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SPECIAL REPORT

Risk Factors for AIDS Associated Kaposi's Sarcoma

An European Multicentre Case Control Study

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EURO-SHAKS, European study on HIV associated Kaposi's sarcoma, is a BIOMED 1 project financed by the European Union (DG XII). The Spanish side of the project has been financed by FISS (Fondo de Investigaciones Sanitarias). The aims of this study are to identify possible genetic, behavioural, biological and environmental risk factors for HIV associated Kaposi's sarcoma through a multicentre case-control study. An extensive personal questionnaire, a clinical data form and blood sample is required from all participants. In addition, a cutaneous biopsy is request from KS patients. The presence of several European groups in this project implies a large and diverse sample size and will allow to correlate the behaviour, clinical and biological data in different geographical areas, and therefore study the possible transmission routes as well as the natural history of the putative causal agent of KS. One of the main objectives of EURO-SHAKS is to create a European Bank of AIDS biological samples for possible future investigations. (Pathology Oncology Research Vol 3, No 1, 74–77, 1997)

Key words: AIDS, Kaposi's sarcoma, risk factors, case-control study

Introduction

Kaposi's sarcoma (KS) was first described almost 125 years ago, however its etiology is still unknown. Several agents, most of them infectious, have been associated with the occurrence of KS in all of its presenting forms (classical, endemic and epidemic). Up to date, several viral agents such as cytomegalovirus, hepatitis B, herpes 6 and papillomavirus 16 have been suspected of causing KS. More recently a novel human herpes virus, named HHV8, with high homology to other gammaherpesvirus has been identified in KS tissue by polymerase chain reaction (PCR)-based techniques.[1] HHV8 DNA sequences have been identified in all forms of KS.[2] Moreover, HHV8 DNA sequences have been reported in different clinical forms of KS and histological patterns (patch stage and nodular stage).[3]

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Several epidemiological studies report a high prevalence of KS among homosexuals leading to the hypothesis that KS is a sexually transmitted disease.[4] Furthermore, some particular sexual practices have been related to the occurrence of KS.[5] However, there are still some epidemiological gaps regarding possible sexual transmission. Firstly, there is a lack of evidence that HHV8 is vertically transmitted to explain the high prevalence of endemic KS among children (regardless of sex). Secondly, there is an absence of a clear increase of KS in countries where heterosexual transmission has increased based on contacts from African countries. Finally, very few clusters among sexual partners have been reported. Genetic factors have also been suggested to be involved in the development of KS since high frequency of HLA-DR5 has been observed in these patients.[6]

Recent studies on HHV8 seroprevalence found rates of 80–90% in AIDS-KS patients, and almost non existing in HIV-positive haemophilic and normal blood donors in the US.[7,8,10] Within the general population, HHV8 seroprevalence rates vary from less than 5% in Britain and the US, slightly higher in some Mediterranean countries (4–12%) and reach around 50% in the Ugandan control population. A recent serological study differs in the rates for the non-

Table 1. **Participating countries**

GREECE	*BELGIUM*	*ITALY*	*SPAIN*
National University of Athens Division of Infectious Diseases St.Pierre Hospital Brussels	National AIDS Reference Centre Department of Dermatology "A.Sygros" Hospital Athens	C.I. Malattie Infettive H.San Raffaele Milan	Centre on Epidemiological Studies on AIDS in Catalonia Hospital Universitari Germans Trias i Pujol, Badalona, Barcelona
Co-ordinator: Prof. J. Stratigos *Collaborating Staff:* Prof. N. Stavrianeas Dr. D. Polydorou Dr. C. Botsis *Laboratory:* Dr. M. Vounatsou Dr. M. Hadjivassiliou-Pappa *Interviewer:* P. Damascos	*Co-ordinator:* Dr. P. Hermans Dr. N. Clumeck *Interviewer:* H. Colpin	*Co-ordinator:* Prof. A. Lazzarin *Collaborating Staff:* Dr. A. Saracco Dr. M. Moro Dr. M.G. Viganó *Laboratory:* Dr. G. Tambussi *Interviewer:* M. Vicinanza	*Co-ordinator:* Dr. J. Casabona Dr. M. Vall *Collaborating Staff:* Dr. G. Gambús Dr. A. Rodés Dr. J. Cadafalch Dr. B. Clotet Dr. J.M. Gatell Dr. D. Podzamczer Dr. L. Colomer Dr. R. Rubio *Interviewer:* R. Munoz

Project proposers: Dr. P. Hermans, Dr. J. Casabona. *Principal Investigator:* Dr. J. Casabona. *Field Co-ordinator:* Dr. G. Gambús. Administration: T. Yates

risk population.[11] These authors report an age-dependent ratio from 2–8 % in children up to 25% HHV8 seropositivity in healthy adults which in their opinion, suggests another infectiousn route besides through sexual transmission. The confirmation of the distribution will be a crucial issue to ascertain the specificity of this virus as a possible aetiologic factor on KS.

Case-control studies provide the strongest results for the association of particular conditions with low prevalence diseases. Their main difficulty is the selection of controls to ensure comparability of all other variables, particularly when some of the co-factors may be unknown. A small number of case-controls studies on HIV associated KS have been carried out, most of the findings are not consistent across studies and some have been produced from weak epidemiological designs. In particular, the definition criteria for controls has been very heterogeneous including non HIV-infected individuals in some studies.

Table 2. **Contents of the questionnaire**

SECTION 1 *Socio-demographic*	*SECTION 2* *Medical records*	*SECTION 3* *Socio-occupational history*	*SECTION 4* *Use of drugs*	*SECTION 5* *Sexual behaviours*
Information Personal data Education Income	Symptoms STDs Surgery Other diseases HIV infection	Travel Exposure to animals Leisure and occupational activities	Use of alcohol Use of tabacco Illegal drug use Use of nitrite inhalants	Sex for men Sex for money Sexual relations with drug users Sexual relations with HIV+ persons Sexual relations with people with KS

Table 3. **Summary of Clinical History (Data)**

Part A	*Part B*	*Part C*	*Part D*
Date of information collection Identification number Centre Medical record number Date of realisation of questionnaire Date of blood sample extraction Date of AIDS diagnosis	Date of diagnosis of HIV infection Date of last anti-HIV negative serology Progression markers	Antiretroviral treatment	Other AIDS indicative diseases CDC classification Karnofsky scale

Table 4. **Characteristics of cases and controls**

	Mean	*Range*
Age at study entry		
Cases (n=111)	32.2 (SD 9.6)	22–78
Controls (n=110)	39.9 (SD 8.0)	22–62
CD4 count at study entry		
Cases (n=55)	118.3	1–662
Controls (n=36)	124.6	3–470

Preliminary data from Spanish branch.

EURO-SHAKS project

Aim

Identify possible environmental, behaviour, biological and genetic factors for HIV associated Kaposi's sarcoma (KS) through a multicentre case-control study. Participating centres until now are shown in *Table 1*.

Objectives

1. To collect information regarding the socio-demographic, clinical, sexual and drug use behavioural aspects associated with HIV infected persons with (cases) and without (controls) KS, using a standardised questionnaire (personal interview and clinical records).
2. To assess the prevalence of different viral markers (i.e. HHV8) from sera of cases and controls and cutaneous fresh frozen tissue samples.
3. To determine the prevalence of the different HLA genotypes among cases and controls by PCR techniques.
4. To estimate the relative risk (odds ratio) with 95% confidence intervals, of the different studied risk factors and KS.
5. To construct a European AIDS bank of serum, plasma, DNA of KS and non KS AIDS patients and cutaneous KS tissue samples.

Design

A hospital based case-control study.

Inclusion criteria

For the purpose of this study cases and controls are defined as follows. Cases: any AIDS patients with KS diagnosed with clinical criteria and, if possible confirmed with microscopy.

To increase the sample size both prevalent and incident cases with a 3 stage on the WHO activity scale are included. For each case two controls will be recruited. Selection criteria of controls will be as follows: any patients with HIV infection without KS, but with another AIDS indicative disease (diagnosed according to the CDC criteria) and with a stage 3 of the WHO activity scale, diagnosed plus-minus 4 months from the date of the case, and matched by sex, age and transmission group. A boarder sequencing criteria explained elsewhere can be applied.

Sample size

Given that the differences on sexual behaviour to be measured are the same for cases and controls, the overall minimum sample size will be 150 cases and 300 controls. Assuming that 60% of the controls may be exposed sexual practices for HIV, this sample size would low to identify a O.R. 2.5 with a 90% probability of detecting a significant increase in the O.R. of the 5% (α=0.05).

Ethical issues

All participants will sign a consent form to be enrolled in the study. Data is being collected on an anonymous basis. In case that patients do not agree to participate in the study a refusal form will be filled in order to analysed possible bias. We intend to achieve a follow-up of the controls a year after their enrolment in the EURO-SHAKS study for the detection of controls that become cases (by developing KS) and a cutaneous biopsy is required. This matter could be the subject of a further study assessing HHV8 predictive value.

Conduct

Besides prevalent cases, new patients have also been recruited prospectively. Soon after KS diagnosis, cases are interviewed and blood sample is taken. Afterwards controls are identified and interviewed in the same manner. The questionnaire includes socio-demographic and clinical information as well as sexual and drug behaviour. *Tables 2.* and *3.* summarise the contents of the personal questionnaire and clinical data necessary. Local interviewers have been trained to pass the questionnaire in a standardised manner.

Table 5. **Form of presentation at study entry and type of KS diagnosis**

		Frequency	*Percent*
Presentation	Primary	32	57%
	Secondary	24	43%
Total		56	100%
Diagnosis	Definitive	52	90%
	Presumtive	6	10%
Total		58	100%

Preliminary data from Spanish branch.

The collection of biological samples includes: a) 35.5 ml of blood for the obtention of serum, blood clots, plasma, whole blood and purified lymphocytes from all participants and b) for KS patients a snap frozen cutaneous biopsy is required. The EURO-SHAKS project considers it a priority to construct a European AIDS bank, for its own purposes and for further research projects. This study will allow us to analyse the new serological test for HHV8 with a strong epidemiological design and the presence of this virus in lymphocytes and in skin KS samples. The collection of plasma although it is not contemplated in this study, is for possible future analysis of chemokines. Blood clots will be kept to have a DNA AIDS (KS and non-KS) sample bank. The HLA analysis will be carried out on whole blood.

Preliminary data recruited from Spain is summarised in *Tables 4.* and *5.* We expect to increase our recruitment to a 210 cases and the corresponding 420 controls.

One of the advantages of EURO-SHAKS is the inclusion of patients from different countries with different transmission patterns. This will provide a large sample size and will enable the study of KS among particular groups such as intravenous drug users. Moreover, the fact that very detailed behavioural data is collected from two different time periods together with clinical data and biological samples, will lead to a better definition of the association of HHV8 with KS and to a description of its possible transmission routs.

Although the project has almost come to an end in Spain, it is now being implemented in Belgium, Greece and Italy. We would like to take this opportunity to inform any group interested in this project that the participant list is open to further additions pending agreement by the present participating groups.

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