Eur J Cancer Clin Oncol, Vol. 25, No. 8, pp. 1145–1150, 1989.
Printed in Great Britain

0277–5379/89$3.00 + 0.00

Neopterin and Alpha-interferon in Patients Affected by Kaposi's Sarcoma from Africa

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Abstract—*The presence of circulating alpha-interferon and neopterin was investigated in sera of 47 patients affected by African Kaposi's sarcoma, both HIV-seropositive (13 patients) and HIV-seronegative (34 patients). For comparison, analyses were also performed in 20 HIV-seropositive symptomatic African subjects as well as in 20 African and 20 Italian healthy individuals.*

Alpha-interferon and neopterin levels appeared significantly higher in comparison with healthy control groups ($P < 0.001$) *but not with HIV-seropositive African individuals without Kaposi's sarcoma. Moreover, alpha-interferon and neopterin levels were significantly higher in progressive Kaposi's sarcoma (27 patients) than in regressive Kaposi's sarcoma (20 patients)* ($P < 0.001$). *A significant correlation between alpha-interferon and neopterin was observed* ($r = 0.57$; $P < 0.01$). *Furthermore, alpha-interferon levels of HIV-seropositive Kaposi's sarcoma patients resulted significantly higher in comparison with the seronegative ones* ($P < 0.05$).

It is concluded that alpha-interferon and neopterin may be reliable prognostic markers in Kaposi's sarcoma patients.

INTRODUCTION

AN ASSOCIATION between the acquired immunodeficiency syndrome (AIDS) and Kaposi's sarcoma has become widely recognized during the past several years [1]. Nevertheless, it is conceivable that AIDS patients with Kaposi's sarcoma represent a different spectrum of the disease. Because of differences in the clinical picture of AIDS in Africa and in Europe/U.S.A., a provisional clinical case definition for African AIDS has been proposed which includes as a 'minor feature' the presence of Kaposi's sarcoma, even if aggressive [2]. Compared to other AIDS diagnoses, in fact, Kaposi's sarcoma is associated with both longer survival times after diagnosis and lower medical costs [3].

Accepted 1 March 1989.
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Supported in part by grants of the Italian Association for Cancer Research (AIRC) and of the Italian Ministry of Health.

Abbreviations used: AIDS = acquired immunodeficiency syndrome; HIV = human immunodeficiency virus type 1; ARC = AIDS related complex; LAS = systemic lymphoadenopathy; CMV = cytomegalovirus; EBV = Epstein–Barr virus; HSV 2 = herpes virus type 2.

Several lines of evidence indicate that cells of mononuclear phagocyte lineage frequently harbor HIV and perhaps they are the first cells in the body to become productively infected with HIV [4]. Moreover, it has been shown that the monocyte-mediated tumoricidal function of patients with Kaposi's sarcoma without opportunistic infections is intact *in vitro* in contrast to the observed reduction in patients with AIDS and opportunistic infections and AIDS/Kaposi's sarcoma and opportunistic infections [5]. Most studies have attributed such findings to the abnormal functions of lymphocytes in these patients; however, many of these same studies have not thoroughly investigated possible abnormalities in monocyte functions.

Upon activation, human monocytes–macrophages kill various microorganisms and inhibit intracellular microbial replication [6]. Deficient monocyte function has been associated not only with defective cellular immunity but also with an increased susceptibility to infection in humans. In addition to their importance in host defense against microbial and protozoal infections, gamma-interferon-activated macrophages have been shown to play a central role in the defense against the develop-

ment and metastatic spread of neoplasias such as AIDS/Kaposi's sarcoma and lymphomas [7].

Since we observe such striking differences in the mode of spread and evolution of HIV infection, it appears to be very important to identify clinical parameters that might contribute to the increased susceptibility to HIV infection and HIV-related diseases including cancers. Therefore, we decided to analyze a macrophage product, such as neopterin, and a lymphokine, such as circulating alpha-interferon, in sera from African patients affected by Kaposi's sarcoma collected from 1971 to obtain further information about monocytes/macrophages behavior in AIDS and in AIDS-related cancers such as Kaposi's sarcoma.

MATERIALS AND METHODS

Patients

We analyzed 47 sera of patients affected by Kaposi's sarcoma (47 men; mean age 44.06 ± 12.95) collected from 1971 from Northern and Central Africa. The diagnosis was always biopsy-proven [8]. These sera were matched by sera from healthy African subjects according to sex, age, socioeconomic living conditions and geographical location, and also by 20 healthy Italian controls (20 men; mean age 26.7 ± 6 years). A group of 20 HIV-seropositive African subjects (15 ARC, 5 LAS) (20 men: mean age 30.1 ± 9.7) formed a further control group to investigate the markers studied in HIV-seropositive African patients without Kaposi's sarcoma.

The patients were screened for concomitant diseases (particularly second malignancies, lymphomas and tuberculosis) and only those with Kaposi's sarcoma alone as the primary malignancy were entered into the study.

Staging was performed according to the clinical criteria proposed by Krigel *et al.* [9], grouping together Stage I and II as regressive Kaposi's sarcoma and Stage III and IV as progressive Kaposi's sarcoma. On this basis, 20 patients had regressive Kaposi's sarcoma and 27 progressive Kaposi's sarcoma.

Serologic studies

The sera were kept at −80°C until analyzed. The constant value of anti-CMV, anti-EBV and anti-HSV 2 antibody titers suggested a good preservation of the sera examined [10].

All sera were tested at dilutions of 1:5 and 1:10 for the presence of antibodies to HIV by a standard indirect immunofluorescence assay using HUT-78 cells chronically infected with HIV-SF2 (formerly ARV-2). The sera were also tested by an immunoblot technique and, in addition, by an ELISA procedure using infected HUT-78 cells.

Since antibodies to HIV were not detected by any of these three techniques in the African sera obtained prior to 1975, as we have previously reported [10], the sera of Kaposi's sarcoma patients from this study were grouped as follows:

— African Kaposi's sarcoma sera (1971–1978) HIV-seronegative; 20 (four regressive Kaposi's sarcoma and 16 progressive Kaposi's sarcoma);
— African Kaposi's sarcoma sera (1987) HIV-seronegative: 14 (nine regressive Kaposi's sarcoma and five progressive Kaposi's sarcoma);
— African Kaposi's sarcoma sera (1987) HIV-seropositive: 13 (seven regressive Kaposi's sarcoma and six progressive Kaposi's sarcoma).

Analysis of circulating alpha-interferon was performed by the Boots Celltech Diagnostic SUCROSEP interferon-alpha immunoradiometric assay (IRMA) (Boots Celltech, U.K.). Results were expressed as Units/ml [11, 12]. Detection limit of the assay was <1 U/ml. Neopterin was determined by means of RIA kits obtained from Henning Berlin Laboratories, Berlin, F.R.G. Results were expressed as nmol/l [13].

Statistical analysis

Data were expressed as means ± S.D. (standard deviation). For multiple group comparisons the significance of differences between the means of different groups was assessed by one-way analysis of variance. For comparisons of means of two groups, Student's *t* test for unpaired observations was performed. Spearman's rank correlation test and Pearson's correlation coefficient between neopterin and alpha-interferon values were calculated in Kaposi's sarcoma patients. The cut-off for statistical significance was defined as $P < 0.05$.

RESULTS

A significant difference of mean values in neopterin levels between African and Italian HIV-seronegative healthy controls was seen (Table 1) ($P < 0.05$). In fact, raised amounts of alpha-interferon and neopterin were found in some apparently healthy individuals. Furthermore, increased amounts, stage-related, of alpha-interferon and neopterin were observed in the population of African Kaposi's sarcoma patients studied (Table 1).

Alpha-interferon and neopterin appeared directly and significantly correlated in the 47 African Kaposi's sarcoma patients evaluated (Fig. 1) (Pearson's correlation coefficient = 0.36; $P < 0.05$; Spearman's correlation coefficient = 0.57; $P < 0.01$). This correlation was more significant when we evaluated only the HIV-seronegative patients (34 patients) (Fig. 2) (Pearson's correlation coefficient = 0.66; $P < 0.001$; Spearman's correlation coefficient = 0.61; $P < 0.01$), whereas it was not significant in the 13 HIV-seropositive Kaposi's sar-

Table 1. *Alpha-interferon and neopterin levels in patients affected by African Kaposi's sarcoma related to clinical stage*

Patients	(No.)	Alpha-interferon Mean ± S.D. (Units/ml)	Raised/total	(%)	Neopterin Mean ± S.D. (nmol/l)	Raised/total	(%)
Kaposi's sarcoma 'regressive'	(20)	1.48 ± 1.17	2/20	(10)	8.72 ± 14.24	1/20	(8)
Kaposi's sarcoma 'progressive'	(27)	3.38 ± 2.63**	27/27	(100)	50.40 ± 24.92***	27/27	(100)
Healthy African controls	(20)	1.54 ± 1.41	4/20	(20)	6.48 ± 2.60*	1/20	(8)
Healthy Italian controls	(20)	1.20 ± 0.04	0/20	(0)	4.96 ± 1.20	0.20	(0)

*$P < 0.05$; **$P < 0.001$; ***$P < 0.0001$. P values refer to comparison of Kaposi's sarcoma patient groups and African controls with the Italian control group.

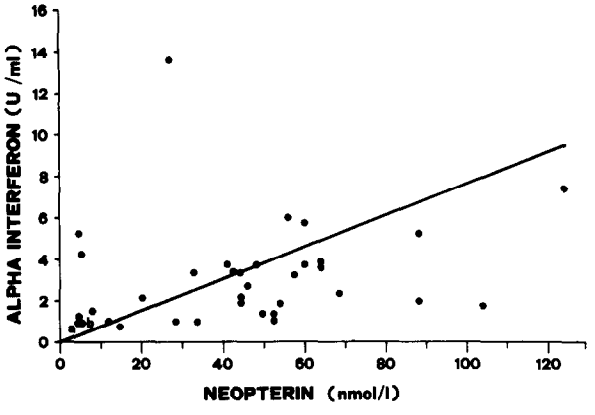

Fig. 1. *Correlation between circulating alpha-interferon and neopterin levels in* 47 *African Kaposi's sarcoma* (*a*: *six points*; *b*: *eight points*).

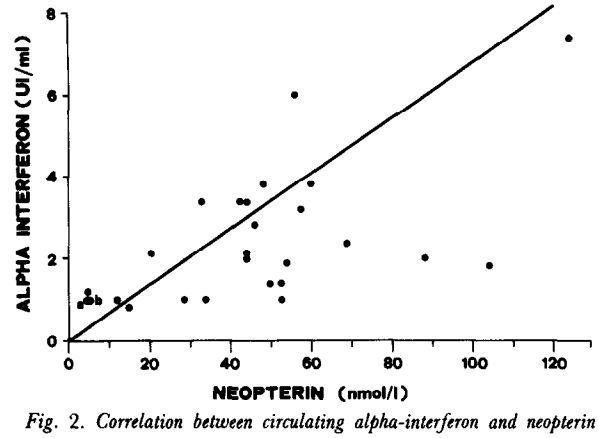

Fig. 2. *Correlation between circulating alpha-interferon and neopterin levels in* 34 *HIV-seronegative African Kaposi's sarcoma* (*a*: *five points*; *b*: *five points*).

coma patients (Spearman's correlation coefficient = 0.36; NS; Pearson's correlation coefficient = 0.27; NS). In this group, raised circulating alpha-interferon levels and low neopterin levels in two patients with regressive Kaposi's sarcoma were observed. Indeed, the 13 HIV-seropositive Kaposi's sarcoma patients showed alpha-interferon and neopterin levels significantly higher than African and Italian HIV-seronegative controls, but not significantly different with respect to the 20 HIV-seropositive symptomatic African individuals (Table 2).

Moreover, Table 2 shows that the mean values of alpha-interferon in HIV-seropositive Kaposi's sarcoma patients also appeared significantly raised with respect to the HIV-seronegative ones. On the contrary, no significantly raised neopterin levels in HIV-seropositive Kaposi's sarcoma patients were observed as compared to the HIV-seropositive ones. The lack of significance may be probably due to the fact that 21/34 (61%) of Kaposi's sarcoma HIV-seronegative patients had a progressive Kaposi's sarcoma.

DISCUSSION

The individual factors which determine evolution to AIDS of HIV-seropositive subjects are various and not yet defined, but certainly T-cell activation together with monocytes–macrophages imbalance play an important role [4]. Consequently, the search for prognostic markers of HIV infection must take into account not only the study of the CD4+ lymphocyte subpopulation but also of other parameters able to inform us about virus replication in the host organism in cells other than lymphocytes [14, 15]. Therefore neopterin, a product of gamma-interferon-activated macrophages [16], has been suggested as prognostic marker in AIDS [17, 18].

Alpha-interferon has been already shown to be present in sera of patients with AIDS and to be a significant *quoad vitam* parameter in patients affected by AIDS/Kaposi's sarcoma [19–21]. Furthermore, it has been hypothesized that the decreased production of gamma-interferon from cells from AIDS/Kaposi's sarcoma patients *in vitro* is caused by the continuous endogenous exposure to interferons *in vivo* [22]. The clear and direct correlation between

Table 2. Alpha-interferon and neopterin levels in HIV-seropositive and HIV-seronegative patients affected by African Kaposi's sarcoma

Patients	(No.)	Alpha-interferon Mean ± S.D. (Units/ml)	*P* value	Neopterin Mean ± S.D. (nmol/l)	*P* value
(a) Healthy African controls HIV-seronegative	(20)	1.54 ± 1.41		6.48 ± 2.60	
(b) African Kaposi's sarcoma HIV-seronegative	(34)	2.06 ± 1.52	b vs. a < 0.01	36.20 ± 30.64	b vs. a < 0.001
(c) African Kaposi's sarcoma HIV-seropositive	(13)	3.90 ± 3.42	c vs. a < 0.001 c vs. b < 0.05	23.28 ± 24.76	c vs. a < 0.001 c vs. b N.S.
(d) African LAS-ARC HIV-seropositive	(20)	3.43 ± 4.18	d vs. a < 0.001 d vs. c N.S.	27.20 ± 32.44	d vs. a < 0.001 d vs. c N.S.

N.S. = not significant.

alpha-interferon and neopterin observed in our African/Kaposi's sarcoma patients appears to support this hypothesis.

Moreover, recent reports about the biologic role of neopterin, within gamma-interferon activated macrophages, may elucidate the prognostic value of this marker: they provide some evidence that, at least for some intracellular obligate pathogens, oxygen-independent mechanisms are important in gamma-interferon-mediated inhibition of growth, within both macrophages and other cell lines [6]. Gamma-interferon-induced tryptophan catabolism appears to be mainly responsible for such inhibition [23, 24], a mechanism also involved in the antiproliferative effects of interferon-activated macrophages [25].

Since reduced pteridines have been well established to be essential cofactors for the hydroxylases also needed for tryptophan metabolism, it has been suggested that the tetrahydroneopterin and tryptophan pathways may be related during the 'priming' of monocytes–macrophages [26].

A continuous activation of these types of cells, as suggested by the constantly raised neopterin levels, could give the host organism a better resistance to Kaposi's sarcoma and/or development of other 'opportunistic' neoplasia, but also make them unable in time (exhaustion? Loss of negative feedback?) to interrupt replication of the obligate intracellular pathogens in HIV-seropositive individuals or to avoid neoplastic diffusion.

Raised neopterin levels are more commonly observed in hemophiliacs than in homosexual men [27]; in our studies we have observed a significant increase in neopterin excretion in HIV-seropositive symptomatic individuals respect to HIV-seronegative hemophiliacs, but the latter group showed also significantly raised neopterin levels as compared to normal controls, suggesting pre-activation of monocytes–macrophages as a consequence of an overload of transfusion antigens [28]: indeed, the appearance of a generalized Kaposi's sarcoma or other 'opportunistic' neoplasias is a very rare and terminal event in hemophiliacs. Neopterin levels in the normal range were instead observed in classic Kaposi's sarcoma patients, as well as in epidemic Kaposi's sarcoma but without opportunistic infections [29, 30]. In fact, in our series of 47 African Kaposi's sarcoma patients, we have not observed significant differences in neopterin levels between HIV-seropositive and HIV-seronegative subjects, instead their levels were correlated to the clinical stage. On the other hand, in the same group of African Kaposi's sarcoma patients, we have observed that the presence of circulating alpha-interferon was significantly correlated with the HIV-seropositivity.

Recently it has been reported that, in HIV infection transmitted by sexual or skin contact, the cells first involved are the CD4+ Langerhans cells of the skin [31]. HIV-infection of these cells leads to a dramatic loss of MHC-II antigens. Since both the antigen-presenting and the immunostimulatory functions of dendritic (accessory) cells correlate with the expression of MHC-II [32], the primary infection of dendritic cells with HIV greatly reduces stimulation of the T-cell system, thus explaining the lower neopterin levels observed in homosexual men and patients affected by AIDS/Kaposi's sarcoma without opportunistic infections [27, 29]. Such an immune system impairment may account for the infections but also for the presence of accompanying neoplasias such as Kaposi's sarcoma.

The loss of the accessory function of Langerhans' cells could explain our observation that the significant correlation between alpha-interferon and neopterin observed in HIV-seronegative African Kaposi's sarcoma disappears in HIV-seropositive ones and the observation of two regressive Kaposi's sarcoma patients with raised alpha-interferon levels

(index of virus replication) but neopterin levels in the normal range.

Individuals having already activated T-lymphocytes and monocytes–macrophages, such as hemophiliacs, will immediately replicate HIV on becoming infected [33].

Therefore, together with the CD4+ lymphocyte count, the assays of circulating alpha-interferon and neopterin, although not specific, appear to be simple and sensitive tests for monitoring the virus replication and activation/exhaustion of cells like monocytes–macrophages. These cells could represent the primary source of virus persistence and diffusion *in vivo* (the 'Trojan horse theory') [4] but also the last defense in host protection against major opportunistic infections and the metastatic spread of 'opportunistic' neoplasias in HIV-seropositive individuals.

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