Commentary

Multifactorial etiology of Kaposi's sarcoma: a hypothesis

Kaposi's sarcoma (KS) is the most common cancer among AIDS patients in North America and Europe, and also occurs among elderly Mediterranean men, sub-Saharan African adults and children, and organ transplant recipients. In 1994 Chang *et al* identified a new herpes virus, HHV-8, as the cause of KS. However, the abrupt onset followed by a steep decline in AIDS-associated KS in the USA suggests HHV-8 is insufficient to induce KS and that additional factors are required. Immunosuppression via HIV, malnutrition, and chemotherapy appears to play a role. In addition, vasoactive agents have been linked to KS in epidemiologic studies and anecdotal reports. Ziegler *et al* suggest that aluminosilicates from African volcanic soils activate HHV-8 to induce KS. Dermatologists have reported a few patients developing KS after initiating captopril or lisinopril therapy and regression of lesions when therapy discontinued. Several epidemiologic studies, but not all, have demonstrated significant associations between development of AIDS-related KS and use of large quantities on nitrite inhalants; furthermore, nitrites are mutagenic. It may be that KS results from a complex interaction between HHV-8, immunosuppression, and vasoactive agents. More research is needed to evaluate these hypotheses to elucidate the cause(s) of KS.

1. Introduction

In 1981, the first reports of Kaposi's sarcoma (KS) and opportunistic infections among previously healthy homosexual (gay) men and others led to a search for an infectious cause of an underlying immunodeficiency, now known as AIDS (CDC 1981; Haverkos and Curran 1982). In 1983, a retrovirus was identified by a French group led by Luc Montagnier and named lymphadenopathy-associated virus (LAV; Barre-Sinoussi *et al* 1983). In 1984, a virus derived from the French virus was reported by a US group led by Robert Gallo and named human T-lymphadenopathic virus type 3 (HTLV-3; Popovic *et al* 1984). Both are now known as human immunodeficiency virus (HIV).

In 1985, we postulated a multifactorial hypothesis to explain the various manifestations of AIDS based on the results of a case comparison study evaluating 87 homosexual men with Kaposi's sarcoma and/or Pneumocystis carinii (now known a *P. jirovecii*) pneumonia (Haverkos *et al* 1985). The natural history of AIDS begins with immune dysfunction resulting from HIV infection, an initiator. One or more cofactors then determine which, if any AIDS-defining illnesses, the patient will manifest. Promoters for the development of AIDS-associated tuberculosis, *Pneumocystis jirovecii* pneumonia, or toxoplasmosis involve activation of new or latent infection with specific infectious agents. At that time we suggested that nitrite inhalants might serve as a promoter for the development of KS (Haverkos *et al* 1985). The etiology of KS appears to be complex and multifactorial. Co-carcinogenesis is the phenomenon of additive or synergistic effects of two or more agents, such that the combination causes cancer in a manner that none can do alone. Oncologists describe this phenomenon as a two-step process of "initiation" and "promotion" of carcinogens (Southam 1963; Rous 1965; Haverkos 2004).

In this paper, we will review and update virologic and epidemiologic data regarding the etiology of KS. We will report our understanding of data linking human herpesvirus – 8 **(**HHV-8) and HIV to the cause of KS, revisit our original work linking nitrite inhalants to KS, and present data on KS in Africa and

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Abbreviations used: CMV, cytomegalovirus; HHV-8, human herpesvirus – 8; HIV, human immunodeficiency virus; HTLV-3, human T-lymphotrophic virus type 3; IFN-γ, interferon-gamma; IL-1β, interleukin-1 beta; KS, Kaposi's sarcoma; LAV, lymphadenopathy-associated virus; MACS, Multicenter AIDS Cohort Study; PCP, *Pneumocystis carinii* pneumonia; TNF-α, tumour necrosis factor – alpha

among elderly men suggesting a role for additional vasoactive agents. Finally we will present our current hypotheses on the etiology of KS.

2. Moritz Kaposi and multiple idiopathic pigmented hemangiosarcoma

In 1872, Moritz Kaposi described three fatal cases of multiple idiopathic pigmented hemangiosarcoma in elderly men at the University of Vienna (Kaposi 1872). KS has since been defined as a malignant neoplasm of blood vessels manifested primarily by multiple vascular nodules in the skin or other organs. The disease is multifocal, with a course ranging from indolent, with only skin manifestations, to fulminant, with extensive visceral involvement. Four distinct types of KS have been described by clinicians since Kaposi's description of the "classic type" (*see* table 1). Although the "classic type" remains more prevalent among elderly men of Mediterranean origin, it has been diagnosed worldwide and typically follows a benign course. The African endemic form of KS was first described in 1914, and occurs predominantly among young Black adult men 25 to 40 years of age (Oettle 1962). There is also a lymphadenopathic subvariant of the African form that affects children at a mean age of 3 years. In the 1970s, a third form associated with immunosuppressant treatment was described among recipients of organ transplantation, patients on long-term corticosteroids for various disorders, and patients immunosuppressed as a results of other therapeutic regimens or malignancy (Friedman-Kien 1989).

3. Human immunodeficiency virus

Gallo, co-discoverer of HIV, initially proposed that HIV alone was necessary and sufficient to cause AIDS-associated KS. Gallo and his associates explored the effects of various proteins expressed by HIV and the various cytokines released as a result of HIV infection using cells lines, and in animal models. They found that HIV-1 Tat protein induced angiogenesis *in vitro*, and that inflammatory cytokines, such as tumour necrosis factor – alpha (TNF-α), interferon-gamma (IFN-γ) and interleukin-1 beta (IL-1β) were increased in the sera and lesions of KS patients. They also showed that these same inflammatory cytokines, TNF-α, IFN-γ, and IL-1β, induced endothelial cells to produce and release Fibroblast Growth Factor and promote KS-like lesions in nude mice (Gallo 1991; Ensoli *et al* 1994; Albini *et al* 1995; Samaniego *et al* 1997).

HIV infection is not found in most patients with other forms of KS, i.e., KS in elderly men, African KS (at least those patients diagnosed before 1980), and KS among organ transplant recipients. Furthermore, the striking epidemiology of KS among AIDS patients, with its sudden increase and then rapid fall of incidence, and its occurrence almost exclusively among gay men, led many to believe in the existence of cofactors acting in conjunction with HIV to induce KS (Haverkos 2004). The search for potential cofactors focused on two areas – factors associated with non-HIV associated forms of KS (Chang *et al* 1994; Ziegler 1993; Ziegler *et al* 2003), and factors associated with the gay male lifestyle (Haverkos *et al* 1985; Lo, 1986; Polk *et al* 1987; Beral *et al* 1990; Armenian *et al* 1993).

Table 1. Characteristics of Kaposi's sarcoma variants

Type	Population	Clinical	Course
Classic	Older men (50-80 years)	Usually confined to lower extremity	Usually indolent, survival 10–15 years
Endemic (African)	Young black males, 15-40 yrs, and children	Localized nodular lesions or large exophytic, aggressive lesions	Nodules indolent; aggressive lesion survival 3–5 years
Iatrogenic	Immune suppressed (e.g., renal transplant)	Localized or widespread involvement	May regress when immune suppressants discontinued
Epidemic (AIDS-related)	Primarily gay men in U.S., Europe; adults in Africa	Head, face, neck, GI and lung most common	Fulminant, survival 1-3 years without effective HIV therapy

AIDS, acquired immunodeficiency syndrome; GI, gastrointestinal system; HIV, human immunodeficiency virus.

4. Human herpesvirus – 8

Gaetano Giraldo and his colleagues first observed herpes-type viral particles in five of eight tissue culture cell lines from African patients with KS (Giraldo *et al* 1972). Later, nucleic acid segments from those tumors were found to be consistent with cytomegalovirus (CMV) by RNA-DNA reassociation, RNA-DNA in situ cytohybridization, and anticomplement immunofluorescence techniques (Giraldo *et al* 1980; Boldogh *et al* 1981). Both African KS patients and controls had high CMV antibody titers (Giraldo *et al* 1975; Giraldo *et al* 1978).

In 1994 a major breakthrough in the etiology of KS was reported. Chang, Moore, and co-workers identified a new human herpesvirus, HHV-8, by representational difference analysis and detected this virus in more than 90% of KS lesions, including those unrelated to HIV (Chang *et al* 1994). Subsequently, human herpesvirus – 8 **(**HHV)-8 has been documented in more than 95% of KS patients from all four types of KS (Moore and Chang 1995). Found in saliva and semen, HHV-8 appears to be spread through sexual activity, kissing, and contact with saliva - similar to routes of transmission of other herpes viruses (Pauk *et al* 2000; Casper *et al* 2006).

The prevalence of HHV-8 antibodies increases with age, shows wide fluctuations geographically, and is dependent on the detection assay, i.e. antibodies to latency-associated nuclear antigens (LANA) or lytic-cycle antigens (Straus 2000). Low rates (less than 5%) of HHV-8 seroprevalence are reported in North Europe and the Americas (Duker et al, 2003), 35% in Sicily (Whitby et al, 1998), and 87% in Botswana (Engels *et al* 2002) and generally seem related to the prevalence of KS (Simpson *et al* 1996). Nevertheless, there are regions of the world, such as Brazil, Gambia, Ivory Coast, and Thailand, with high HHV-8 seroprevalence in which KS is rare (Szajerka *et al* 2007).

In the United States, HHV-8 infection is more common among gay men (prevalence 15-60%) than among heterosexuals (0-9%). Most epidemiologists agree that HHV-8 infection was already prevalent among gay men when the AIDS epidemic began, but do not agree about trends of HHV-8 infection thereafter. Prevalence of HHV-8 infection was 26.5% among gay men in San Francisco in 1978-1979 and 20.4% among gay men in New York City and Washington, DC in 1982 when the HIV epidemic began (O'Brien *et al* 1999; Osmond *et al* 2002). Prevalence of HHV-8 infection has been reported as stable at 25-30% from 1978-1996 among gay men in San Francisco (Osmond *et al* 2002), but a peak of infection during the early 1980s is reported among gay men on the East coast with a subsequent decline in new infections thereafter (O'Brien *et al* 1999).

5. Aluminosilicates in Africa

The African endemic form of KS, first described in 1914, occurs predominantly in black males between 25 and 40 years of age but also children at a mean age of 3 years (Oettle 1962; Friedman-Kien 1989). Among adults KS commonly presents as a chronic nodular condition affecting the arms and legs arising in the lymphatic endothelium and associated with chronic lymphoedema (Ziegler 1993). Among children with KS, lymphadenopathic tumors are most common, predominantly in the oro-facial areas and less commonly in the inguinal-genitals (Ziegler and Katongole-Mbidde 1996). The introduction and spread of HIV infection led to KS becoming the most common cancer in several areas of Africa and an increase in disease among both men and women (Dal Maso 2001). John Ziegler and others have studied the epidemiology of KS in Africa and have conducted case-control studies among both HIV-seropositive and HIV-seronegative patients.

In 1993 John Ziegler reported the increased prevalence of KS among rural peasants and cultivators toiling highland soils containing volcanic clay minerals. He notes similarities between KS and podoconiosis (non-filarial elephantiasis) (Ziegler 1993). In one study among HIV-positive adults in Uganda, he compared 458 adults with KS to 568 HIV-positive adults who had not developed KS. KS patients included 295 men (64%) and 163 women (36%), whereas the HIV-positive control group without KS included 227 men (40%) and 341 women (60%). Study participants were interviewed regarding social and lifestyle factors. In this HIV-positive group, KS was associated with several variables including better education, higher income, urban address, travel, monogamous marriage (as compared to polygamous marriage), self-reported sexually transmitted disease, to be from tribal groups other than the Baganda

(the dominant tribe in Uganda) and exposure to water (Ziegler *et al* 1997). In 2003, Ziegler reported the results of another study of KS this time among HIV-negative adults in Uganda. Social and lifestyle factors from 117 HIV-negative adults with KS (91 males and 26 females) were compared with 1,282 HIV-seronegative patients with a provisional diagnosis of cancer other than KS (control group). KS was associated with higher income, leaving home at an earlier age, owning goats or pigs, and to rarely or never wearing shoes. HHV-8 antibody status was available for 80 (68%) of cases and 607 (45%) of controls; 79% of cases tested had anti-HHV-8 antibodies compared to 50% of controls (Ziegler *et al* 2003). In 1996, Ziegler and colleagues also provided limited clinical and laboratory data on 100 children with KS under age 15 years treated in Kampala 1989-1994. Of the 100 children with KS reported, 63 were boys, 37 girls, median age at diagnosis was four years, median age on onset was 33 months. These tumors presented as lymphadenopathic and mucocutaneous tumors, most commonly in the oro-facial area (79 cases) and inguinal-genital region (13 cases). Of 63 children with KS tested for HIV antibodies, 49 (78%) were positive; 8 of 8 children tested for HHV-8 antibodies were positive (Ziegler and Katongole-Mbidde 1996).

As a result of those studies, Ziegler proposed that KS results from the activation of latent HHV-8 in cells by immune suppression or inflammation to an oncogenic state. Walking barefoot in volcanic soils exposes pores and sweat glands in bare feet and sweat glands to abrasions and allows aluminosilicates and possibly iron oxides to be taken up by lymphatics, inflaming lymph nodes and initiating KS lesions in the feet and legs (Ziegler 1993; Ziegler *et al* 2001). Sam Mbulaitaye, a co-investigator of Ziegler, and colleagues suggested that the exposure to soil and water may further allow parasitic infections leading to immune suppression and/or local inflammation of tissues (Lin *et al* 2008).

6. Nitrite inhalant abuse among gay men

Our group previously analyzed data from three epidemiologic studies conducted by CDC, comparing patients by AIDS disease outcome (Haverkos *et al* 1985). Eighty-seven gay patients (47 with KS, 20 with *Pneumocystis carinii* pneumonia (PCP), and 20 with both KS and PCP) had participated in the earlier CDC studies, and their interviews and laboratory results were available. The interviews were conducted face-to-face by CDC physicians and collected lifetime behavioral information prior to the onset of AIDS prodromal symptoms. Compared with patients who had PCP only, patients with KS and those with both diseases reported a larger number of male sexual partners, more recreational drug abuse, higher incomes and higher rates of non-B hepatitis. Multivariate analysis showed that the variable most strongly associated with KS was the use of large quantities of nitrite inhalants (greater than 383 days of nitrite use lifetime prior to onset of symptoms – OR=12.5 in univariate analysis). Other variables significant on multivariate analysis included income greater than $20,000 in year prior to onset of symptoms (OR=7.5), any receptive anal intercourse in year before onset (OR=5.1), and greater than 99 male sexual partners in the year before onset of illness (OR=3.3) (Haverkos *et al* 1985).

There are several reasons to consider nitrite inhalants as an etiologic factor in KS. First, the epidemiology of nitrite use among gay men in the US parallels that of KS (Haverkos *et al* 1994). Second, some, but not all, epidemiologic studies have shown a statistical association between the use of large quantities of nitrite inhalants among gay men with AIDS who developed KS compared with gay men with AIDS who did not develop KS (Haverkos and Drotman 1995). Third, anecdotal clinical reports of increased frequency of AIDS-related KS on the chest and face, especially the nose, and in the lungs are consistent with body areas most heavily exposed to nitrite vapors when inhaled (Haverkos *et al* 1994). Fourth, plausible biological mechanisms of action have been proposed for nitrites and their metabolites, such as cholesteryl nitrite and nitrosamines, to be carcinogenic, and mutagenesis has been demonstrated in the Ames test (Mirvish and Haverkos, 1987; Dunkel *et al* 1989; Mirvish *et al* 1993). Finally, nitrites are known to specifically affect small blood vessels thought to give rise to KS (Haverkos *et al* 1994).

Nitrite inhalant use might also contribute to the development of KS through immune suppression. National Institute of Health investigators studied the effects of nitrite inhalation on the immune system of 18 healthy, HIV-negative male volunteers in a secured research medical facility at the National Institute on Drug Abuse, Baltimore, Maryland. There were two groups of subjects: nine subjects inhaled three doses of amyl nitrite per day for three consecutive days (total dose 3.06 ml amyl nitrite); 9 subjects participated

in 13 inhalation sessions over 18 days (total dose 4.5 ml amyl nitrite). Nitrite inhalation caused decreases in absolute numbers of CD3+ T lymphocytes and in natural killer (NK) cell activity against K562 target cells. CD3+ T lymphocytes returned to pre-treatment levels within one week of cessation of the drug, and NK cell numbers returned to pre-inhalation or greater levels after nitrite discontinuation (Dax *et al* 1991).

NIH-funded investigators in the Multicenter AIDS Cohort Study (MACS) compared behavioral and laboratory evaluations of 24 gay men who developed KS in the 15 months following entry with a subset of 295 gay controls selected from 1,176 HIV-positive men who had not developed AIDS within 15 months of entry. In multivariate analysis, a decreased number of T helper lymphocytes < 600 per ml. (OR=0.01), hemoglobin greater than 15.5 g/dl. (OR=0.13), and immunoglobulin A levels greater than 370 mg/dl (OR=7.04) were independently associated with subsequent KS. No significant differences were found between cases and controls for behavioral measures: use of nitrites during sex in previous two years (OR=0.42), more than 100 sexual partners in previous two years (OR=1.74), anal receptive intercourse with most partners in previous two years (OR=0.39) (Polk *et al* 1987).

What explains the huge differences in reports of sexual behavior and drug abuse in the two studies? There were several differences in the design, such as, definitions of cases and controls, and in implementation between the CDC case-control compared to the NIH-funded cohort studies. NIH researchers asked about nitrite use only within two years of entry and did not attempt to quantify drug usage in their analysis. The NIH investigators employed a pencil and paper interview format which could conceivably introduce reporting bias, especially when the behaviors of interest involve sex and drug abuse. Interviewing patients and controls regarding sexual and drug behaviors during the two years prior to interview with no regard to onset of prodromal symptoms (as was done in the CDC studies), or the effects such symptoms might have on sexual behavior and drug usage, is a also potential source of bias (Haverkos *et al* 1985; Polk *et al* 1987; Armenian *et al* 1994).

In 1994 the MACS investigators reported an updated analysis comparing the behavioral data reported by 316 men who had developed Kaposi's sarcoma with that from 510 men who had developed AIDS, but no evidence of cancer. More of the KS patients were from Los Angeles, used a higher number of recreational drugs, and were more sexually active than the patients with AIDS but not KS. On multivariate analysis the following variables were significant: West coast sexual partner (OR=1.86), history of 5 or more STDs (OR=3.16), number of male partners in the previous two years (1.68), and use of nitrites in the previous two years (1.68) (Armenian *et al* 1994).

7. ACE inhibitor use among elderly men and renal transplant recipients

Elderly Mediterranean men and patients receiving immunosuppressive therapy are at increased risk for KS. In 2003, I was consulted by a regulatory division at FDA to provide an update on nitrite inhalants and KS. At about the same time, Dr. Scott Norton, a dermatology colleague at Walter Reed Army Medical Center provided me with three case reports of patients with KS in whom lesions had begun after captopril or lisinopril (angiotensin-converting enzyme [ACE] inhibitors) therapy was initiated; and, lesions disappeared or improved when therapy was stopped (Puppin *et al* 1990; Labre *et al* 1991; Bilen *et al* 2002). On the other hand, another KS case report among a renal transplant patient in which KS appeared to regress with the institution of captopril therapy (Vogt and Frey 1997).

French clinicians have reported the case study of a 70-year old heterosexual male with essential hypertension treated for six years with captopril (75 mg daily) and acebutolol (200 mg daily) who presented with a 2-month history of three nodular angiomatous lesions on his left arm. Histopathology was typical of KS. HIV testing was negative; CD4 count was 802 cells per ml. Captopril was stopped and KS lesions were noted to be diminished one month later and absent 3 months later. Biopsy of skin of the arm at an unspecified time showed residual features of KS (Puppin *et al* 1990).

A second group of French investigators reported similar observations in a 70 year old Algerian woman with rheumatoid arthritis and KS. Cutaneous and gastric KS lesions appeared 8 months after start of captopril and cleared when captopril was stopped (Labre *et al* 1991).

Turkish clinicians reported KS in a 78 year old man with hypertension for 28 years and diabetes mellitus for 6 years. He was being treated with lisinopril, *Pentaerythritol tetranitrate*, digoxin, dipyridamole,

ginkgo glicozids, carbamazepine, and insulin. He presented with a 6 month history of multiple skin lesions on his right shoulder, left axilla, presternum, left groin and gluteus, with lymphedema of the lower extremities, lymphadenopathy on the submandibular, axillary, and inguinal areas; and hemorrhagic lesions were noted in the esophagus on endoscopic examination. HIV antibody testing was negative. Histopathology of esophageal and submandibular lymph nodes was consistent with KS. Because the lesions appeared 6 months after the start of lisinopril and the two case reports cited above were noted by the clinicians, lisinopril therapy was discontinued. Marked improvement was noted at 4 months for cutaneous lesions on the upper parts of the body; complete resolution of the upper body and esophageal lesions were noted at 8 months after stopping lisinopril. Lesions on the thighs persisted and were treated with cryotherapy and surgery. The authors suggested that lisinopril might play a causal role for KS in their case (Bilen *et al* 2002).

8. Discussion

We propose that KS results from a myriad of complex interactions between HHV-8 infection, immunosuppression, and selected vasoactive substances (table 2). AIDS-related KS in the USA and Europe appears to result from a complex interaction of HHV-8, HIV, and perhaps nitrite inhalant abuse (Haverkos 2004). In Africa HHV-8 infection may be reactivated by HIV and/or by inflammatory agents in the soil, such as aluminosilicates (Ziegler *et al* 2001). Anecdotal clinical reports further suggest that HHV-8 may be activated by ACE inhibitors and other vasoactive substances among classical KS patients, renal transplant recipients, and other immunosuppressed, HHV-8-infected patients. More research is needed to test these hypotheses.

Wide acceptance of the "single agent, single disease" paradigm has led to the prevention and control of many previously baffling and devastating illnesses. Many scientists have invoked that paradigm to establish the cause of KS. Initially HIV and later HHV-8 were proposed as the single agent cause of KS (Gallo 1991; Chang and Moore 1996; Ganem 2006). However, those single agent hypotheses do not explain the occurrence of KS in the various populations. In this paper we propose another paradigm, a co-carcinogenic or multifactorial hypothesis for the etiology of KS.

According to Thomas Kuhn, "the research worker is a solver of puzzles, not a tester of paradigms. … Like the chess player … he tries out various alternate moves in the search for a solution. These trial attempts, whether by the chess player or by the scientist, are trials only of themselves, not of the rules of the game. They are possible only so long as the paradigm itself is taken for granted. Therefore, paradigm-testing occurs only after persistent failure to solve a noteworthy puzzle has given rise to crisis. And even then it occurs only after the sense of crisis has evoked an alternate candidate for paradigm" (Kuhn 1962). In this paper we propose a co-carcinogenic or multifactorial hypothesis for the etiology of KS.

In 1963 Chester Southam noted that co-carcinogenesis was not a new phenomenon but that it was worthwhile to consider that most cancers were due not to single causative agents, but rather to a complex of multiple factors each of which contributes something to the whole process. These co-carcinogens

Table 2. Multifactorial hypothesis – Etiology of Kaposi's sarcoma (KS)

KS variant	Herpes virus	Immune system	Vasoactive agent
Classic	HHV-8	Senescent T- cell depression	ACE inhibitors
Endemic (African)	HHV-8	Environment (parasites, diet, herbs)	Aluminosilicates, Iron oxides
Iatrogenic	HHV-8	Steroids, other immuno-suppressants	ACE inhibitors
Epidemic (AIDS-related)	HHV-8	T-cell defect due to HIV	Nitrite inhalants (Aluminosilicates in Africa)

ACE inhibitors, angiotensin converting enzyme inhibitors, i.e. captopril, lisinopril; AIDS, acquired immunodeficiency syndrome; HHV-8, human herpes virus type 8; HIV, human immunodeficiency virus; KS, Kaposi's sarcoma.

may act simultaneously or sequentially; continuously, repeatedly, or rarely. They may act directly on the potentially cancer cell or indirectly by affecting other tissues of the host. This complex etiology of cancer is particularly challenging in that it promises no single solution. Nevertheless it fits the epidemiology of KS incidence better than any single etiology and it is appealing because it offers the possibility that prevention of exposure to any one of the postulated co-carcinogens might significantly reduce cancer incidence, and that treatment directed at any one carcinogen might slow progression of disease (Southam 1963; Haverkos 2004). In conclusion, we encourage cancer researchers to re-evaluate the paradigm of co-carcinogenesis when deciphering the etiology of KS.

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HARRY W HAVERKOS*

Captain US Public Health Service (Retired) Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA
**Address for correspondence: 15328 Bitterroot Way, Rockville, MD 20853, USA (Fax, 301 402-0817; Email, hhaverkos@usuhs.mil)*

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