ARTICLE

Haptoglobin-related Protein as a Serum Marker in Malignant Lymphoma*

Ron EPELBAUM,[1] Channa SHALITIN,[2] Ruth SEGAL,[1] Clari VALANSI,[2] Ida ARSELAN,[3] David FARAGGI,[3] Michelle LEVIOV,[1] Menahem BEN-SHAHAR,[1] Nissim HAIM[1]

[1]Department of Oncology, Rambam Medical Center; [2]Department of Biology, Technion-Israel Intitute of Technology; [3]Department of Statistics, University of Haifa; Haifa, Israel

A novel serum 21 kDa haptoglobin-related protein (Hpr) was investigated in patients with malignant lymphoma, to evaluate its correlation with clinical and histologic features at presentation and its possible role as a tumor marker for patient outcome. One hundred fifty eight serum samples were taken from 88 patients with non-Hodgkin's lymphoma (n=58) and Hodgkin's disease (n=30) at presentation and in the course of follow-up. Sera from 61 healthy volunteers served as normal controls. Serum Hpr levels in the lymphoma patients (median $430x10^3$ u/ml, range 0-$4000x10^3$) were significantly higher than in the control group (median $68x10^3$ u/ml, range 0-$180x10^3$) (p=0.0001). Higher median Hpr values were detected in patients with advanced disease (p=0.013), "B" symptoms (p=0.029) and in males (p=0.053). There was also a significant correlation between Hpr and erythrocyte sedimentation rate (p=0.028). Serial determinations showed a significant decrease of the initial Hpr values obtained after treatment in 41 patients, 38 of whom achieved complete remission. In the follow-up period additional Hpr measurements were taken from 17 patients. Three of them eventually relapsed, and showed increased Hpr levels at the time of relapse. Hpr levels remained low in 11 of 14 patients who maintained complete remission, and increased in three. In conclusion, serum Hpr is a new serum tumor marker of potential use in the clinical setting of lymphoma. (Pathology Oncology Research Vol 4, No 4, 271–276, 1998)

Key words: Haptoglobin-related protein, 21-kDa protein, ELISA, lymphoma

Introduction

The outlook for lymphoma patients is heterogeneous. Although a substantial proportion of patients achieves complete remission, some of them may either do not response to treatment or experience relapse. The evaluation of the effectiveness of induction therapy is important, as well as early detection of relapse, since proper first and second line treatments can lead to cure or can have a significant impact on the course of disease. In this respect, laboratory evaluation may serve as additional tool for the clinical assessment of patients. However, the biochemical monitoring of lymphoma poses many problems due to the lack of any specific markers. Serum concentration of lactate dehydrogenase (LDH) and β2 microglobulin, as well as various cytokins, has been claimed to be associated with adverse disease features and to have prognostic significance.[1-3] Longitudinal studies have demonstrated that they might be used for monitoring response to treatment and detection of relapse. Yet, their utility in the clinical setting of lymphoma is limited.

We have detected a novel 21 kDa protein in sera of patients by enzyme-linked immunosorbent assay (ELISA), using polyclonal anti-p21 antibodies. While only 4.6% of

Received: August 12, 1998; *accepted:* Sept 30, 1998

Correspondence: Dr. Ron EPELBAUM, Department of Oncology, Rambam Medical Center, Haifa 31096, Israel; tel: 972-4-8543128, fax: 972-4-8542929

*This work is dedicated to the memory of Dr. Arie H. Bartal, a dedicated oncologist and friend. This work was supported by Chemotech Thechnologies Ltd., by grant no. 3676 from the Chief Scientist's Office of the Ministry of Health, Israel, and by the Fund for Promotion of Research in the Technion.

 1219-4956/98/040271+06 $ 12.00/0

the healthy donors showed p21 serum levels higher than their mean ± 33 to 80% of the 94 cancer patients with various tumors exceeded this value and were considered positive in the ELISA test. In particular, patients with malignant lymphoma, urogenital and gastrointestinal tumors had a 2.8- to ninefold increase in p21 serum levels.[4] Western blots of haptoglobin-hemoglobin complexes, isolated from representative sera of these patients, immunoreactive with anti-haptoglobin antibodies revealed a novel haptoglobin (Hp) complex which does not bind hemoglobin. Using high-resolution two-dimensional electrophoresis, it was demonstrated that this Hp-related protein (Hpr) contained an α chain which has the same molecular weight as normal α2 chains – 21kDa, but with a more basic pI.[5] These findings are in accord with the amino acid sequence deduced from Hpr cDNA sequence previously described by Tabak et al.[6]

The Hp locus is located on chromosome 16. 2.2 kb downstream of the Hp gene another gene, Hp-related, was described.[7] In the hypothetical coding region of Hp-related gene, the major difference from Hp is to be noted in the first intron, which contains remnants of a retrovirus-like element.[7] Nuclear run-on experiments have shown that Hp-related gene is not transcribed in either fetal or adult liver.[8] Tabak et al has recently shown that the Hp-related gene is expressed in tumor cells.[6] Microsequencing of the 21kDa band isolated from ascites fluid of a colon cancer patient revealed two peptides, Hp and Hp-related α chains, comigrating on first dimension SDS-PAGE. The N-terminal amino acid sequence of Hp-related α chain, detected only after CnBr cleavage,[5] coincided with the amino acid sequence deduced from the Hp-related cDNA sequences previously determined by Tabak et al.[6]

In the current study, we have extended our work on lymphoma patients, and examined the correlation between serum Hpr levels and clinico-pathologic features at presentation, as well as response to treatment and follow-up of patients.

Materials and Methods

Patients and sera

One hundred fifty eight samples were taken from 88 patients with non-Hodgkin's lymphoma (NHL) (n=58) and Hodgkin's disease (HD) (n=30) at presentation and in the course of follow-up, between 1992 and 1994. Patients' pretreatment characteristics are summarized in *Table 1*. Forty-two (48%) were males and 46 (52%) were females, with a median age of 48 years (range, 18-87 years). Thirty-six (41%) patients had bulky disease (10 cm, or mediastinal lymphadenopathy ≥ 1/3 maximal width of chest), 26 (30%) had systemic symptoms and 34 (39%) had extra-nodal involvement. All patients were staged according to the Ann-Arbor staging system.[9] To determine the extent of disease each patient underwent a complete history and physical examination, routine laboratory tests, imaging procedures (computed tomography of chest, abdomen and pelvis, and whole-body gallium scan) and bone-marrow biopsy. Forty-one (47%) of the patients had advanced stage III–IV disease at presentation. Histopathologic grading of NHL patients and classification to low-grade versus aggressive intermediate and high-grade lymphoma was done using the criteria of the International Working Formulation.[10] Seventy-six percent of NHL patients had aggressive subtypes of disease. Treatment varied according to type of disease and stage. The treatment of HD patients was based mainly on the hybrid combination of mechlorethamine, vincristine, procarbazine, prednisone and doxorubicin, bleomycin and vinblastine, with or without irradiation. Cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) or CHOP-like regimens were used for patients with aggressive NHL, while those having low-grade NHL received single agent or various chemotherapy regimens. Complete response (CR) was defined as disappearance of all clinical evidence of active disease. Patients achieving less than CR were considered non-responders. Serum samples from 61 healthy

***Table 1.* Clinical features of 88 patients with lymphoma**

	Patients	
	No.	*%*
Disease		
Hodgkin's	30	34
Non-Hodgkin's	58	66
Age		
Median	48	
Range	18–87	
Sex		
Male	42	48
Female	46	52
Stage		
I	18	20
II	29	33
III	14	16
IV	27	31
"E" Site		
Yes	34	39
No	54	61
B Symptoms		
Yes	26	30
No	62	70
Size		
Bulky	36	41
Non-Bulky	52	59

normal volunteers similar for age (median age 43 years, range 20-87) and sex (43% males and 57% females) were collected over a similar period of time. Sera were stored at –20°C until used. Just before the assay, sera were heated 30 minutes at 56°C to inactivate complement and a serum interference factor.

Anti-p21 polyclonal antibodies

Anti-p21 polyclonal antibodies (pAb) were produced by immunization of rabbits using p21 protein (Hp-related α chain) isolated from cancer patients by SDS-polysacrylamide electrophoresis under reducing conditions.[11] Anti-p21 pAb were shown to be immunoreactive with recombinant Hp-related α chain (Shalitin et al, data not shown).

ELISA method

Polyvinyl microtiter plates (Costar) were coated with 200 µl/well anti-Hpr antiserum as capture antibodies (at a 1000-fold dilution in PBS pH 7.4 containing 2% (wt/vol) nonfat dry milk (Cadbury's Marvel) at 4°C overnight. Plates were washed three times with TBST (200 µl/well) (50mM Tris-HCl pH 8.0, 150 mM NaCl containing 0.05% Tween 20) and the excess detergent was removed by further washing the plates with TBS (200 µl/well) (50mM Tris-HCL pH 8.0, 150 mM NaCl) three additional times. Normal and cancer patients' sera were serially diluted (1/2000-1/10,000) with carbonate buffer (25 mM Na_2CO_3, 55 mM $NaHCO_3$, pH 9.6, containing 1 mM PMSF) and incubated overnight at 4°C. Plates were washed twice with phosphate buffered saline (pH 7.4). Plates were coated for 90 minutes at room temperature with PBS containing 3% (wt/vol) nonfat dry milk (Cadbury's Marvel). Antigen capture plates were incubated 60 minutes at 37°C with 100 µl/well of anti-p21 pAb at 1/1000 dilution in PBS containing 3% (wt/vol) nonfat dry milk. Then the plates were washed three times with TBST (200 µl/well). Antigen-antibody complexes were detected by incubation with biotinylated goat anti-rabbit IgG (from Sigma) (1/1000 in TBST) for 30 minutes at room temperature. The plates were washed three times with TBST. Finally, ExtrAvidin Peroxidase (from Sigma) 1/500 in TBST was added for 30 minutes at room temperature and the plates were washed three times with TBS. The solid phase bound enzyme was detected using a chromogenic substrate (100 µl/well) containing 0.05% o-phenylenediamine and 0.05% hydrogen peroxide in 0.1M phosphate citrate buffer pH 5.0. After 10-30 minutes, the reaction was stopped by the addition of 50 µl of 2N H_2SO_4 and color yield was assessed at 492 nm using an Anthos Labtech 2001 reader. Negative controls included buffer instead of sera to measure nonspecific binding of avidin-biotinylated peroxidase complex which yielded a reading of 0.1-0.2 A492. Serum Hpr values were calculated from a linear calibration curve obtained from a positive serum sample yielding a reading of 0.8-0.9 A492 for 100 arbitrary units of Hpr per ml at a dilution of 1/10,000.

Statistical Analysis

Results are reported as median Hpr serum levels. Differences between groups of patients were compared using Wilcoxon 2-sample test. The Wilcoxon sign-rank test for paired observations was used to analyze Hpr values in different disease phases. Correlations were calculated using the Spearman rank-sum correlation coefficient. Survival analysis was performed using the log-rank test.

Results

Pretreatment sera

As shown in *Figure 1*, patients with lymphoma had significantly higher Hpr concentrations (median, $430x10^3$ u/ml; range, 0 to $4000x10^3$) than normal controls (median, $68x10^3$ u/ml; range, 0 to $180x10^3$; p=0.0001). We examined the Hpr levels of patients within various prognostic categories *(Table 2)*. Significantly higher median Hpr values were detected in patients with stages II to VI (stage II-VI vs stage I, 565 vs $225x10^3$ u/ml, p=0.012), in patients with "B" symptoms ("B" vs "A", 600 vs $275x10^3$ u/ml, p=0.029) and in male patients (males vs females, 610 vs $395x10^3$ u/ml, p=0.053). Hpr levels did not correlate with size of disease, although a trend towards more elevated values was observed in patients with bulky disease (median, $577x10^3$ u/ml) compared with non-bulky disease (median $330x10^3$ u/ml, p=0.086). Serum Hpr levels were not significantly different between patients aged under or over 60 years, in patients with or without extranodal involvement and in the various histologic subtypes, whether HD vs NHL

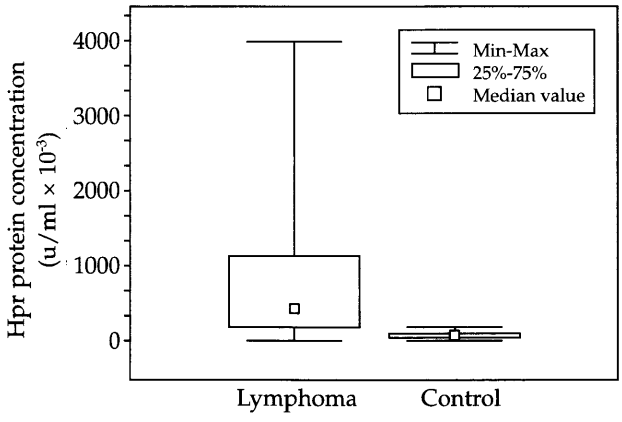

Figure 1. *Serum haptoglobin-related protein in untreated patients with lymphoma (n=88) and in healthy controls (n=61).*

Table 2. **Hpr levels and disease characteristics**

Category	*No. of Patients*	*Hpr (u/ml x 10^{-3})*		*P*
		Median	*Range*	
Stage				
I	18	225	26–1520	0.012
II–IV	70	565	0–4000	
B-symptoms				
Absent	62	275	0–3600	0.029
Present	26	600	155–4000	
Sex				
Female	46	395	0–3025	0.053
Male	42	610	40–4000	
Size				
Non-bulky	52	330	0–4000	0.086
Bulky	36	577	60–3600	
E-site				
No	54	517	0–4000	0.31
Yes	34	395	26–3100	
Disease				
Hodgkin's	30	615	40–3600	0.35
Non-Hodgkin's	58	410	0–4000	
Age				
< median	44	330	40–4000	0.71
> median	44	552	0–3100	

or low-grade vs aggressive NHL. Pretreatment Erythrocyte Sedimentation Rate (ESR) values were available in 66 patients. ESR and Hpr levels in these patients showed a significant correlation (p=0.028). Seventy-three patients had a serum LDH level determined at the time of serum collection for the Hpr assay. No correlation was found between LDH and Hpr levels. We also analyzed Hpr levels according to treatment results in 71 patients who were evaluable for response. The difference between the median Hpr value observed in patients who either failed to achieve CR or subsequently relapsed ($480x10^3$ u/ml; range 60 to $3100x10^3$) and that of patients who obtained a persistent CR (Median, $345x10^3$ u/ml; range O to $4000x10^3$) was not statistically significant. Univariate analysis demonstrated that few factors had a significant effect on survival: tumor size (p=0.0006), age (p=0.015), systemic symptoms (p=0.02) and stage of disease (p=0.03). Pretreatment level of Hpr did not affect survival, neither sex, extranodal involvement , ESR and LDH levels.

Post-treatment sera

Forty-one of 88 patients were monitored for Hpr levels after treatment, 38 of whom achieved CR. Their initial post-treatment Hpr values were obtained at a median of 4 months from end of treatment, and showed a significant decrease as compared to Hpr levels at presentation (230 vs $430x10^3$ u/ml, p=0.0001) *(Figure 2)*. In the follow-up period, additional Hpr determinations were performed for 17 of 41 patients. Out of 14 patients who maintained the CR, the level of Hpr remained decreased in 11, and increased in 3, without evidence of recurrent at the time of evaluation. Three patients eventually progressed, 2 from CR and one from partial response. *Figure 3*. shows longitudinal studies of these 3 patients and a fourth patient described below. Cases 1 and 2 represent patients with aggressive NHL who were treated with CHOP chemotherapy and achieved CR, but had a relapse few months later. Hpr values appear to reflect the state of disease. Case 3 is an example of patient with low-grade lymphoma who slowly and partially responded to chlorambucil and steroid therapy, and then escaped. This was quite well mirrored by the decreasing and increased values of Hpr. Case 4 is a patient with advanced HD who refused to receive any treatment and continued steadily to progress. The Hpr serum level continued to increase during disease progression.

Discussion

Measuring Hpr levels in sera of lymphoma patients, we found that pretreatment values were high and in correlation with several prognostic factors. Hpr levels were also correlated with treatment results, as they decreased after successful therapy and increased again on relapse. This suggests that Hpr in the serum of patients with HD and NHL is released by the neoplastic cells. Previous investigators have examined the levels of acute phase proteins (APP), including Hp, which are commonly elevated in cancer patients. APP are the products of the acute phase response, which is an indication of the immune status of the host as well as tumor extent and activity. The degree of rise of Hp appears to correlate with the extent and activity

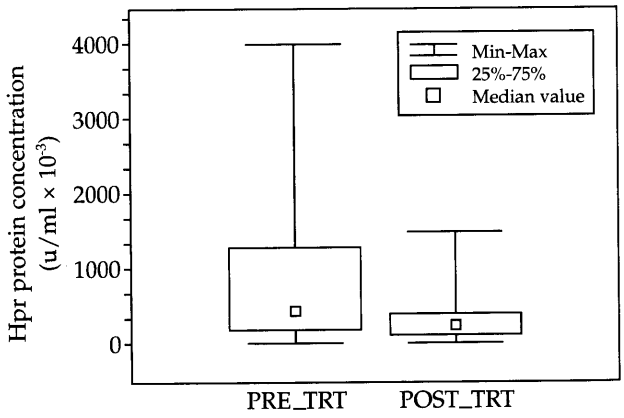

Figure 2. *Serum haptoglobin-related protein in 41 patients with lymphoma evaluated before and after treatment.*

of various tumors.[12] Hp level was found to be a significant prognostic factor of survival in patients with renal cell carcinoma,[13] lung cancer[14] and recurrent rectal carcinoma,[15] using univariate analysis. However, when multivariate Cox analysis was performed Hp was not identified as an independent prognostic factor. Onizuka et al found Hp to be positively associated with survival in patients with uterine cervical carcinoma undergoing radiotherapy, by multivariate regression analysis.[16] Conflicting data exist regarding Hp in patients with lymphoma. Shah et al found a significant elevation of Hp level in the active disease, as compared to remission, in patients with NHL.[17] Hp had a significant relationship to survival in patients with indolent and aggressive lymphoma, using univariate analysis, but not in multivariate analysis.[18] In a group of patients with low-grade lymphoma Hp could not predict the symptom-free period.[19] Desai et al undertook a large study to assess the relationship of Hp levels with different stages of malignant lymphoma and also to see the effect of treatment on the Hp level. Hp level was found to increase 2-3 fold in 176 patients with NHL and HD as compared to controls, and there was a continuous rise in Hp level as the stage advanced. Significant reduction in Hp level was observed when the disease regressed due to chemotherapy but it again increased with the relapse of disease.[20] Since the above methods used for the quantitative determination of Hp did not specifically assay the Hpr, the pretreatment levels were increased 2-3 fold as compared with a six fold increase in the median serum Hpr level compared to the control group described in this report.

A few studies have shown Hpr to be implicated as a tumor marker. Oh et al[21] identified in malignant ascites derived from ovarian and lung cancer patients an immunosuppressive substance which they called suppressive E-receptor-like factor (SER). SER represents a variant of Hp which does not bind Hb.[21] Serum levels of SER before and after treatment were measured using SER specific mAb in patients with renal cell carcinoma receiving autolymphocyte therapy (ALT). Pretreatment levels elevated above normal or increases over the pretreatment levels during the course of therapy were associated with disease progression. Low levels of SER prior to treatment or decreased to below normal levels following therapy were associated with positive response to ALT.[12] Using antibodies generated against a synthetic peptide, corresponding to the 34 N-terminal residues of the hypothetical Hp-related gene product, for immunohistochemical analysis of paraffin-embedded tissue, Kuhajda et al[22] found that nearly 30% of early breast cancer express proteins bearing Hp-related epitopes. These Hpr positive breast cancers were more likely to recur after primary resection and were associated with shorter disease-free intervals.[22,23] The prognostic value of Hpr was also evaluated in patients with prostate cancer. Tissue samples of prostate carcinoma were examined for the Hpr designated Oncogenic Antigen OA-519. Its expression correlated with higher tumor grade, larger tumors and advanced stage.[24] However, the antibodies used by Kuhajda et al specifically detected OA-519 as fatty acid synthase.[25] Our previous data have shown for the first time that Hpr containing a totally different chain is in fact a tumor antigen. Tabak et al have recently shown that the Hp-related gene is expressed in the human hepatoma G2 and leukemia molt-4 cell lines, and have cloned and sequenced the cDNA of the Hp-related gene. It was observed that the 140 amino acid polypeptide representing the Hp-related chain is totally different from the normal Hp chain at the N-terminus. The deduced amino acid sequence of Hp-related cDNA exhibited only 63% identity to the human Hp 1F chain.[6] The amino acid sequence deduced from this Hp-related cDNA coincided with the internal amino acid sequence near the N-terminus of the 21 kDa protein isolated from ascites fluid of a colon carcinoma patient.[5] The sequences of Hp and Hpr are highly homologous for the β chain, with only 16 changes in 245 amino acids.

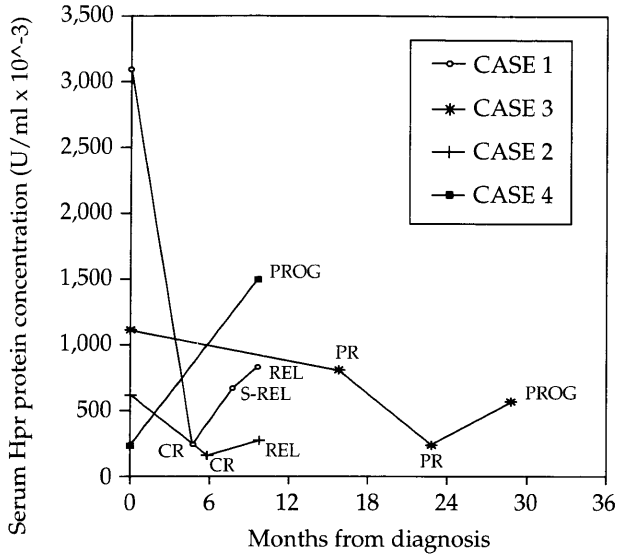

Figure 3. Time-course analysis of serum haptoglobin-related protein in 4 patients according to clinical phases of disease. CR = complete response; PR = partial response; PROG = progression; REL = relapse; S-REL = suspected relapse.

The production of the specific anti-p21 pAb enabled us to develop the ELISA test for measuring Hpr levels in serum of cancer patients, and to evaluate its clinical value. Increased levels of Hpr were found in the circulation of tumor bearing patients. These included patients with malignant lymphoma, urogenital, gastrointestinal and testicular germ cell tumors.[4,26] We found Hpr levels to be an indicator of response to therapy as well as of progression of disease in patients with urogenital tumors.[27-29] In the current study we found that pretreatment serum concentra-

tions of Hpr are markedly increased in patients with NHL and HD as compared with normal controls, and that they are correlated with several clinical and laboratory characteristics which may reflect tumor burden, including stage of disease, "B" symptoms and ESR. A significant decline of Hpr levels was observed in patients responding to therapy, while those with increasing levels of this protein during follow-up had a relapse, in most cases. Higher, though not statistically significant, pretreatment levels of Hpr were observed in patients who failed treatment, as compared with those who had no evidence of disease. Initial levels of Hpr did not predict patients' outcome. However, the evaluation of the prognostic value of Hpr might be biased by the heterogeneity of clinico-pathologic features and treatment approaches.

In conclusion, Hpr appears to be a new serum marker which might be useful for monitoring the clinical course of lymphoma. Hpr determination may provide useful additional information to the conventional initial work-up and evaluation of response and relapse, yet its optimal use should be further investigated. Hpr is probably released by the lymphoma cells, as it was shown by our previous results to be expressed in various tumor cell lines.[6,30] Determination of the biological function of this Hp-related gene product may well contribute to the understanding of tumorigenesis.

References

1. *Child JA, Spati B, Illingworth S, et al:* Serum beta 2 microglobulin and c-reactive protein in the monitoring of lymphoma. Cancer 45:318-326, 1980.
2. *Swan F, Velasquez WS, Tucker S, et al:* A new serologic staging system for large-cell lymphomas based on initial 2-microglobulin and lactate dehydrogenase levels. J Clin Oncol 7:1518-1527, 1989.
3. *Blay JY, Farcet JP, Lavaud A, et al:* Serum concentrations cytokines in patients with Hodgkins disease. Eur J Cancer 30A:321-324, 1994.
4. *Shalitin C, Epelbaum R, Moskovitz B, et al:* Increased levels of a 21-kDa protein in the circulation of tumor-bearing patients. Cancer Detection and Prevention 18: 357-365, 1994.
5. *Shalitin C, Valensi C, Admon A, et al:* Elavated levels of haptoglobin-related protein in sera of cancer patients. J Tumor Marker Oncol 13:15-25, 1998.
6. *Tabak S, Lev A, Valansi C, et al:* Transcriptionally active haptoglobin-related (Hpr) gene in hepatoma G2 and leukemia molt-4 cells. DNA and Cell Biology 15:1001-1007, 1996.
7. *Maeda N:* Nucleotide sequence of the haptoglobin and haptoglobin-related gene pair. J Biol Chem 260:6698-6709, 1985.
8. *Oliviero S, Morrone G, Cortese R:* The human haptoglobin gene: transcribtional regulation during development and acute phase induction. EMBO J 6:1905-1912, 1987.
9. *Carbone PP, Kaplan HS, Musshoff K, et al:* Report of the committee on Hodgkins disease staging classification. Cancer Res 31:1860-1861, 1971.
10. *Rosenberg SA, Berard CW, Brown BW, et al:* National cancer institute sponsored study of classifications of non-Hodgkins lymphomas: Summary and description of a Working Formulation for clinical usage. Cancer 49:2112-2135, 1982.
11. *Shalitin C, Epelbaum R, Moskovitz B, et al:* Enzyme-linked immunosorbent assay for the detection of a novel 21 kDa protein associated with the clinical course of patients with urogenital tumors. Int J Oncol 1:107-112, 1992.
12. *Knee TS, Osband MS, Carpinito GA, et al:* Acute phase reactants and suppressive E-receptor factor in various groups of patients receiving autolymphocyte therapy. Cancer Detection and Prevention 15:189-197, 1991.
13. *Ljungberg B, Grankvist K, Rasmuson T:* Serum acute phase reactants and prognosis in renal cell carcinoma. Cancer 76:1435-1539, 1995.
14. *Buccheri G, Ferrigno D:* Prognostic significance of the serum protein content in lung cancer. Eur Respir J 7 (Suppl 18): 389s, 1994.
15. *Hannisdal E, Tveit KM, Theodorsen L, et al:* Host markers and prognosis in current rectal carcinoma treated with radiotherapy. Acta Oncol 33:415-421, 1994.
16. *Onizuka K, Migita S, Yamada H, et al:* Studies on serum protein fractions of patients with uterine cervical cancer undergoing radiotherapy: relationship between changes in serum protein fractions and prognosis. Nippon Igaku Hoshasen Gakkai Zasshi 54:1007-1017, 1994.
17. *Shah I, Lewkow LM, Khilanani U:* Correlation of hypercupremia with other acute phase reactant in malignant lymphoma. Cancer 51:851-854, 1983.
18. *Hagberg H, Glimelius B, Gronowitz S, et al:* Biochemical markers in non-Hodgkins lymphoma stages III-IV and prognosis: A multivariate analysis. Scan J Haematol 33:59-67, 1984.
19. *Martinsson U, Glimelius B, Hagberg H, et al:* Primarily asymptomatic low-grade non-Hodgkin lymphoma: prediction of symptom-free survival and total survival. Eur J Haematol 43:332-338, 1989.
20. *Desai SS, Ishwad CS, Balakrishnan V, et al:* Serum haptoglobin level in patients with malignant lymphoma and its clinical significance. Indian J Cancer 27:46-54, 1990.
21. *Oh SK, Very DL, Walker J, et al:* An analogy between a potent I immunosuppressant in cancer and fetal haptoglobin. Cancer Res 47:5120-5128, 1987.
22. *Kuhajda FP, Piantadosi S, Pasternack GR:* Haptoglobin-related protein (Hpr) epitopes in breast cancer as a predictor of recurrence of the disease. N Engl J Med 321:636-641, 1989.
23. *Kuhajda FP, Katuprutuwa AI, Pasternack GR:* Expression of haptoglobin-related protein and its potential role as tumor antigen. Proc Natl Acad Sci USA 86: 1188-1192, 1989.
24. *Shurbaji MS, Kuhajda FP, Pasternack GR, et al:* Expression of oncogenic antigen 519 (OA-519) in prostate cancer is a potential prognostic indicator. Am J Clin Pathology 97:686-691, 1992.
25. *Kuhajda FP, Jenner K, Wood FD, et al:* Fatty acid synthesis: A potential selective target for antineoplastic therapy. Proc Natl Acad Sci USA 91:6379-6383, 1994.
26. *Grunder-Culemann E, Bokemeyer C, Neubauer V, et al:* Analysis of a non-ras 21 kDa protein in patients with metastatic testicular germ-cell tumors. J Cancer Res Clin Oncol 119:685-688, 1993.
27. *Shalitin C, Epelbaum R, Valansi C, et al:* Detection of a 21 kDa protein in sera of patients with urogenital tumors. Cancer J 74-78, 1990.
28. *Shalitin C, Epelbaum R, Valansi C, et al:* A novel 21 kDa protein as a serum marker for benign and malignant urogenital tumors: preliminary communication. Int J Cancer 49:861-866, 1991.
29. *Shalitin C, Moskovitz B, Livne PM, et al:* Serial levels of p21 before and after surgery in patients with renal cell carcinoma. in Guiliani L, Puppo P, eds. Urology 1992. Monduzi Editore, Bologna, Italy, 325-329, 1992.
30. *Shalitin C, Valanci C, Lev A, et al:* Cell-cycle inhibition in human BE-13 T-cell leukemia cells by haptoglobin-related (HPR) antisense cDNA. Anticancer Res 18:1745-1750, 1998.