Koch's postulates & Kaposi's sarcoma

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> Man's mind cannot grasp the causes of events in their completeness, but the desire to find those causes is implanted in man's soul. And without considering the multiplicity and complexity of the conditions any one of which taken separately may seem to be the cause, he snatches at the first approximation to a cause that seems to him intelligible and says: "This is the cause!"
>
> —*Leo Tolstoy*[1]

What is Kaposi's sarcoma (KS) and what role did this otherwise obscure cancer play in recognition of what would be named AIDS? What prompted its change from an indolent and rare sarcoma of elderly Mediterranean men to become an aggressive neoplasm killing substantial numbers of younger gay men? For that matter, what causes KS?

Faced with questions like these, I recalled my initial exposure to Koch's postulates, which have been taught to every medical student for a hundred years. Robert Koch formulated these criteria to establish a causative relationship between a microbe and a disease:

1. The microorganism must be found in abundance in all organisms suffering from the disease.
2. The microorganism must be isolated from the diseased host and grown in pure culture.
3. The microorganism should cause disease when introduced into a healthy organism.
4. The microorganism must be re-isolated from the inoculated, diseased host and shown to be the same as the originally inoculated agent.

In the spring of 1981, the Centers for Disease Control and Prevention (CDC) became aware of an apparent increase in two rare diseases, KS and *Pneumocystis carinii* pneumonia (PCP). Immunologists at the University of California, Los Angeles (UCLA) reported five cases of PCP in gay men without known underlying disease.[2] At the same time, dermatologists in New York City, San Francisco, and Los Angeles identified 26 gay men with KS in skin, lymph nodes, and/or other organs. The men ranged in age from 26 years old to 51 years old; eight had died.[3]

On July 6, 1981, I entered the Epidemic Intelligence Service (EIS) at the CDC and was immediately recruited by Dr. James Curran (ΑΩΑ, University of Michigan, 2002 Alumnus) to construct a case definition, find cases systematically, and orient the data by time, place and person.

Kaposi's sarcoma

In 1872, Moriz Kaposi, a Hungarian-borne dermatologist described five cases of multiple idiopathic pigmented hemangiosarcoma in men at the University of Vienna.[4] The disease that bears his name has been reported worldwide:

Moriz Kaposi, MD, circa 1872.

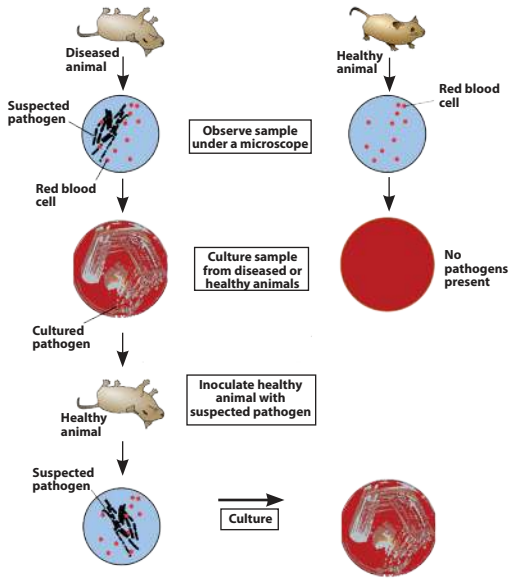
- A man in his 60s, presents with dark red to violaceous plaques or nodules on the extremities, lives another eight to 10 years before dying of some unrelated cause.
- African KS presents variably among young Black men and children. Benign, nodular lesions limited to the extremities form one end of the spectrum; florid, disseminated lesions were at the other end; survival ranged from three to 10 years following presentation.
- In the 1970s, oncologists noticed KS appearing among immunocompromised patients, such as renal transplant recipients, patients on long-term corticosteroids, and those immunosuppressed as a result of some other therapeutic regimens or malignancy.[5]

In 1981, data on KS in the United States were available at the National Cancer Institute (NCI) and from several case

Robert Koch (1843–1910)

Koch's postulates

1. **The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.**
2. **The microorganism must be isolated from a diseased organism and grown in pure culture.**
3. **The cultured microorganism should cause disease when introduced into a healthy organism.**
4. **The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.**



reports. The NCI's Surveillance, Epidemiology, and End Results (SEER) program surveyed 10 areas of the country (the states of Connecticut, Hawaii, Iowa, New Mexico, and Utah, and the cities of Atlanta, Detroit, New Orleans, San Francisco, and Seattle) for the incidence and outcome of various cancers. The SEER population represented slightly more than 10 percent of the U.S. population and was reasonably representative of the nation. From 1973 to 1977, approximately 30 cases of KS were reported to NCI each year. The male-to-female ratio was three to one.

Investigators at Memorial Sloan-Kettering Cancer Center in New York City reported their experience treating 92 patients with KS from 1949 to 1975. Seventy-six percent were men with an average age of 63 years. More than one third of those KS patients had at least one other primary cancer during the period of study.[5]

Italian oncologist, Gaetano Giraldo, and his colleagues observed herpes-type particles on electron microscopy in five of eight tissue culture cell lines from African patients with KS.[6] At that time, there were five known human herpes viruses: herpes simplex types 1 and 2; Epstein-Barr virus; varicella-zoster virus; and cytomegalovirus (CMV). Giraldo noted that nucleic acid segments from those tumors resembled CMV.[7]

While I was setting up surveillance, Curran charged Dr. Harold Jaffe (ΑΩΑ, David Geffen School of Medicine at University of California, Los Angeles, 1971) with listing hypotheses of causation and designing a study to test them. Jaffe listed his leading hypotheses:

1. Cytomegalovirus
2. An environmental toxin, most likely nitrite inhalants
3. Immune overload caused by exposure to multiple infectious agents
4. A "new" infection agent, most likely related to herpes or hepatitis viruses

Cytomegalovirus was on the top of everyone's list. UCLA immunologist, Michael Gottlieb found evidence of CMV in his initial five cases, and Giraldo found evidence for CMV in KS tissues in Africa. But why should CMV be causing an epidemic now? Could it be a new or mutated strain now circulating among gay men? And what was its relationship to immunosuppression? Was the virus causing immunosuppression or taking advantage of another immunosuppressive cause?

In October 1981, we conducted a case-control study of KS and PCP among gay men in Atlanta, Los Angeles, New York City, and San Francisco. Fifty cases and 120 controls were included; 39 men with KS, eight with PCP, and three with both diagnoses. The leading risk factors were lifetime number of sexual partners, and meeting partners in bathhouses, both suggesting a novel sexually transmitted agent.

CMV was ruled out as the cause as CMV antibodies were found in 100 percent of cases and 98 percent of controls. Levels of antibodies to CMV were higher among cases than controls, but the differences were not

Characteristics of Kaposi's sarcoma variants

Type	Population	Clinical	Course
Classic	Older men (50 years-80 years old)	Usually confined to lower extremity	Usually indolent, survival 8-10 years
Endemic (African)	Young Black males (15 years-40 years old), and children in Africa	Localized nodular lesions or large exophytic, aggressive lesions	Nodules indolent; aggressive lesions survival 3-5 years
Iatrogenic	Immune suppressed (e.g., renal transplant)	Localized or widespread involvement	May regress when immune suppressants discontinued
Epidemic (AIDS-related)	Gay men in New York and California	Head, face neck, gastro-intestinal and lung most common	Fulminant, survival 1 year-3 years

statistically significant. Cultures of urine and throat swabs yielded CMV in 25 percent of cases and nine percent of controls. DNA restriction endonuclease analyses, a way to fingerprint each isolate of CMV, were performed on 10 samples from cases and 10 from controls. Different patterns were found for each—there were no matches.[8,9]

Additional studies were implemented. First, we identified AIDS cases who were sexual partners and developed a network of 40 sexual contacts spanning 10 cities. Second, we evaluated interviews and laboratory results of cases reporting exclusively heterosexual behaviors. Finally, we compared gay men with KS and those with PCP.

In June 1983, Giraldo hosted the first workshop of a European study group on AIDS and Kaposi's sarcoma in Naples, Italy. Jean Claude Chermann, a virologist at Institute Pasteur, Paris, reported the isolation of a new retrovirus, named Lymphadenopathy-associated virus (LAV), from a lymph node of a gay male with multiple lymphadenopathy. The virus was propagated in cultures of T-lymphocytes from a healthy blood donor and umbilical cord blood. At 15 days in culture, reverse transcriptase activity was detected in the supernatant. A retrovirus was observed on electron microscopy, its morphology was different than that of HTLV-I and HTLV-II, and its major core proteins were immunologically distinct.[10] I was convinced that this was the virus everyone sought!

KS versus PCP study

When I returned from the European conference, the CDC statisticians presented their analysis of the KS versus PCP study. It included 47 gay men with KS alone, 20 with PCP alone, and 20 with both KS and PCP; 57 from Jaffe's study, and 30 interviewed later. Men with KS or both diseases had significantly higher incomes; were more sexually active as measured by the number of partners and STD rates; used more recreational drugs such as marijuana and nitrite inhalants; and had more non-B hepatitis than patients with PCP alone. In logistic regression, nitrite inhalant use was the best variable to distinguish men who developed KS versus those who developed PCP, and suggested that the causes of KS and PCP were not the same.[11]

I was startled by those results; up to that point, I thought we were looking for a new virus to explain the epidemic. Were there two epidemics? Or was there one epidemic of immune deficiency, with cofactors to explain the various manifestations of AIDS? Were nitrites a cause of KS or simply a marker of something else, e.g., another virus or toxin?

I presented our findings at the next staff meeting and was met with much skepticism. Curran had a number of concerns. How does one rule out selection bias when studying patients from multiple cities and at different times? Did fatigue disproportionately affect PCP patients? Was a case-comparison study the optimal design to test the hypothesis?

Jaffe raised "Ockham's razor" to explain away our findings. William d'Ockham was a 14th century English Franciscan friar known for the law of parsimony. "Simpler explanations are, other things being equal, generally better than more complex ones"—the Law of Parsimony, attributed to William d'Ockham (c1285–1349). One should opt for an explanation in terms of the fewest possible causes, factors, or variables. Jaffe felt we should focus on finding the new virus, and not get waylaid by side hypotheses.

To address Curran's concerns, we re-analyzed the data by various subsets of patients—those from NYC versus those from other sites; by length of interviews; by male and female interviewers; by individual interviewers; by numbers of sexual partners reported. To our amazement, every single analysis supported the hypothesis that nitrite use was greater among patients with KS than those with PCP.[11]

Nitrite inhalants

Alkyl nitrites [RONO] (e.g., amyl, butyl, isopropyl) are colorless or yellow liquids at room temperature, and are highly volatile. They are esters of nitrous oxide that have a fruity odor (often described as unpleasant), and have

been nicknamed "poppers" because of the sound made when glass capsules containing amyl nitrite are crushed. A vasodilatory effect following inhalation of amyl nitrite vapors was described in 1859 and led to the first report by T. Lauder Brunton, a Scottish medical student, of its clinical application to provide relief for angina pectoris in 1867.

Amyl nitrite was marketed by prescription starting in 1937, and use became so widespread that the Federal Drug Administration (FDA) allowed over the counter purchases starting in 1960. About that time, it was discovered that gay men, adolescents, and young adults in the U.S. and Europe were using amyl nitrite as an aphrodisiac to prolong penile erection and ease anal intercourse. In 1968, the FDA reinstated amyl nitrite as a prescription only medication. Soon thereafter, an underground market for pirated amyl nitrite and other nitrite congeners, predominately butyl nitrites, emerged. Those products were sold as "room odorizers" such as Rush® and Hardware.®[12]

A role for nitrites as a cause of KS made sense. Nitrites were used more often by gay men than heterosexuals, and KS occurred much more commonly among gay men with AIDS than among injection drug abusers, hemophiliacs, or Haitians. Also, the occurrence of AIDS-related KS on the chest, face, and especially the nose, was consistent with body areas most heavily exposed to nitrite vapors. And, nitrites were known to be carcinogenic in laboratory testing, and to affect blood vessels—KS is a blood vessel cancer—so the association was biologically plausible.

We postulated a multifactorial model to explain the various manifestations of AIDS. We proposed that the natural history of the new syndrome began with immune dysfunction, most likely as a result of infection of T-helper cells by a novel human retrovirus, an initiator. One or several cofactors then determined which, if any, opportunistic infections or cancers each patient would manifest. We suggested that nitrite inhalants, or another variable positively correlated with their use, promoted development of the cancer, KS. Promoters for PCP, tuberculosis and toxoplasmosis were likely new or reactivated infections with those respective agents. The paper, "Disease Manifestation Among Homosexual Men with Acquired Immunodeficiency Syndrome: A Possible Role of Nitrites in Kaposi's Sarcoma" was published.[11] It generated little scientific interest.

In June 1984, I transferred to the National Institutes of Health as a Health Science Administrator to supervise grants and contracts to study treatments for opportunistic infections; define the full spectrum or natural history of HIV infection; and develop animal models of infection for testing vaccines, immune modulators, and antiviral therapies.

In March 1985, the HIV antibody tests became available and researchers found that most cases of KS among elderly men, organ transplant recipients, and Africans were negative for HIV.[13] Most gay men with KS were positive, but not all. In one report, dermatologists at New York University reported six HIV-negative gay men with KS between the ages of 32 years and 62 years; five of the six reported nitrite inhalant use.[14]

By Koch's criteria, HIV was neither necessary nor sufficient to cause KS. Those results encouraged me to continue searching for another cause, or causes, of KS.

In December 1986, I transferred to the National Institute on Drug Abuse (NIDA), Rockville, MD, as a medical officer in the Division of Clinical Research to initiate an AIDS research program among drug abusers. Within two weeks at NIDA an opportunity was presented to explore KS etiology.

I attended an emergency meeting to develop a response to the U.S. Congress, Public Law 99–570, enacted October 27, 1986. Section 4015 of the bill called for the Director of the NIDA to conduct a study on alkyl nitrites to determine:

1. The extent and nature of the use of alkyl nitrites products by the public;
2. The extent to which the use of such products conform to the advertised uses of the products; and
3. The extent to which the sale of such products to the public presents a health risk and the nature of such risk.[12]

I volunteered to organize the review.

On March 31, 1987, NIDA sponsored a technical review entitled "The extent of use and health hazards of nitrite inhalants" attended by approximately 25 scientists. Highlights: The acute toxicity of nitrites in animals and man was reviewed by several investigators. Skin and tracheobronchial irritations (especially around the nose and lips), burns from accidental ignition, headaches, hypotension, cyanosis, methemogloginemia, intoxication, and the development of habitual use patterns were listed as adverse effects of nitrite inhalation. Several presentations focused on pharmacologic mechanisms by which nitrites may be involved in the genesis of KS in AIDS: carcinogenicity and immunosuppression.

I reviewed the six epidemiologic studies of nitrite use and KS in gay men and found inconsistent results. Three described a strong association between larger quantities

of nitrite inhalant use and KS, but three did not confirm the association. I discussed the difficulties of interpreting questionnaire data when sample sizes are small, and methods, populations, and questions vary.

The 10 presenters provided a manuscript of their findings. NIDA monograph, number 83, entitled *Health Hazards of Nitrite Inhalants*, was printed in August 1988.[15]

I had met Hank Wilson, a gay activist from San Francisco, in 1981. He created the Committee to Monitor Poppers, and compiled volumes of research on nitrite inhalants. Wilson mailed a copy of the NIDA monograph to all 535 members of the U.S. Congress and asked his Congressman, Mel Levine (CA) to propose a bill to outlaw popper abuse.

The U.S. Congress enacted a ban on the manufacture and retail of butyl nitrites (except when used in legitimate commercial purposes) in the Anti-Drug Abuse Act (Public Law 100–690, Section 2404—November 18, 1988). The law specified that the Consumer Product Safety Commission (not the FDA) would enforce the ban.[12]

Rates of KS among Caucasian men peaked in 1989 and decreased thereafter; rates of KS among Black men peaked shortly thereafter before declining. Of course, changes in KS incidence cannot be solely attributed to the ban on nitrites, as timing of the decline is confounded by the emergence of azidothymidine (AZT) and antiretroviral therapies.

To circumvent the clear intent of the law, nitrite manufacturers began to sell other alkyl congeners, such as isopropyl nitrite, as new and improved room odorizers and as video head cleaners.

In 1990, Congress attempted to close a loophole in the law by banning all "volatile alkyl nitrites" (Public Law 101–647, Section 3202—November 29, 1990). Furthermore, nitrite manufacturers rapidly developed a cyclohexyl nitrite, deemed legal (not an alkyl nitrite) by the Consumer Product Safety Commission in 1992.[12] As they saw lower profits in the U.S., they moved to aggressively market nitrites in the Caribbean, Europe and Asia—anywhere they were still legal.

Human herpes virus-8 (HHV-8)

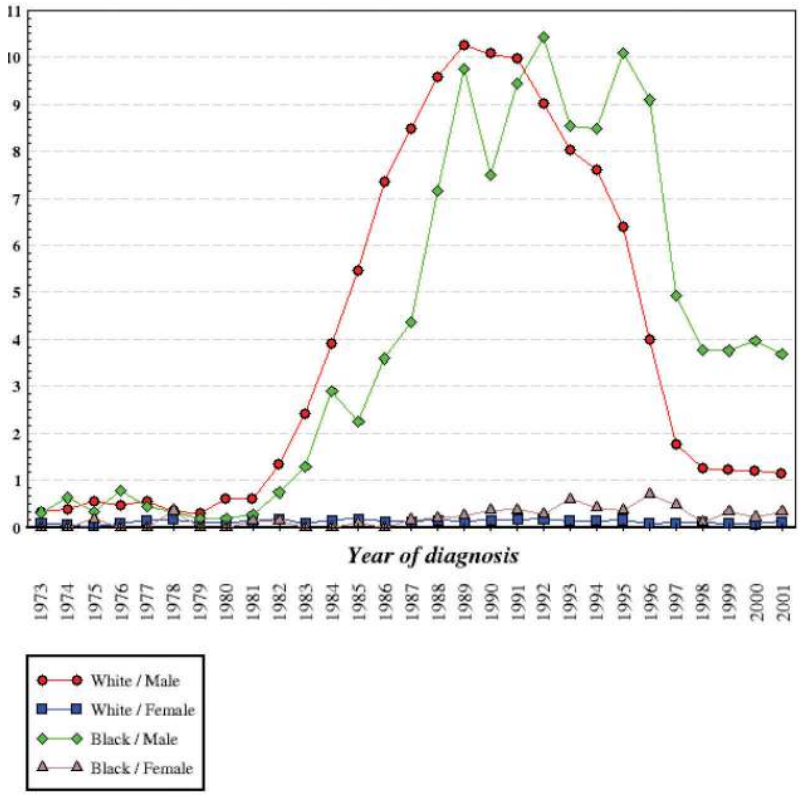
In 1994, a major breakthrough in the etiology of KS was reported. Investigators at Columbia University in New York, identified a new human herpes virus, HHV-8, by representational difference analysis, and detected the virus in more than 90 percent of KS lesions, including those unrelated to HIV, i.e., elderly men, Africans, and renal transplant recipients.[16] HHV-8 appeared to be spread through sexual activity, kissing, and contact with saliva—similar to routes of other herpes viruses. The prevalence of HHV-8 antibodies increases with age, showed wide fluctuations geographically, and was dependent on the detection assay employed.[17]

HHV-8 did not fulfill Koch's postulates; it may be necessary but is not sufficient to cause KS.

Vasoactive substances

Meanwhile Dr. John Ziegler was conducting epidemiologic studies among both HIV-seropositive and HIV-seronegative KS patients in Uganda. He proposed that KS resulted from the activation of latent HHV-8 by immune suppression or inflammation to an oncogenic, lytic state in some endothelial cells. Walking barefoot among volcanic soils, whereby chemicals

Age-adjusted rates of Kaposi's sarcoma by gender and race, 1973–2001



Source: Surveillance, Epidemiology and End-Results (SEER), Incidence and age-adjusted rates of KS, nine registries, 1973-2001.[22]

Multifactorial causes of Kaposi's sarcoma

KS variant	Herpes-virus	Factors affecting immune system functions	Vasoactive agents
Classic	HHV-8	Immunosenescence, other cancers	Exposure to volcanic soils, amyl nitrite, ACE inhibitors
Endemic (African)	HHV-8	Environment (parasites, diet, herbs) Drugs (antimalarials)	Aluminosilicates/ iron oxides taken up by lymphatics
Iatrogenic	HHV-8	Steroids, other immunosuppressants	ACE inhibitors
Epidemic (AIDS-related)	HHV-8	T-cell defect due to HIV	Nitrite inhalants

Abbreviations: ACE-angiotensin converting enzyme; HHV-Human herpes virus.

such as iron and aluminosilicates, aided by quartz abrasions, caused trauma, inflammation, and blocked lymphatics drainage, thus predisposing HHV-8 infected Africans to KS on the extremities.[18,19]

On medical rounds in 2003, a dermatology colleague at Walter Reed Army Medical Center, provided me with information about yet another vasoactive agent associated with KS. He shared several case reports of patients with KS in whom lesions had begun after captopril or lisinopril (angiotensin-converting enzyme [ACE] inhibitors) therapy was initiated. Lesions disappeared or improved when therapy was stopped. Those cases of KS included a 70 year old French heterosexual male with essential hypertension, a 70 year old Algerian woman with rheumatoid arthritis, a 78 year old Turkish man with hypertension and diabetes, and a renal transplant recipient.[20]

With those insights, I was able to hypothesize a multifactorial etiology for all four types of KS.

What caused KS among elderly men in Vienna in the 1800s? I don't think we will ever know, but I reviewed those early cases for clues. Kaposi's first case was a 69-year-old married master smith from Brodes, a town in Lower Austria. He was admitted in July 1868 complaining of tension in his hands and feet that had been going on for more than a year. He stopped working six months earlier as he could no longer stand. On physical exam he had numerous painful, brown-red to blue-red plaques and nodules on his hands, feet, and face; pitting edema was noted in dependent areas. After two months in the hospital, he left to die among his family and neighbors.

Kaposi's second case was a 66-year-old married distiller from Cracow, who was admitted on April 5, 1869 after suffering from the disease for 14 months. The skin of his feet showed sharply outlined violet-red, coarse, nodular infiltrates, which were painful to the touch. The upper extremities and both eyelids were also affected. On May 12 he developed profuse bloody diarrhea and fever, and died on May 21. At autopsy, disseminated disease was observed including extensive lesions of the GI tract.[4]

What would review of those medical records indicate about pre-existing medical conditions, prior medications, travel history and more? If preserved blood samples were available for those patients, one could measure immune function, and test for antibodies to HHV-8 and HIV. If even tissue (biopsy) fragments or histopathology slides were available to science, one could today perform molecular marker studies or searches for HHV-8 genome fragments (e.g., via polymerase chain reaction).

When did HHV-8 infection enter the human condition? Could those men have been exposed to aluminosilicates or iron oxides living and working in Eastern Europe? Or, could they have been patients with chest pains treated with nitrite of amyl? Medical student, T. Lauder Brunton first described amyl nitrite as a treatment for angina pectoris in 1867. After graduating from the University of Edinburgh in 1868, Brunton did post-graduate work at the University of Vienna under Dr. Ernst von Brucke, professor of physiology.[21]

In the 19th century, Robert Koch and others developed, and conclusively proved, the germ theory of disease causation. The acceptance of the single agent, single disease concept led to the sciences of infectious diseases and microbiology, and to the prevention, control and even elimination of several devastating illnesses.

However, simple explanations for the etiology of KS have proven incomplete. KS results from myriad interactions between HHV-8 infection, immunosuppression, and vasoactive agents. Coronary artery disease is an example of a disease with multiple risk factors including cigarette smoking, genetics, hypertension, obesity, and lack of exercise. Reye's syndrome appears to result from a complex etiology involving salicylates, varicella zoster virus/influenza B viruses, and genetic errors of metabolism. From experience exploring causation for KS, we have developed multifactorial hypotheses for the etiologies of cervical cancer, hepatocellular carcinoma, multiple sclerosis, schizophrenia, and type 1 diabetes mellitus.[22-25]

Just as the etiology of the European War of 1812 appears to be complex and multifactorial, so may be the causes of several chronic diseases, and conditions such as

health disparities and the opioid crisis. Or, as the late H.L. Mencken said, "For every complex problem there is an answer that is clear, simple and wrong."

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