Table 4 and Figs. 1–3). However, significantly more patients in CR had elevated cytokine levels than did healthy persons.

Eighteen of 52 patients in CR had elevated levels of one or more cytokines. One patient with elevated sIL-2R and IL-6 levels suffered from tuberculosis. Another patient with an elevated IL-8 level had a relapse 7 months later. Five patients without clinical symptoms had only borderline increased values. In 9 cases the reason for the elevation in cytokines remained unclear, and in 2 cases clinical data were not evaluable. Sera from 9 patients with relapse of HD were obtained before subsequent treatment of the relapse. Increased serum levels were observed in 4 of 9 cases (sIL-2R), 4/7 cases (IL-6) and 1/5 cases (G-CSF).

Discussion

This study demonstrates that many patients with untreated HD have increased levels of circulating sIL-2R, IL-6, IL-7, IL-8 and G-CSF. In contrast, detectable concentrations of IL-1α, IL-1β, IL-2, IL-3, IL-4, TNFα, TNFβ and GM-CSF were observed only in single cases. Serum levels of sCD-23 were detectable but not elevated over those of normal controls.

Soluble IL-2R is a released form of the light chain (CD-25) of the high affinity IL-2 receptor. Elevated serum levels of soluble IL-2R mainly indicate T-cell activation and were found in various hematological malignancies such as hairy cell leukemia [32], B-CLL [33], T-CLL [34], lymphoblastic lymphoma [35], ALL [36], non-Hodgkin's lymphoma [37–39] and in HD [21–24].

IL-6 is a pleiotropic cytokine (review in [40]) with effects on numerous cells, e.g., B-cells, T-cells and myeloma cells [41, 42]. Elevated serum levels of IL-6 have been found in various inflammatory diseases and malignant tumors [43–48]. Our results confirm and extend previous studies [26, 27] which described elevated IL-6 levels in HD but which were based on much lower patient numbers.

IL-7 (review in [49]) supports the proliferation of B-cell precursors in the bone marrow. Enhanced IL-7 expression was reported in leukemic cells of CLL

Serum levels of G-CSF

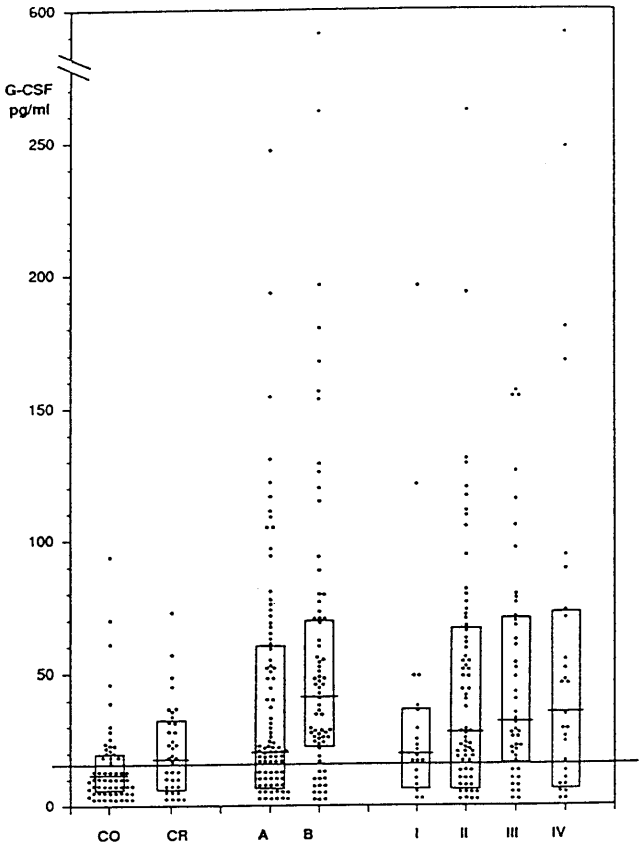

Fig. 3. Serum levels of G-CSF in patients with HD and in control sera (= CO). CR = patients in complete remission; A = pt. without B-symptoms; B = pt. with B-symptoms; I/II/III/IV = pt. in stages I, II, III or IV; horizontal lines represent the median values; boxes mark the 25% and 75%-quartiles. Detection limit = 16 pg/ml.

Serum levels of Interleukin-7

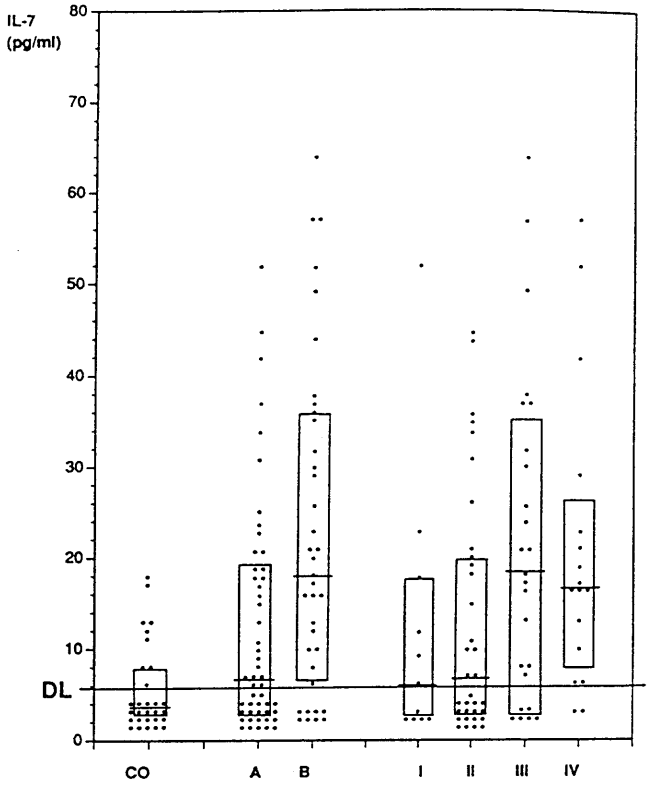

Fig. 4. Serum levels of IL-7 in patients with HD and in control sera (= CO). A = pt. without B-symptoms; B = pt. with B-symptoms; I/II/III/IV = pt. in stages I, II, III or IV; horizontal lines represent the median values; boxes mark the 25% and 75%-quartiles. Detection limit = 6 pg/ml.

patients [50]. G-CSF is a hematopoetic growth factor which stimulates proliferation and differentiation of granulocyte progenitor cells. Increased circulating G-CSF levels have been observed in infections [51], congenital neutropenia [52], various hematological disorders [53] and during reconstitution after chemotherapy [54] own data).

IL-8 (review in [55]) is a chemotactic factor for neutrophils, basophils and T-lymphocytes. Recent studies have shown elevated levels of IL-8 in serum of patients with systemic infection and septic shock [56].

Thus, all cytokines which are elevated in sera of HD are not specific for HD but are also detected in other malignant and non-malignant diseases. H- and RS-cells and established Hodgkin's cell lines have been shown to produce IL-2R [1, 5–7], IL-6 [1, 2, 4, 10] and IL-8 [1, 2] which were detected in serum of HD patients. Moreover, IL-1α, IL-3, IL-4, IL-5, IL-6R, IL-9, TNFα, TNFβ, GM-CSF, M-CSF, TGFβ and IFNγ are expressed by tumor cells [1–17]. It has been suggested that the characteristic histological pattern of HD with a small number of malignant H- and RS-cells surrounded by many reactive cells might be due to the release of cytokines by the tumor cells. It is also possible that the reactive non-malignant cells are the main producers of the circulating cytokines. In support of this hypothesis is the fact that elevated levels of cytokines were also observed in patients with a complete remission after therapy of HD.

Many patients with HD (46.2% in our study) suffer from systemic symptoms such as fever, night sweats and weight loss. It has been proposed that clinical symptoms in HD may be caused by cytokines produced by H/SR or reactive cells. In our study, a significant correlation was found between elevated IL-6 levels and the presence of B-symptoms. IL-6 is considered to be involved in the regulation of the acute-phase response [43]. Since IL-6 induces fever and chills [57] we hypothesize that IL-6 plays a central role in the generation of systemic symptoms of HD patients. It is likely, however, that additional factors are involved, since some patients without B-symptoms had very high IL-6 levels and others with B-symptoms had normal IL-6 levels. In addition, the response to IL-6 in different patients may vary. The correlation between IL-6 and B-symptoms was also described by Kurzrock et al. [27] in a small group of patients. In our study only a few patients had detectable serum levels of IL-1 and TNF, which are also known to be pyrogenic.

Furthermore, this analysis confirms the significant

Table 3. Correlation of cytokine levels with laboratory parameters.

	IL-2R	G-CSF	IL-6	IL-7
ESR (1 h)	0.383 $p<0.001$	W	0.445 $p<0.001$	W
Neutroph.	W	0.375 $p<0.001$	0.304 $p<0.001$	n.s.
Lymphoc.	n.s.	n.s.	W	−0.338 $p=0.004$
Platelets	W	n.s.	0.320 $p<0.001$	0.333 $p=0.003$
Hb	−0.328 $p<0.001$	W	−0.393 $p<0.001$	W
Albumin	−0.377 $p<0.001$	n.s.	W	−0.321 $p=0.009$
α2-globulin	W	n.s.	0.312 $p<0.001$	n.s.
Alk. phos.	0.353 $p<0.001$	W	0.317 $p<0.001$	n.s.
LDH	0.396 $p<0.001$	W	W	n.s.

Boxes show Spearman's correlation coefficient and p-values. n.s. = not significant; W = significant but weak correlation ($-0.3 < r(s) < 0.3$).

Table 4. Analysis of cytokine levels in HD patients in complete remission.

Cytokine	n	% Above normal range	Median U/ml pg/ml	Comparison with pt. before therapy p =	Comparison with normal controls p =
sIL-2R	52	19	742	0.0001	0.0001
IL-6	38	24	<1	0.0001	0.0120
G-CSF	36	8	18	0.0016	0.0130
IL-8	23	22	<100	0.0340	0.0260

correlation between sIL-2R and the presence of B-symptoms [21–24]. IL-7 and G-CSF were correlated with B-symptoms to a lesser degree. The functional association between these cytokines and B-symptoms, however, remains unclear. Serum levels of sIL-2R, IL-6 and IL-7 were significantly correlated with advanced stages. This suggests an association between progression of HD and higher levels of these cytokines. Our results are in agreement with findings of previous studies [30, 32, 33] in which higher sIL-2R levels were also observed in patients with advanced stages of HD. Our analysis did not show a significant correlation of cytokines with overall survival or treatment failure. Pui et al. [21] and Gause et al. [24] reported a better long-term prognosis in patients with lower sIL-2R values, whereas Ambrosetti et al. [23] found no significant differences. Kurzrock et al. [27] observed a better survival in patients with relapsed HD with lower IL-6 levels.

From our results we conclude that a variety of cytokines seems to be involved in the biology of HD and the development of clinical symptoms. Moreover, cytokines are correlated with disease activity and tumor spread but the prognostic value is low compared to parameters such as stage or B-symptoms. Simultaneous investigation of cytokine expression in tumor tissue and in serum might help to elucidate the origin and significance of cytokines in HD.

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