Eur J Cancer Clin Oncol, Vol. 24, No. 9, pp. 1391–1396, 1988.
Printed in Great Britain

0277-5379/88$3.00 + 0.00
© 1988 Pergamon Press plc

Urinary Neopterin and Immunological Features in Patients with Kaposi's Sarcoma

G. SANTELLI,* G. MELILLO,† A. MARFELLA,‡ M. NAPOLITANO,† V. D'ALESSIO,† R.A. SATRIANO,§ E. BETH-GIRALDO,‖ G. GIRALDO,‖ M. PERNA‡ and G. CASTELLO†¶

**Sezione di Farmaco e Chemioterapia Sperimentale, †Sezione di Ematologia e Immunologia Clinica, ‡Laboratorio di Patologia Clinica, ‖Divisione di Virologia Oncologica, Istituto Nazionale dei Tumori, Via M. Semmola* 80131, *Napoli, Italy and §Clinica Dermatologica, I Facoltà, Università di Napoli, Napoli, Italy*

Abstract—*We studied neopterin excretion levels and immunological features of* 20 *patients affected by Kaposi's sarcoma (KS), compared to* 30 *normal controls. Eighteen patients had the classic form of Kaposi's sarcoma (CKS), while two patients were anti-human immunodeficiency virus (HIV) seropositive and affected by the epidemic form associated with the acquired immunodeficiency syndrome (AIDS). In CKS patients, a trend of an increase of neopterin levels with more advanced stages appeared from our data whereas a significant reduction in CD3+ and CD4+ lymphocytes subsets was observed already at early stages* ($P < 0.01$). *CD8+ cells did not show significant variations. A significant increase in serum IgA immunoglobulins* ($P < 0.05$) *was also observed.*

Comparative analysis of the two patients affected by AIDS/KS showed the profound deficit in T-cell immunity but also the prognostic value of neopterin monitoring. Furthermore these findings seem to confirm Kaposi's sarcoma as an 'opportunistic neoplasia' and indicate neopterin as a useful prognostic marker.

INTRODUCTION

BEFORE the recognition of the acquired immunodeficiency syndrome (AIDS), Kaposi's sarcoma (KS) was considered a rare and unusual dermal tumor, occurring mainly in elderly men, generally of Mediterranean or Jewish ancestry (classic KS, CKS).

Recently, an epidemic of the fulminant lymphoadenopathic variety of KS (epidemic KS) appeared particularly among young and middle-aged homosexual men in the United States [1]. Furthermore, an association of the various forms of KS with human cytomegalovirus has been repeatedly found [2].

Concerning the immunological profile, few studies are reported in the recent literature on patients with CKS, while several papers have been published on epidemic KS.

Neopterin is a metabolite of an intermediate in the biosynthesis of tetrahydrobiopterin (BH4), an essential cofactor for the hydroxylases needed for biosynthesis of catecholamines and serotonin [3]. Neopterin is released by monocyte-macrophages and seems to be related to the activation of the cell-mediated immune response. Constantly raised neopterin levels are associated with a persistent activation of immune system and are well correlated with the clinical status of autoimmune diseases, neoplasias and infections by viruses such as human immunodeficiency virus (HIV) [4].

Therefore neopterin monitoring has been proposed as a useful prognostic marker in some types of cancer [5] and also has been suggested that it could help in identifying anti-HIV seropositive subjects at high risk to develop full blown AIDS [6].

The aim of this work was to evaluate the immune status of KS patients, emphasizing the relationship between neopterin and immunological parameters.

MATERIALS AND METHODS

Patients

Eighteen patients (13 men and five women; mean age 64.4 ± 10.8 years) affected by CKS and two affected by AIDS/KS, all untreated, were studied. Urine and blood samples were initially obtained at time of the first observation.

Accepted 16 February 1988.
¶To whom correspondence should be addressed.
This work was supported in part by grants from Ministero della Sanità and Associazione Italiana per la Ricerca sul Cancro, Italy.

Two CKS patients were affected by a second primary malignancy: a gastric adenocarcinoma stage III and a Hodgkin's disease stage IIIA.

Staging was performed according to Krigel's classification [7], except that stage III and IV were grouped together.

On this basis, among the sixteen patients with CKS alone, six patients had stage I, six stage II and four stage III disease. One patient with AIDS/KS was a hemophiliac; his neopterin excretion levels together with immunologic and virologic monitoring were evaluated starting from March 1985, about 20 months before the appearance of the specific skin lesions. The other patient affected by AIDS/KS and opportunistic infections (OI) at the first observation was a homosexual man.

A group of 30 normal subjects, matched by sex and age, formed the control group.

Neopterin quantitation

The first urine of the morning was obtained from patients and controls. Samples were either analyzed immediately in dim light or stored at −20°C in darkened containers until used.

A Varian model LC5500 liquid chromatograph was used in conjunction with a Varian Vista 402 computerized data system (Varian Ass. Inc., Palo Alto, CA, U.S.A.). Owing to the physiologically variable concentrations of urine samples, urinary neopterin was related to urinary creatinine. In fact, earlier studies [8] showed that the neopterin/creatinine ratio remains relatively constant during a 24 h period.

Use of such a neopterin/creatinine ratio makes the analysis almost independent of the physiological variations in the urinary concentration and eliminates possible errors due to sample preparation or sample injections. HPLC separation was achieved according to Hausen's method [8] by means of the Varian Neopterin System. Neopterin was quantitated by peak height with the computerized data system using the external standard method. Results were expressed as μmol/ml creatinine.

Immunological parameters

T cell and T cell subsets were identified, without separation, by incubation with FITC-conjugate monoclonal antibodies to the CD3, CD4 and CD8 antigens (Ortho Diagnostic System, Raritan, NJ, U.S.A.), as phenotypic markers for total mature T cells, helper-inducer and suppressor-cytotoxic subsets, respectively. Their subsequent enumeration was obtained with a Spectrum III flow cytometer (Ortho Diagnostic System).

Serum immunoglobulin levels (IgA, IgG, IgM) were determined by nephelometry with an immunochemistry system (ICS Analyzer II, Beckman, Fullerton, CA, U.S.A.).

Assay for HIV antibodies

Screening of coded serum samples was initially performed by an indirect immunofluorescence (IIF) assay. Moreover, all positive sera were confirmed by the Western blot technique using commercial kits as well as infected cellular extracts or purified virus from E11 cell (HUT-78) cells which are 100% persistently infected by HIV strain ARV-2 [9], grown in our laboratory for 2 years. Both cell lines, E11 and HUT-78, were kindly supplied by J.A. Levy (Cancer Research Center, San Francisco, U.S.A.). IIF was carried out as previously described for other viral antibody titrations [10]. E11 and HUT-78 cells were mixed at a ratio 1:2 before seeding on multispot slides, thus including an internal specificity control for each spot [11]. Slides were fixed in acetone at −20°C and stored at −80°C until further use.

Antigens prepared in this manner were used within the following 4 weeks and no fluctuation of expression was observed, except in very early passage of E11 cells. All sera were tested twice at appropriate dilution (generally at 1:10) and read double blind. Borderline positive sera in IIF, initially considered as positive, at retesting were considered negative. All IIF-positive sera gave concordant results in Western blot analysis.

Statistical analysis

Statistical analysis was performed when appropriate using the Student's t test for unpaired observations and linear regression. Values were expressed as mean ± S.D. according to conventional statistical methods.

RESULTS

Table 1 shows clinical features, immunological parameters and neopterin excretion levels of patients affected by KS considered in this study. The values for anti-HIV seropositive patients were determined at the time of the appearance of the specific skin lesions. All CKS were anti-HIV seronegative, patients 19 and 20 (AIDS/KS) were seropositive with high anti-HIV titers (1:3200) in IIF.

A trend of an increase of neopterin levels with more advanced stages appears from our data (Fig. 1).

Considering the 12 patients affected by CKS alone at stages I or II, neopterin values (166 ± 72 μmol/mol), did not differ significantly from the normal range observed in controls, whereas the CD3+ cell population percentage already showed a significant reduction (61.8 ± 7.1; $P < 0.001$).

Comparative results between the 16 patients affected by CKS alone and normal controls are summarized in Table 2.

A significant reduction in the percentage of

Table 1. *Clinical and immunological characteristics and urinary neopterin levels of patients with Kaposi's sarcoma*

Patients Sex Age	CD3+ (abs/mm³)	CD4+ (abs/mm³)	CD8+ (abs/m³)	CD4/CD8 ratio	IgA (mg/dl)	IgG (mg/dl)	IgM (mg/dl)	Neopterin (μmol/mol creatinine)	Diagnosis and clinical stage
1. M 45	969	560	547	1.02	308	1130	103	67	CKS I
2. M 58	830	549	464	1.18	174	1080	84	129	CKS I
3. F 56	2065	1085	980	1.10	152	900	92	145	CKS I
4. M 70	1410	1048	237	4.42	316	854	99	162	CKS I
5. F 59	1725	1315	392	3.84	192	1250	92	230	CKS I
6. M 63	1349	989	550	1.78	153	1160	47	250	CKS I
7. M 54	1339	880	535	1.64	198	2710	97	63	CKS II
8. M 76	1830	1210	760	1.59	318	1530	120	107	CKS II
9. M 56	868	642	345	1.85	138	954	92	133	CKS II
10. M 69	1206	908	317	2.86	220	1150	88	182	CKS II
11. M 62	1483	1064	419	2.54	368	1120	113	234	CKS II
12. F 63	990	576	504	1.14	218	961	154	285	CKS II
13. M 79	1050	723	519	1.39	314	1490	165	126	CKS III
14. M 82	2196	866	712	1.21	461	1710	134	178	CKS III
15. M 83	881	492	609	0.80	238	1210	77	397	CKS III
16. F 68	829	451	378	1.19	588	1080	97	457	CKS III
17. F 50	827	404	492	0.82	256	3170	206	449	CKS + gastric ca.
18. M 65	535	380	259	1.46	N.D.	879	67	681	CKS + Hodgkin d.
19. M 16	543	97	413	0.23	323	1230	99	1830	AIDS/KS + hemophilia A
20. M 31	901	114	647	0.17	298	1580	122	4058	AIDS/KS + O.I.

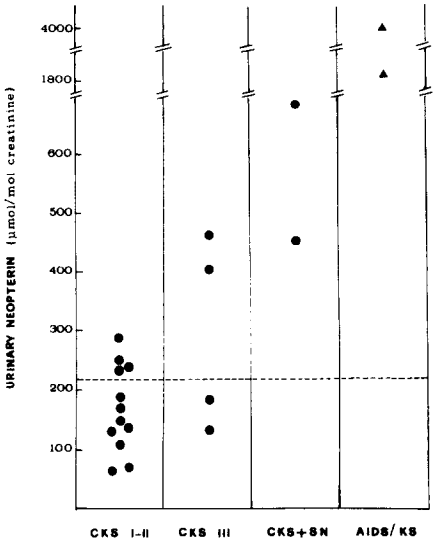

Fig. 1. *Urinary neopterin levels in patients affected by Kaposi's sarcoma. Abbreviation used: CKS = classic Kaposi's sarcoma. Stage I–II–III; SN = second primary malignancy; AIDS/KS = epidemic Kaposi's sarcoma.*

CD3+ and CD4+ cells was found in these patients; on the other hand, the CD8+ cell population percentage was not significantly decreased.

Neopterin excretion levels in these patients, comprehensive of CKS stage III, appeared slightly raised ($P < 0.02$).

Nevertheless, a weak correlation between reduction of CD3+ and CD4+ cells and raised neopterin values appeared when considering all the CKS patients, including the patients with second primary malignancy ($r = 0.50$ and $r = 0.54$ respectively), but the associations were statistically significant ($P < 0.05$).

The analysis of the serum immunoglobulin levels did not show significant changes, except for a slight but significant increase in the IgA class ($P < 0.05$). This result was likely due to the IgA increase in 43.7% (7/16) of CKS patients, while IgG were increased only in two patients. IgA levels were higher than normal in both epidemic KS. Finally, 50% of CKS individuals had at least one class of elevated immunoglobulins (and IgA was the most commonly elevated isotype).

Immunologic monitoring including neopterin excretion values of the hemophilic patient with AIDS/KS is shown in Fig. 2.

Raised neopterin levels in the last 2 years (higher than 400 μmol/mol) accompanied a severe reduction of CD4+ cells and development of AIDS/KS and OI as a terminal event. Both AIDS/KS patients died within 2 months of the last neopterin evaluation.

DISCUSSION

The natural history and clinical course of KS are strongly associated with host immunologic status. At present, in fact, KS is considered an opportunistic neoplasia developing in immunologically compromised individuals [12, 13], in whom an un-conventional oncogenic virus (CMV?) is probably acting in the initiation of the transformation process [2].

This is supported by several pieces of evidence:

(a) the incidence of KS is higher in individuals receiving immunosuppressive treatments, such as those undergoing organ transplantation or affected by autoimmune disorders [14];

Table 2. Immunological features and neopterin excretion levels in patients affected by classic Kaposi's sarcoma

	Patients* No 16	Controls* No. 30	*P* value†
CD3+ cells %	62.1 ± 7.9	71.8 ± 5.3	<0.001
CD4+ cells %	39.8 ± 8.2	46.4 ± 4.8	<0.01
CD8+ cells %	25.5 ± 8.6	26.9 ± 3.5	N.S.
CD4+/CD8+	1.81 ± 1.0	1.74 ± 0.3	N.S.
Neopterin (μmol/mol creatinine)	197 ± 110	134 ± 42	<0.02
IgA (mg/dl)	272 ± 122	194 ± 92	<0.05
IgG (mg/dl)	1268 ± 449	1173 ± 259	N.S.
IgM (mg/dl)	103 ± 29	150 ± 89	N.S.

*Mean ± S.D.
†*P* values were obtained with Student's *t* test for unpaired observations.
N.S.—not significant.

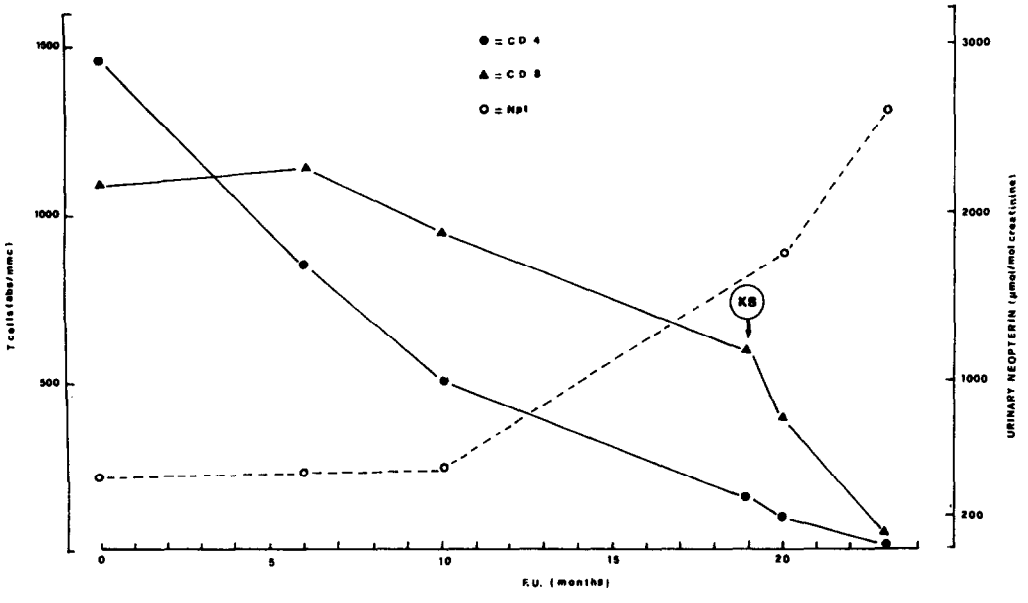

Fig. 2. T cell subsets and neopterin excretion levels in the 2 years follow up (F.U.) of a patient affected by hemophilia A, anti-HIV seropositive, who developed a Kaposi's sarcoma as a terminal event. The patient died 2 months after the last neopterin evaluation (March 1987).

(b) the disease may frequently resolve with discontinuation or reduction of immunosuppressive therapy [15];
(c) the primary KS is often associated with tumors of immunologically committed cells [16];
(d) the recent arising of an epidemic, lymphadenopathic variety of KS in young subjects with acquired immunodeficiency syndrome, where prognosis and survival are well-correlated with T cell immunity [17, 18].

However, the published data about the immune responsiveness in CKS patients are scarce and somewhat contradictory. For example, Stahl *et al.* [19] did not find any significant difference in the absolute number of T and B cells and T subsets compared to the normal range, while La Nasa *et al.* [20] reported a decrease of T cell number and a strong reduction of the CD4+ subset, an increase of the CD8+ subset and an abnormal CD4/CD8 ratio. Finally, Marinig *et al.* [21] observed a marked decrease of T suppressor cells with a significant increase of the CD4/CD8 ratio.

The data reported here—a slight increase of serum IgA with a significant reduction of T lymphocytes and T helper cells—suggest an underlying abnormal immunoregulation in this disease rather than a profound immunodepression. We do not know the reason for the conflicting reports in the literature, although the different methodology, and the rarity and heterogeneity of the neoplasia may play a role.

Neopterin monitoring is a simple but sensitive parameter of the monocyte/macrophage activation and constantly raised neopterin levels specifically

reflect activation of these cells by interferon gamma derived by stimulated T cells [4]. Patients affected by indolent CKS, in our series, did not show raised neopterin levels until more advanced stages and/or second primary malignancies appeared. This finding may reflect the maintenance of a functional reserve in the monocyte/macrophage function and suggest therefore a better prognosis.

Low levels of neopterin are associated indeed with favorable prognosis also in other neoplasias [22, 23].

On the contrary, immunological data of patients affected by AIDS/KS and OI reported in the literature are in good agreement among themselves, showing a profound deficit in T cells with a reduced number of CD4+ cells, an inverted CD4/CD8 ratio and raised urinary neopterin levels [24]. In our patient affected by hemophilia A, we observed a progressive increase in neopterin excretion correlated with a fall in T cell number long before the appearance of the aggressive form of AIDS/KS. The persistent activation of the monocyte-macrophage axis suggested by the raised neopterin levels in anti-HIV seropositive patients could allow for the development of the opportunistic infections and of the aggressive behavior of neoplasias [25].

In conclusion, our results are in favor of the hypothesis that the different evolution of CKS and AIDS/KS is more related to the immunological status of the host rather than to a possible different etiopathogenetic mechanism and stress the role of neopterin monitoring as a useful marker of KS progression.

Acknowledgements—The authors thank E. Leonardi, F. Martucci and F. Nitsch for expert technical assistance.

REFERENCES

1. Friedman-Kien AE. Disseminated Kaposi-like sarcoma syndrome in young homosexual men. *J Am Acad Dermatol* 1981, **5**, 468–471.
2. Giraldo G, Beth E. The involvement of cytomegalovirus in acquired immune deficiency syndrome and Kaposi's sarcoma. In Klein E, ed. *Progress in Allergy*. Basel, Karger, 1986, Vol. 37, 319–331.
3. Kaufman S. The structure of phenylalanine hydroxylation cofactor. *Proc Natl Acad Sci USA* 1963, **50**, 1085–1092.
4. Huber C, Bachtelor Jr, Fuchs D *et al.* Immune respone-associated production of neopterin-release from macrophages primarily under control of interferon gamma. *J Exp Med* 1984, **160**, 310–316.
5. Reibnegger GJ, Bichler AH, Dapunt O *et al.* Neopterin as a prognostic indicator in patients with carcinoma of the uterine cervix. *Cancer Res* 1986, **46**, 950–955.
6. Perna M, Nitsch F, Santelli G *et al.* Urinary neopterin: a useful marker for AIDS? *Lancet* 1985, **i**, 1048.
7. Krigel RL, Labenstein LJ, Muggia FM. Kaposi's sarcoma: a new staging classification. *Cancer Treat Rep* 1983, **67**, 531–534.
8. Hausen A, Fuchs D, Konig K, Wachter H. Determination of neopterin in human urine by reversed-phase HPLC. *J Chromatogr* 1982, **227**, 61–70.
9. Levy JA, Hoffmann AD, Kramer SM *et al.* Isolation of lymphocytopathic retrovirus from San Francisco patients with AIDS. *Science* 1984, **225**, 840–842.
10. Giraldo G, Beth E, Haemmerling U *et al.* Detection of early antigens in nuclei of cells infected with cytomegalovirus or herpes simplex virus type 1 and 2 by anticomplement immunofluorescence, and use of a blocking assay to demonstrate their specificity. *Int J Cancer* 1977, **19**, 107–116.
11. Kaminsky LS, McHugh T, Stites D *et al.* High prevalence of antibodies to acquired immune deficiency syndrome (AIDS)-associated retrovirus (ARV) in AIDS and related conditions but not in other disease states. *Proc Natl Acad Sci USA* 1985, **82**, 5535–5539.
12. Cairns RJ. Opportunistic auto-immune disease and opportunistic malignancy from impaired cell-mediated immunity. *Br J Dermatol* 1974, **91**, 601–602.
13. Costa J, Rabson AS. Generalised Kaposi's sarcoma is not a neoplasm. *Lancet* 1983, **i**, 58.
14. Gange RW, Wilson Jones E. Kaposi's sarcoma and immunosuppressive therapy: an appraisal. *Clin Exp Dermatol* 1978, **3**, 135–146.
15. Penn I. Kaposi's sarcoma in immunosuppressed patients. *J Clin Lab Immunol* 1983, **12**, 1–10.
16. Safai B. Kaposi's sarcoma: a review of the classical and epidemic forms. *Proc NY Acad Sci* 1984, **437**, 373–382.
17. Schroff RW, Gottlieb MS, Prince ME, Chai LL, Fahey JF. Immunological studies of homosexual men with immunodeficiency and Kaposi's sarcoma. *Clin Immunol Immunopathol* 1983, **27**, 300–314.
18. Mitsuyasu RT, Taylor J, Glaspy J, Fahey JL. Heterogeneity of epidemic Kaposi's sarcoma. *Cancer* 1986, **57**, 1657–1661.
19. Stahl RE, Freidman-Kien A, Dubin R, Marmor M, Zolla-Pazner S. Immunologic abnormalities in homosexual men. *Am J Med* 1982, **73**, 1712–1718.
20. La Nasa G, Carcassi C, Di Tucci A *et al.* Immunologic findings in classic Kaposi's sarcoma.

Abstract to International Meeting on causes, pathogenesis, management and treatment of AIDS and related conditions, Cagliari, 1–3 May 1984, 30, Passamonti, Cagliari, Italy.
Marinig C, Fiorini G, Boneschi V, Melotti E, Brambilla L. Immunologic and immunogenetic features of primary Kaposi's sarcoma. *Cancer* 1985, **55**, 1899–1901.
Santelli G, Marfella A, Abate G, Comella P, Nitsch F, Perna M. Urinary neopterin levels in hematologic malignancies. *Tumori* 1986, **72**, 139, 143.
Reibnegger G, Hetzel H, Fuchs D *et al.* Clinical significance of neopterin for prognosis and follow-up in ovarian cancer. *Cancer Res* 1987, **47**, 4977–4980.
Abita JP, Cost H, Milstien S, Kaufman S, Saimot G. Urinary neopterin and biopterin levels in patients with AIDS and AIDS-related complex. *Lancet* 1985, **ii**, 52–53.
Wachter H, Fuchs D, Hausen A, Reibnegger G, Werner ER, Dierich MP. Who will get AIDS? *Lancet* 1986, **ii**, 1216–1217.