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Research Protocol

A Matched Case-Control Study of Soft Tissue Sarcoma

Agent Orange Projects Office
Department of Medicine and Surgery
Veterans Administration

and

Department of Soft Tissue Pathology
Armed Forces Institute of Pathology

March 1983

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I. Introduction

There is much concern in the United States that many veterans report health problems that possibly stem from their military service in Vietnam. Their complaints include a wide variety of medical problems such as psychological, dermatological and physiological illnesses, reproductive disorders and even cancer. Agent Orange was the herbicide most commonly applied in Vietnam by the United States Air Force between 1965 and 1971. It was a mixture of the two commercial herbicides, 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid). The 2,4,5-T contained minute amounts of an extremely toxic chemical, dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin or TCDD), which contaminated the herbicide during the manufacturing process. TCDD is teratogenic and carcinogenic in experimental animals (Poland and Knutson, 1982; Kociba et al., 1978; NCI, 1980).

The possibility that exposure to the herbicide may induce rare forms of cancer in humans such as soft tissue sarcoma (STS) has been suggested from recent studies in Sweden (Hardell and Sandstrom, 1979; Hardell, 1981). The Swedish studies have shown that persons reporting exposure to phenoxy herbicides have a 5 to 6 fold higher risk of developing STS compared to persons without such exposure. A similar risk was reported by one of the Swedish investigators for malignant lymphoma (Hardell et al., 1981).

These significant observations have not yet been replicated by other research teams and studies by Finnish (Riihimaki, 1982) and New Zealand investigators (Smith et al., 1982) failed to show an association of STS with exposure to phenoxy herbicides. However, several confirmed cases of STS have been reported among workers involved in the manufacturing or use of phenoxy herbicides (Cook, 1981; Honchar and Halperin, 1981; Moses and Selikoff, 1981). These industrial workers, in contrast to the herbicide applicators, are believed to be exposed to relatively high levels of the TCDD contaminant.

Soft tissue sarcomas are a complex and diverse group of malignant neoplasms that originate in nonepithelial extraskeletal supporting structures of the body, excluding the hematopoietic-lymphatic system, the glia and supporting tissues of specific organs and tissues (Enzinger, et al., 1969). Soft tissue sarcomas account for about 1% of all malignant neoplasms and for about 2% of all cancer deaths. The average annual age-adjusted incidence rate is 3.89 per 100,000 and it is estimated that about 8,000 patients are diagnosed with STS each year in the United States (Cutler and Young, 1975). The most common histologic types are malignant fibrous histiocytoma, leiomyosarcoma, sarcoma not otherwise specified, liposarcoma and fibrosarcoma in that order.

Little is known about the etiology of STS. The epidemiologic study of STS has been especially difficult because of uncertainties in the morphologic classification of this diverse group of neoplasms. In addition, the International Classification of Disease (ICD), being site-oriented, does not distinguish between the heterogeneous types of sarcoma.

A small proportion of cases are probably related to Mendelian syndromes and the familial multiple-cancer syndrome (Tucker and Fraumeni, 1981; Blattner et al., 1979). An excess of STS has also been reported in patients receiving threapeutic immunosuppression for renal transplantation and other conditions (Hoover and Fraumeni, 1973; Kinlen et al., 1979). Some cases are associated with genetically determined immunodeficiency syndromes (Spector et al., 1978). Patients with chronic lymphocytic leukemia are also prone to STS (Greene et al., 1978).

There is very limited information on environmental risk factors for STS. A small fraction of STS is induced by heavy external radiation therapy for various benign disorders and malignant tumors. Nearly all cell types of STS have been described following radiation, the most common being fibrosarcoma (Kim et al., 1978; Czesnin and Wronkowski, 1978). Some radioactive materials used for diagnostic or therapeutic purposes may induce sarcomas at or near sites of deposition (Falk et al., 1979a; McKillop et al., 1978). The best known examples of associations between specific chemicals and STS of specific cell types is angiosarcoma of the liver and exposure to vinyl chloride or inorganic arsenical compounds. Among 168 deaths from hepatic angiosarcoma during 1964-1974 in the United States, 37 deaths were associated with vinyl chloride, thorotrast, or

inorganic arsenic (Falk et al., 1979b). The increased risk of developing STS among Swedish workers exposed to phenoxy herbicides or chlorophenols was described earlier.

In view of the concern raised by many veterans that their contact with Agent Orange during Vietnam service may increase the risk of developing STS and conflicting research findings in the scientific literature regarding association between exposure to phenoxy herbicides and STS, we have decided to conduct an independent epidemiologic study.

II. Research Questions

A. Phase I: Study based on the existing records

1. Does service in Vietnam during 1965-1971 increase or decrease the risk of developing STS among veterans?

2. Is there a trend in the odds of developing STS with increasing probability of exposure to Agent Orange?

3. Does the histopathology and anatomic site of STS among Vietnam veterans differ from those of non-Vietnam veterans and non-veterans (i.e., individuals who never served in the military)?

B. Phase II: Study based on the existing records and information obtained from interviews

4. What are the other host or environmental risk factors for the development of STS? Factors to be considered are:

(a) Occupational and non-occupational exposure to phenoxyacetic acids herbicides and other chlorophenols;

(b) Exposue to phenoxyacetic acid containing drugs such as clofibrate;

(c) Other factors such as genetic syndromes, immunologic deficiency, lymphedema, trauma and exposure to ionizing radiation, asbestos, arsenic, vinyl chloride and steroids.

III. Study Design

A matched case-control study design will be used, in which individuals with STS (cases) are compared with individuals without STS (controls) with respect to Vietnam service, probable Agent Orange exposure and other possible risk factors. The case-control method is chosen primarily because it is well suited to the study of rare diseases (annual incidence of STS is 3.9/100,000), it is relatively quick and inexpensive, and it allows the study of multiple potential causes of the disease.

1. Cases

Cases will be drawn from accession lists of the Armed Forces Institute of Pathology (AFIP). The AFIP offers a unique resource to contribute to this study. The AFIP routinely provides consultation services for pathologists throughout the United States, especially for conditions such as STS which present special diagnostic problems. One quarter to one third of the STS's occurring in the United States are sent to AFIP for review. Thus, the AFIP is one of the largest single registries in the world for this group of tumors. The uniformity and high quality of diagnoses at AFIP give it an added advantage as a resource for epidemiologic studies.

Selection will be restricted to males, who were diagnosed as STS patients sometime between January 1, 1975 and December 31, 1980 and were aged 20 to 40 at the time of diagnosis. These eligibility criteria are established 1) to restrict the study to persons who were potentially at risk of exposure to Agent Orange; that is, persons would have been aged 18 to 23 sometime during 1965 and 1971, the period when Agent Orange was most heavily used in Vietnam, 2) to allow a minimum of 4 years of latency period, and 3) to reduce selection bias by restricting the cases to those referred to AFIP before the recent publicity on Vietnam service (or Agent Orange exposure) and the risk of developing STS.

2. Controls

Controls will be selected from the patient logs of referring pathologists or their pathology department. This is to duplicate the selective factors (e.g., socioeconomic status, area of residency, etc.) which bring people to these hospitals or clinics. Excluded from consideration as controls will be diagnoses of STS, non-Hodgkins lymphoma and Hodgkins disease. The latter two conditions have been associated with exposure to phenoxyacetic acid herbicides, chlorophenols, or their contaminants (Hardell, 1981). A contact person in each referring pathology unit, usually a medical assistant or nurse, will be asked to select the two sequential patients who matched the case by race, sex and age ($\pm$ 5 years): one with a malignant neoplasm, and one with a non-malignant disease in the log book following the STS case. A pilot study is needed to test the feasibility of this control selection method.

There are several reasons for choosing two controls per case: one control from other cancer patients and the other control from non-cancer patients. First, 2:1 matching will increase the statistical power of the study. This will be discussed in the following section. Second, possible recall bias and interviewer bias can be minimized by selecting other cancer patients as controls. The cancer patient may try harder to remember his exposure to well publicized chemical carcinogens and radiation. Recall bias may, therefore, occur when these patients are compared with individuals with no cancer. In addition, the interviewer may tend to probe the cancer patients or their families more intensively for histories of exposure than they might for the control subjects or their families. Third, on the other hand, it is also possible that exposure to phenoxy herbicides and chlorophenols may cause some of the cancers in the control group and this would mask an association between an exposure to these chemicals and STS. Having non-cancer patient controls would eliminate this possibility.

Recall bias or interviewer bias will not be a problem for determining military service status because this will be verified by records kept by the Veterans Administration* and the National Personnel Records Center**.

* VA BIRLS (Beneficiary Identification and Records Locator Subsystem). The Veterans Administration maintains a file of nearly 40 million computerized records known as the BIRLS file. It contains the veteran's name, date of birth and social security number and/or service numbers. Although Vietnam service is one of the information categories represented, this information is not provided on most of the records. Prior to 1972 only veterans who filed a claim for VA services were placed in the BIRLS file. Since January 1973 the names of all discharged veterans have been listed.

** National Personnel Records Center (NPRC). The NPRC is the major repository for records of veterans who have been discharged from the service. This is believed to be the best records center for determining the Vietnam service status of veterans. Absence of the study subject's record in the center would indicate that he did not serve in the military or that he was still on active duty.

IV. Statistical Considerations

1. Sample size determination

The number of people to be selected for the study depends on the specifications of four values: (1) the relative frequency of risk factor among controls in the target population, P_o, (2) a hypothesized relative risk associated with the risk factor that would have sufficient public health importance to warrant its detection, R, (3) the desired level of significance, alpha; that is, the probability of making an error of claiming that the risk factor under investigation is associated with disease when in fact it is not; (4) the desired study power, 1-beta; that is, the probability of claiming that the risk factor is associated with disease when in fact it is.

Sample size for the study was determined under the following conditions:

(1) alpha = 0.05
(2) beta = 0.2 or 1-beta = 0.8
(3) R = 2
(4) P_o = 0.05
(5) two sided test
(6) two matched controls per case

Under these conditions we will need a total of 500 cases and 1,000 controls. Please see attachment 1 for detailed calculation. Assuming about an 85% success rate for obtaining appropriate records for other condidtions, we will start with 600 cases of STS from the AFIP file. Preliminary data provided by the AFIP indicate obtaining 600 cases will not be a problem. (Please see attachment 2) As of the end of 1980 about 1,100 cases had already met the criteria for cases.

2. Analyses of Data

The data will be analyzed using conventional epidemiologic and biostatistical methods including the following:

a. When a single univariate binary risk factor is considered, the following matched analysis with two controls per case is used:

$$X^2 = \left[\frac{P_2 - P_1}{s.e.(P_2 - P_1)}\right]^2 = \frac{[(m-1)\ B-mA]^2}{mB - \sum_{i=1}^{N} n_i 2}$$

$P_1 = \frac{A}{N(m-1)}$, The proportion of controls having risk factor

$P_2 = \frac{B - A}{N}$, The proportion of cases having the risk factor

where, A = the total number of controls with the risk factor

B = the total number of either cases or controls having the risk factor

N = the total number of matched triples

m = 3 (1 case + 2 controls)

n_i = number of either case or controls having the risk factor within a given triple

x_i = number of controls with the risk factor within a given triple

The odds ratio is calculated as follows:

$$0 = \frac{(m-1)\ (B-A) - \sum_{i=1}^{N} x_i(n_i - x_i)}{A - \sum_{i=1}^{N} x_i\ (n_i - x_i)}$$

b. An attempt to explore the individual and joint effects of a number of variables will be made using multivariate statistical analysis based on the linear logistic model. This technique enables one to investigate the effect of several variables simultaneously in the analysis while allowing for the matched design (Holford et al., 1978; Breslow et al., 1978).

V. Proposed Study Strategies

A. Phase I: A study based on the existing records

1. Case selection

a. Tabulate all male STS cases referred to AFIP during January 1, 1975 and December 31, 1980 by specific diagnosis and by age.
b. Identify from the AFIP records the males aged 20-40 at the time of diagnosis.
c. Randomly select a total of 600 cases among all eligible cases.
d. Obtain necessary information from AFIP records (name, age, name of pathologist and his location, etc.) for each case.

2. Control selection

a. Secure the consent of the pathologist whose patients will be approached to participate in the study.
b. Select controls from pathology log books with the cooperation of referring pathologists or their assistants. Controls will be be matched to case by sex, race, age ($\pm$5 years). The first two eligible patients (one with cancer excluding STS, non-Hodgkin's lymphoma, and Hodgkins disease and one with non-malignant disease) filed immediately after the case will be selected for controls.

3. Determination of military service status

a. Provide the National Personnel Records Center (NPRC) in St. Louis a listing or computer tape containing full names of both cases and controls, social security number and other identifying information obtained from the AFIP, referring pathologists and primary care physicians.

b. The NPRC will search and pull military personnel records for on-site review by VA contractor employees.

c. The contractor will review and extract necessary information from the file.

d. Cases or controls from non-military hospitals whose records are not kept in the NPRC could be either non-veterans (never served in the military), or still on active duty. However, if one assumes that active duty servicemen use military hospitals especially for the treatment of illness that requires referral to pathologists and since these cases and controls are from non-military hospitals it would be almost certain that they did not serve in the military or that they are on reserve duty.

e. The cases and controls from military hospitals will be referred to the military personnel records centers of each branch of service for ascertaining active duty status and obtaining appropriate military records.

f. The names and social security number (SSN) of cases and controls will be cross checked with the VA BIRLS file. The BIRLS file contains a record for each VA beneficiary and as of January 1973, BIRLS began including records for all veterans at separation from military service.

g. Develop an Agent Orange exposure ranking scheme based on MOS data and other information extracted from military records.

4. Initial analysis of data obtained from the available records.

The first three research questions listed on page 5 can be addressed with information obtained from the records.

B. Phase II

1. Locate cases and controls with help from pathologists, the surgeon's office and/or primary physicians. Since cases and controls medical records go back only a maximum of 7 years, it may not be insurmountable to locate them. However, this effort will be complemented by the following tracing mechanisms:

   a. IRS-NIOSH-SSN
   b. Telephone directory
   c. Post Office
   d. State motor vehicle department
   e. Credit bureau

2. Develop a questionnaire

3. Prepare introductory and informed consent letters and obtain consent of cases and controls, or their next-of-kin prior to conducting interviews, in accordance with existing regulations.

4. Develop an interview schedule. Conduct a pretest and make necessary revisions.

5. Conduct telephone interviews of the cases and controls, or their next-of-kin.

6. Review, edit and code all completed interviews.

7. Analyze data.

8. Final Report.

VI. Confidentiality

Confidentiality of all records pertaining to individuals in the study will be carefully protected. Names of individuals will be used solely to locate persons for the purpose of determining their military service status and of interviewing. Personal identifiers will not be retained on any data record used for analysis, nor will they be included in any publication or other presentation of study results. Records with personal identifiers will be under the control of VA and AFIP investigators or their agents and will not be accessible to other individuals or groups.

Acknowledgements

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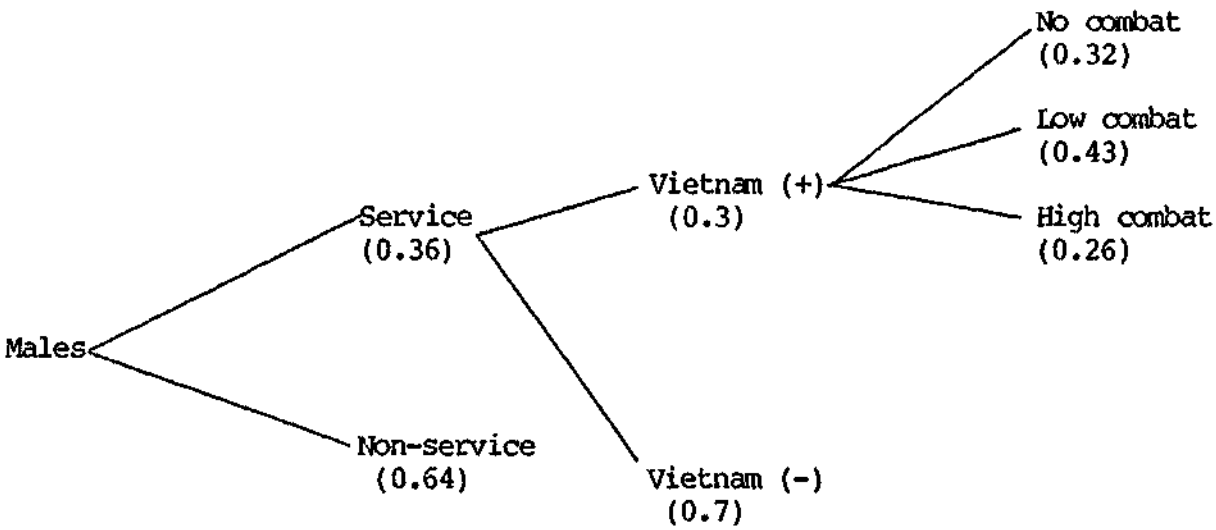
Attachment 1

a. The relative frequency of the risk factor among controls in the target population, P_O



(1) risk factor = Vietnam service/high combat duty
Assuming the servicemen in the Vietnam/High combat category were most likely exposed to Agent Orange, the P_O was calculated as follows:
$P_O = 0.36 \times 0.3 \times 0.26 = 0.029$

(2) risk factor = Vietnam service
$P_O = 0.36 \times 0.3 = 0.11$

(3) risk factor = Vietnam service/combat (high + low)
$P_O = 0.36 \times 0.3 \times (0.43 + 0.26) = 0.07$

(4) risk factor = Occupational and non-occupational herbicide exposure NCI assumes $P_O = 0.1$

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Attachment 1 - Continued

We have chosen a conservative number $P_o = 0.05$ for the study.

b. Sample size with two controls per case.

(1) $P_o = 0.05$, alpha = 0.05, beta = 0.10, R=2

$$n = \left[Z_\alpha \sqrt{(1 + 1/c)\ \bar{P}\bar{q}} + Z_\beta \sqrt{P_1 q_1 + P_o q_o / c} \right]^2 / (P_1 - P_o)^2$$

where, $P_1 = P_o R/[1 + P_o (R-1)]$

$\bar{P} = (P_1 + CP_o) / (1 + C)$

$q_1 = 1 - p_1$, and $\bar{q} = 1 - \bar{p}$

c = number of controls per case

$$n = [1.96\sqrt{(1 + 1/2)\ 0.065 \times 0.935} + 1.28\sqrt{0.095 \times 0.905 + (0.05 \times 0.95)/2}]^2$$

divided $(0.095 - 0.052)^2 = 509$

(2) $P_o = 0.05$, alpha = 0.05, beta = 0.20, R=2

n = 372

c. Sample size with two matched controls per case

(1) $P_o = 0.05$, alpha = 0.05, beta = 0.10, R=2

$m = [Z_{\alpha/2} + Z_\beta \sqrt{P(1-P)}]^2 / (P-1/2)^2 = 90$

$P = R/(1+R) = 2/3 = 0.667$

$M \approx m/(P_o q_1 + P_1 q_o) = 90/0.135 = 666$

(2) $P_o = 0.05$, alpha = 0.05, beta = 0.20, R=2

m = 67.7

M = 501.8

Attachment 2

SOFT TISSUE SARCOMAS

AFIP
1975 -- 1980
Ages 20 to 90 years
(Excludes military and dependents)

	Number	%	M	F	Ages 20-	30-	40-	50-	60-	70-	80-
Malignant Fibrous Histiocytoma	1921	24	1114	803	80	119	208	388	513	416	197
Leiomyosarcoma	1285	15	592	692	56	124	206	293	327	216	63
Sarcoma NOS	987	12	520	466	238	158	138	154	140	118	41
Liposarcoma	800	10	485	314	53	104	130	185	195	105	28
Fibrosarcoma	510	6	288	221	86	77	71	102	91	58	25
Malignant Schwannoma	463	6	250	211	95	73	73	69	77	58	18
Atypical Fibroxanthoma	411	5	290	119	8	13	21	63	89	124	93
Hemangiopericytoma	356	4	144	212	59	61	70	78	45	29	14
Dermatofibrosarcoma Protruberans	341	4	170	169	75	96	66	59	28	14	3
Poorly Differentiated Sarcomas	340	4	192	147	96	47	43	57	54	33	10
Angiosarcomas	254	3	139	115	30	24	37	43	57	42	21
Synovial Sarcomas	247	3	134	113	82	65	37	33	24	3	3
Rhabdomyosarcomas	138	2	88	50	72	14	15	17	11	5	4
Myxofibrosarcomas	27		15	12	1	3	5	3	6	6	3
Neurogenic Sarcomas	5		2	3	1	1	1	2	0	0	0
	8085		4423	3647	2011						

aged 20-40 a total of 2011 STS
expected number of male cases = $2011 \times \frac{4423}{8070} = 1102$