

Cancer risk among women sterilized with transcervical quinacrine hydrochloride pellets, 1977 to 1991*

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Objective: To determine whether a cluster of eight cancers among 572 women who had received transcervical quinacrine hydrochloride was a random occurrence or evidence of an increased risk of cancer.

Design: Retrospective cohort study using interviews and reviews of medical records. Cancer cases were evaluated using cohort analyses and space-time cluster methods.

Setting: Santiago and Valdivia, Chile.

Subjects: Fourteen hundred ninety-two women who received transcervical quinacrine pellets for sterilization between 1977 and 1989.

Main Outcome Measure: Age- and site-specific incidence of invasive cancers.

Results: Eight hundred two women were interviewed. From 1 to 14 years of data were available on 600 of the noninterviewed women from clinic records. During 7,852 woman-years of follow-up, 17 invasive cancers were identified, compared with 11.8 expected, based on age-specific rates from the Cali, Colombia cancer registry. Five cases of cervical cancer were observed, compared with 3.96 expected. Only one other uterine cancer was observed, a leiomyosarcoma, compared with 0.2 or 0.3 other uterine cancers expected.

Conclusions: The occurrence of an unusual cluster was confirmed, but no evidence was found of excess cancer risk associated with quinacrine pellet sterilization. However there was a single provocative observation (the leiomyosarcoma), and surveillance of the cohort is continuing. *Fertil Steril* 1995; 64:325-34

Key Words: Quinacrine, cancer incidence, clinical trials, cohort analysis, space-time cluster

Voluntary sterilization is the most common method of birth control in the world. Since the mid-

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1960s, researchers have been working toward the development of inexpensive nonsurgical methods of female sterilization that involve simple delivery methods and are associated with minimum morbidity. The use of intrauterine quinacrine hydrochloride pellets may be such a method (1, 2).

Family Health International (FHI) was working with Chilean investigators when the identification of a cluster of cancers raised the issue of the possible carcinogenicity of quinacrine. Eight cases of cancer at a variety of sites were identified during the long-term follow-up of 572 women. These women had received two or three transcervical insertions of 250 mg of quinacrine hydrochloride between 1977 and 1981 as part of studies of the use of quinacrine pellets for nonsurgical sterilization.

The procedure used for quinacrine sterilization

has been described and involves the use of an IUD inserter to place seven cylindrical pellets into the uterus, each pellet containing 36 mg of quinacrine. The pellets dissolve and much of the quinacrine either is absorbed systemically or drains back out of the uterus into the vagina. However, enough remains in the uterus to cause inflammation and scarring of the intramural portion of the tubes. To produce reliable scarring, more than one insertion is necessary. Recent studies by Zipper et al. (3) have focused on the use of two insertions and included an adjuvant to improve efficacy. After two insertions at 1-month intervals, three pregnancies were seen in 159 women after approximately 2 years of follow-up.

Quinacrine is an acridine derivative and is mutagenic, probably because of its intercalation into the DNA molecule (4). Despite its widespread use as an antimalarial in the Pacific theater in World War II (5) and its use as a treatment for rheumatic diseases in recent years (6), FHI became concerned that transcervically administered quinacrine might be an unrecognized human carcinogen. For various reasons, including the identification of the cancer cluster described above, FHI halted its work on the licensure of quinacrine under a U.S. Food and Drug Administration (FDA) Investigational New Drug (IND) exemption. At the same time, FHI commissioned a review of the toxicology of quinacrine, and began the retrospective cohort study described below.

MATERIALS AND METHODS

The purpose of this study is the evaluation of a possible relationship between sterilization with quinacrine hydrochloride and a cancer cluster. The study includes a "cluster analysis" to determine whether the observed pattern of cancers occurring in the cohort was a departure from a random pattern within the general population. Fourteen hundred ninety-two Chilean women who had received quinacrine pellet sterilization treatments were identified for inclusion in the study population. The study's procedures involved: [1] clinic record reviews of all women, [2] active tracing of these women and interviews with them to identify cancer occurrences through calendar year 1991, [3] hospital record reviews for all cancer cases identified, and [4] review of pathology slides for all gynecologic cancers.

Field Methods

There were 1,341 women from Santiago and 151 from Valdivia. Interviews were completed for 138 women from Valdivia (9.1%) and for 664 women from Santiago (49.5%). In the case of women who had died, the interview was conducted with the closest available living relative.

The data forms from all clinical and hospital record reviews and personal interviews were sent to FHI in Research Triangle Park, North Carolina. Extensive checking was performed for completeness and accuracy. Cases of invasive cancer that were diagnosed after quinacrine pellet sterilization were identified and reviewed and served as the main outcome measure for the analysis. Histopathology slides were requested for the gynecologic cancers for independent review.

Analysis Methods

Initial descriptive assessments were made using Pearson chi-square tests. The objective of the main analysis was to determine if the observed cluster represented a significant departure from the expected pattern of cancer occurrence among this cohort of Chilean women. If this observed cluster was determined to be statistically significant, an additional objective was to determine if there was any evidence to suggest an excess cancer risk due to sterilization procedures with quinacrine pellets.

We calculated the number of woman-years for each cohort member based on the length of follow-up. For the women who were interviewed, the number of woman-years was defined as the interval from their initial sterilization until the time of the interview. For the cohort members who were not interviewed, the follow-up interval was measured from the date of first insertion until the date of the last visit recorded in the clinic's records. The conventional 6-month rounding rule was applied for the preparation of the woman-years as whole years. If a person contributed ≤ 6 months to a year, they were not included in that year. The comparison of the incidence density of cancer cases between Valdivia and Santiago was performed using a maximum likelihood estimate of the rate ratio.

The estimation of the expected cancer risk for the cohort was calculated using the age- and gender-specific incidence rates from Cali, Colombia (7). The only referent data considered for this analysis were other South American data. We had begun our analysis using volume V of *Cancer Incidence in Five Continents*, which did not include data from several South American registries that were subsequently included in volume VI. To reassess the choice of Cali as the referent data base, we compared Cali's ranking by incidence rate of cervical cancer with the other South American registries in volume VI. Among the six South American registries in volume VI, Cali ranks fourth with an age-standardized rate of 42.2 per 100,000. It was below the rates in Trujillo, Peru; Asuncion, Paraguay; and Goiania, Brazil, and above the rates in Quito, Ecuador and Porto Alegre, Brazil.

The range of rates in the other South American registries was from a high of 54.6 per 100,000 in Trujillo to a low of 31.2 per 100,000 in Porto Alegre.

Data from two sources within Chile also were considered; however, neither source was considered either sufficiently detailed or consistent across age groups. One of the sources was based on a small population and was internally inconsistent, (Cancer Incidence from the Valdivia Tumor Registry, 1985 to 1989, personal communication, Dr. Carlos Martinez, December 9, 1992), while the other source was based only on death certificate data (Anuario de Estadísticas de Mortalidad, 1981 to 1990 from Demographia, personal communication from Dr. Alfredo Dabancens, January 12, 1993).

The pattern of observed and expected occurrences was evaluated with three statistical tests from the computer program CLUSTER (8). Owing to the absence of a conventional spatial distribution, the principle lines of evaluation were temporal. In this process, two time-series techniques developed especially for surveillance of rare health events were used. These techniques evaluate the observed pattern of cancer occurrence against the expected distribution using both the Poisson and Negative Binomial distributions (9). In each case, the null hypothesis is that the observed cases follow a pattern that is reasonable, based on the total cases observed and the amount of time available for their occurrence. The space-time clustering analysis techniques we used have been described in detail (10).

On the basis of the patterns identified by the cluster analyses, cases were selected that represented the greatest departure from the expected pattern (i.e., suspected cluster), based on the location of cases within the woman-years distribution. The characteristics of the individual cases comprising this suspected "cluster" were inspected for cancer risk characteristics on the basis of their personal and/or medical histories (e.g., family history of cancer, prior contraception, and other personal characteristics) (11). We assessed the potential biologic plausibility with regard to generally accepted disease latency considerations, secular trends within the populations represented, and potential toxicologic mechanisms involving quinacrine exposure. This last evaluation was based upon the technique of sentinel health event studies (12, 13).

RESULTS

A thorough review of the data set revealed a total of 36 cancers. Of these, 7 cancers were diagnosed before quinacrine sterilization, 12 were carcinomas in situ of the cervix, and 17 were invasive cancers diagnosed after quinacrine insertion. Carcinomas in

situ of the cervix generally are excluded from cancer registry data (7), and we called Dr. Pelayo Correa, former director of the Cali registry, and verified that they were not included in the Cali registry data. We therefore excluded the 12 carcinoma in situ cases from our analysis, because of a lack of comparison data. However, a separate analysis of cases of carcinoma in situ from Santiago has been performed (see Dabancens et al. [14], this issue). The present analysis concerns only the 17 invasive cancers, 8 from Valdivia and 9 from Santiago. Of the 17 women with invasive cancers, 8 were dead at the time of this study.

This retrospective cohort study has good statistical power for these analyses assessing excess risk for all cancers. There was 76% power (24% chance of a type II error, i.e., of falsely rejecting the hypothesis of an association) for detecting a twofold excess of cancer of all sites in a cohort of approximately 8,000 woman-years. This is acceptable considering both the limited number of women and the relatively rare disease rate (0.00150 cases per woman-year). Further examination found that there was 61% power (39% chance of a type II error) based on the 12 expected cases. With these assessments of power, the criterion evaluated was the ability to detect at least a twofold excess of all cancers.

Descriptive Analyses

From 1977 through 1989, 1,492 women in Chile received voluntary sterilization via transcervical insertion of quinacrine pellets and thus were eligible for the retrospective cohort. Of these, 802 women were able to be located and interviewed (54.6%). It is notable that 138 of the 151 women from Valdivia (91.4%) were interviewed, whereas only 664 (49.5%) of the 1,341 women from Santiago were interviewed.

Comparisons—Interviewed versus Noninterviewed Subjects

A comparison was made between the 664 Santiago cohort members who were interviewed and the 677 who were not interviewed (Table 1). The variables assessed were date of initial insertion of quinacrine hydrochloride, age at sterilization, years of education, number of live births, and previous birth control methods used. The interviewed cohort members were slightly older at their initial sterilization than those who were not interviewed ($\chi^2 = 10.5$, 3 *df*, *P* = 0.015). For education, parity, and previous birth control, the groups were comparable.

The interviewed women received their first quinacrine insertion at an earlier date than the noninterviewed women ($\chi^2 = 89.5$, 12 *df*, *P* < 0.001). The median insertion date for both of these groups was

Table 1 Comparison of Year of Insertion and Demographic Characteristics of Interviewed Versus Noninterviewed Women in Santiago and of All Women in Valdivia*

| Characteristic | Santiago Site | | χ^2 | Valdivia site, all women (n = 151) |
|--------------------------------|--------------------------|-----------------------------|--------------------|---------------------------------------|
| | Interviewed (n = 664) | Noninterviewed (n = 677) | | |
| Year of insertion | | | 89.46 12 <i>df</i> | |
| 1977 | 55 | 35 | | |
| 1978 | 30 | 15 | | |
| 1979 | 23 | 9 | | 151 |
| 1980 | 14 | 8 | | |
| 1981 | 50 | 18 | | |
| 1982 | 18 | 5 | | |
| 1983 | 2 | 4 | | |
| 1984 | 59 | 44 | | |
| 1985 | 19 | 49 | | |
| 1986 | 92 | 165 | | |
| 1987 | 64 | 101 | | |
| 1988 | 100 | 116 | | |
| 1989 | 138 | 108 | | |
| Age at Sterilization | | | 10.49, 3 <i>df</i> | |
| <30 years | 111 | 146 | | 51 |
| 30-34 years | 254 | 253 | | 57 |
| 35-39 years | 205 | 212 | | 38 |
| 40+ years | 94 | 64 | | 5 |
| Total reporting | 664 | 675 | | 151 |
| Years of education | | | 1.25, 2 <i>df</i> | |
| <6 years | 153 | 82 | | 57 |
| 6 to 10 years | 308 | 156 | | 60 |
| >10 years | 75 | 48 | | 34 |
| Total reporting | 536 | 286 | | 151 |
| No. of live births | | | 2.75, 4 <i>df</i> | |
| <3 live births | 92 | 82 | | 53 |
| 3 to 4 live births | 391 | 385 | | 76 |
| 5 to 6 live births | 146 | 171 | | 15 |
| 7 to 8 live births | 28 | 28 | | 6 |
| >8 live births | 7 | 9 | | 1 |
| Total reporting | 664 | 675 | | 151 |
| Previous birth control methods | | | 3.70, 2 <i>df</i> | |
| None | 55 | 72 | | 13 |
| Oral | 271 | 248 | | 34 |
| Others | 333 | 352 | | 98 |
| Total reporting | 659 | 672 | | 145 |

* Discrepancies between characteristic and column totals and the respective group total represent missing data.

1986, yet 18.4% of the interviewed group first received quinacrine insertions before 1981 versus < 10% of those not interviewed.

Table 2 shows the number of years of follow-up of women from time of sterilization until a cancer occurrence, last clinic visit or interview, by study site and by interview status. Among Santiago women not interviewed, 587 had made clinic visits for 1 to 14 years after quinacrine insertion, but could not be located for an interview in 1991. Ninety women were excluded from this table: 87 because their length of follow-up was too short, i.e., they did not have 6 months of follow-up in any calendar year, and 3 because their date of last follow-up was missing. Women who were not interviewed contributed 32% of the total woman-years from Santiago and two of the nine cancer cases (22%) from the Santiago cohort. The two cancers in the noninterviewed women

were a cervical cancer and a bile-duct cancer. Using a one-tailed test, there was no significant difference in cancer incidence per 1,000 woman-years between the Santiago women interviewed and not interviewed (1.69 versus 1.05, respectively, $P = 0.27$). We considered excluding the noninterviewed women from the analysis, but that would not have altered our conclusions.

Cases versus Noncases

More of the cases than expected were residents of Valdivia, based on the woman-years contributed by each site, with a rate ratio of 3.3, $P = 0.015$. The 17 cancer cases were sterilized at slightly older ages than the comparison women ($\chi^2 = 10.3$, 3 *df*; $P = 0.02$). Also, the cases were likely to have had fewer pregnancies and a larger proportion of live births

Table 2 Length of Follow-up by Study Site and Interview Status Until Interview or Loss to Follow-up or Cancer Diagnosis, Quinacrine Cohort, 1977 to 1991

| Study site/status | Years of follow-up | | | | | | | | Total |
|------------------------------|--------------------|--------|--------|--------|---------|----------|----------|----|-------|
| | 1 to 2 | 3 to 4 | 5 to 6 | 7 to 8 | 9 to 10 | 11 to 12 | 13 to 14 | 15 | |
| Valdivia [†] | | | | | | | | | |
| No cancer | 0 | 4 | 1 | 2 | 6 | 104 | 26 | 0 | 143 |
| Cancer | 0 | 1 | 4 | 1 | 1 | 1 | 0 | 0 | 8 |
| Santiago [*] | | | | | | | | | |
| Interviewed? | | | | | | | | | |
| No cancer | 99 | 194 | 125 | 58 | 51 | 45 | 70 | 15 | 657 |
| Cancer | 3 | 1 | 2 | 0 | 1 | 0 | 0 | 0 | 7 |
| Not interviewed [‡] | | | | | | | | | |
| No cancer | 306 | 180 | 39 | 17 | 21 | 8 | 14 | 0 | 585 |
| Cancer | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 2 |

* Total no. of woman-years: 1,626.

† Total no. of woman-years: 6,052.

‡ Total no. of woman-years: 4,140.

§ Total no. of woman-years: 1,912.

from those pregnancies than the remainder of the cohort.

Cohort-Observed versus Expected

A woman-year, time period matrix was prepared to estimate expected values and to examine the age and temporal variation (Table 3). Ninety-one women were excluded from this table: the same 90 who were excluded from Table 2, plus 1 who was excluded because of unknown age. The number of woman-years is slightly greater in Table 3 compared with Table 2, because woman-years were counted from insertion to last follow-up, whereas the woman-years in Table 2 were counted from insertion to last follow-up or to the occurrence of cancer.

In this table, the woman-years from one subject may be included in several cells of the matrix as the person moves from one age category to another or from one time period to another. The observed cancers were entered into the cells according to the age and time of occurrence, and the expected cancers for each cell were calculated based on the number of woman-years and the age-specific rates from the Cali data.

One thousand four hundred one women provided 7,852 woman-years of follow-up from the date of their first quinacrine hydrochloride insertion. Based on the age distribution and woman-years of exposure over the 15-year interval, 11.82 new cancer cases would be expected (12). The 17 cancer cases observed are not unusual (observed:expected ratio = 1.44; $P = 0.094$). Comparing observed and expected by geographic location, there were 9 observed and 9.15 expected in Santiago, for a ratio of 0.98 ($P = 0.5$), and 8 observed and 2.67 expected in Valdivia, for a ratio of 3.00 ($P = 0.007$).

A separate analysis was conducted using only cases that occurred after ≥ 5 years of follow-up; this

adjustment was made to evaluate the relative impact of a restriction for nominal latency considerations. This analysis excluded the first 5 years of follow-up, thus reducing the number of woman-years to 3,174 and the number of observed cases to 12. The expected number of cases for this analysis was 6.25; an observed:expected ratio of 1.92. Both of the cases in the 1977 to 1981 period were dropped with this approach. Three of the excluded cases were taken from the 1982 to 1986 interval (thyroid, gall bladder, and breast); the latter two of these cases were part of the initially reported "cluster." However, none of the cell or marginal results were remarkably different from those shown in Table 3. For the sake of being conservative in our interpretation, the analysis was left unchanged, i.e., using all 17 cases and woman-years.

Temporal Evaluations

Table 4 shows the time elapsed from sterilization for each case. The most frequent cancers were breast (five cases) and cervical (five cases). The women with breast cancer were an average of 44 years of age and time elapsed since their first quinacrine insertion averaged 4 years. This interval is not compatible with a risk of breast cancer from quinacrine exposure in that breast cancer generally is accepted to have a disease induction period of over a decade and a latency of another decade or longer. It is notable that most of the breast cancer cases occurred relatively soon after quinacrine insertion, yet no temporal clustering was found. The mean age of the women with cervical cancer was 45 years and 8 years had elapsed on average after their first quinacrine insertion. These time characteristics are more compatible with reasonable biologic processes for cancers of the cervix where it is believed that induction may occur within a few years of exposure to a carcinogen and

Table 3 Observed and Expected Cancer Cases and Woman-years of Follow-up by Age Group and 5-year Periods Among 1,401 Women Sterilized with Transcervical Quinacrine Hydrochloride

| Age group* | 1977 to 1981 | 1982 to 1986 | 1987 to 1991 | Total |
|-----------------|-----------------|-----------------|-----------------|-------|
| <30 | | | | |
| woman-years | 137 | 176 | 247 | 560 |
| Observed | 0 | 0 | 0 | 0 |
| Expected | 0.05 | 0.07 | 0.09 | 0.21 |
| Ratio O:E | — | — | — | — |
| 30 to 34 | | | | |
| woman-years | 374 | 570 | 841 | 1,785 |
| Observed | 0 | 1 | 0 | 1 |
| Expected | 0.28 | 0.43 | .53 | 1.35 |
| Ratio O:E | — | 2.3 | — | 0.7 |
| 35 to 39 | | | | |
| woman-years | 336 | 870 | 1,281 | 2,487 |
| Observed | 1 | 2 | 0 | 3 |
| Expected | 0.41 | 1.05 | 1.55 | 3.01 |
| Ratio O:E | 2.5 | 1.9 | — | 1.0 |
| 40 to 44 | | | | |
| woman-years | 163 | 615 | 1,227 | 2,005 |
| Observed | 1 | 4 | 2 | 7 |
| Expected | 0.34 | 1.27 | 2.53 | 4.13 |
| Ratio O:E | 3.0 | 3.2 | 0.8 | 1.7 |
| 45 to 49 | | | | |
| woman-years | 19 | 205 | 587 | 811 |
| Observed | 0 | 2 | 3 | 5 |
| Expected | 0.05 | 0.56 | 1.61 | 2.22 |
| Ratio O:E | — | 3.6 | 1.9 | 2.3 |
| 50 to 54 | | | | |
| woman-years | 1 | 23 | 161 | 185 |
| Observed | 0 | 1 | 0 | 1 |
| Expected | — | 0.1 | 0.69 | 0.80 |
| Ratio O:E | — | 10.1 | — | 1.3 |
| 55 to 59 | | | | |
| woman-years | 0 | 1 | 18 | 19 |
| Observed | 0 | 0 | 0 | 0 |
| Expected | — | 0.01 | 0.10 | 0.11 |
| Ratio O:E | — | — | — | — |
| Total | | | | |
| woman-years | 1,030 | 2,460 | 4,362 | 7,852 |
| Observed | 2 | 10 | 5 | 17 |
| Expected | 1.13 | 3.48 | 7.20 | 11.82 |
| Ratio O:E | 1.8 | 2.9 | 0.7 | 1.4 |

* Ratio O:E, observed cases:expected cases.

the disease latency may be a similar interval. The observed cervical cancers vary in terms of the interval from first quinacrine insertion to a cancer diagnosis, and there was no temporal clustering of the cervical cancers within the follow-up period.

Site-specific Evaluation

Table 5 lists the 17 cancers by anatomic site and by treatment center. The observed:expected ratios for breast, cervical, and uterine cancer in Table 6 were obtained using indirect standardization with the Colombia age-specific rates. For breast cancer

Table 4 Diagnoses of Cancer by Anatomic Site and Time Since First Quinacrine Insertion, Quinacrine Cohort 1977 to 1991

| Interval in years | Cancers |
|-------------------|---|
| 1 to 3 | Breast, cervix, thyroid |
| 4 to 6 | Breast (n = 3), bile duct, gallbladder |
| 7 to 9 | Cervix (n = 2), colon, brain, breast, uterine sarcoma |
| 10 to 12 | Cervix (n = 2), gallbladder |

the ratio was 1.64 ($P = 0.18$) and 1.26 for cervical cancer ($P = 0.35$). Based on these results, there was little reason to continue inspection of these cases, as the ratios were not statistically significant and because these two cancers are the most commonly occurring in Chile in this age group.

The single uterine leiomyosarcoma was of particular interest in this analysis; however, there are few data available to evaluate incidence rates. Based on the Cali data, one would expect 0.2 or 0.3 cases of cancer of the body of the uterus, depending on whether or not "unspecified uterine" cancers are included. Based on data from nine U.S. cancer registries, the number of expected uterine leiomyosarcomas would be approximately 0.08 or 0.15, using rates for white or black women, respectively (15). These are not significantly different from the one case observed. We observed no cases of ovarian cancer in this cohort, while 0.49 cases were expected.

Cluster Analysis

The statistical assessment for evidence of a non-random cluster of cancer occurrence in this cohort is shown in Table 7. The "scan" statistic of the 15-year period (with a 3-year window) found 9 of 17 cases in a 3-year period (1985 to 1987) with 3.4 expected ($P < 0.061$). The 1986 occurrence of cases was highly significant ($P < 0.001$) (9). Finally, the direct evaluation of the case distribution by city further accentu-

Table 5 Cancers Diagnosed in the Quinacrine Cohort, 1977 to 1991, by Anatomic Site and Treatment Center

| Site | Santiago | Valdivia | Total |
|-----------------|----------|----------|-------|
| Cervix* | 3 | 2 | 5 |
| Breast | 4 | 1 | 5 |
| Gallbladder | 0 | 2 | 2 |
| Bile duct | 1 | 0 | 1 |
| Colon | 0 | 1 | 1 |
| Thyroid | 1 | 0 | 1 |
| Uterine sarcoma | 0 | 1 | 1 |
| Brain | 0 | 1 | 1 |
| Total | 9 | 8 | 17 |

* Twelve cervical carcinomas in situ were excluded from this evaluation.

Table 6 Observed and Expected Cancer Diagnoses for All, Breast, and Cervical Cancers, Quinacrine Cohort 1977 to 1991

| Site | Observed | Expected* | Ratio | 95% Confidence limits |
|--------|----------|-----------|-------|-----------------------|
| All | 17 | 11.82 | 1.44 | 0.84 to 2.30 |
| Breast | 5 | 3.04 | 1.64 | 0.53 to 3.84 |
| Cervix | 5 | 3.96 | 1.26 | 0.41 to 2.95 |

* Source: Expected numbers were calculated from age- and gender-specific incidence rates from the Cali, Colombia cancer registry.

ated the temporal pattern (it was already marginally significant). These results clearly signal the presence of an unusual occurrence of cancer in 1985 to 1987 in Valdivia, and this is the same observation that led to this study. These results confirm the impression that an unusual cluster of cancers did occur and justify the further evaluation represented by these analyses.

Case Studies

Based on the inspection of cases, there were five cancer diagnoses that were deemed to represent "questionable" occurrences. These cases were ones that [1] were not among the most common sites among Chilean women of these ages (i.e., not breast or cervical cancer) or [2] occurred in years where there were more than the expected number of cancer cases. These two criteria identified two gallbladder cancer cases, and one cancer each of the colon, brain, and soft tissue of the uterus. Generally speaking, these five "questionable" cases represented women both of reasonable ages and sufficient follow-up for valid biologic potential. Some details from the evaluation of these cases follow.

All of these cases were sterilized in Valdivia in 1979 and each received three insertions of quinacrine.

The gallbladder cases are probably part of a wider trend reported in Chile as a whole (Dabancens A, personal communication). Although the female population of Valdivia increased over the decade of the 1980s by 20%, the frequency of gallbladder cancer increased in Chile by 47.5% over the same period.

As isolated occurrences, the colon and brain cancer cases are unlikely to be significant. The uterine sarcoma, however, is curious and worthy of note because of the anatomic location in proximity to the quinacrine insertion. It should be noted that this case was one of the original cases in the cluster that led to this study. The case description follows:

This woman was under 30 years of age at the time of her initial sterilization. Her smoking history and her prior use of birth control pills are unknown. She was mildly obese (height 160 cm and weight 75 kg),

but no information was provided on her dietary patterns. She had menarche at age 15 years. She reported a single sexual partner and her first live birth was at age 19 years. She had three children and two failed pregnancies; she had no pregnancies since sterilization. She had no abnormal Pap smears since sterilization and her family history of cancer was unknown. She had no history of fibrocystic disease. Seven years after sterilization she underwent a total abdominal hysterectomy with a diagnosis of well-differentiated leiomyosarcoma. The pathology report indicated multiple pelvic and peritoneal metastatic nodules and hepatic metastases. She received combination chemotherapy and died 1 year later.

Review of Slides

We were able to obtain the histopathology slides from five of the six gynecologic cancers, including four of the five cervical cancers and the leiomyosarcoma. The slides were reviewed by an independent pathologist. The sarcoma was found to be consistent with leiomyosarcoma, and the four cervical cancers were confirmed.

DISCUSSION

This type of "cluster" analysis is unconventional in form and may be subject to criticism (16, 17). The statistical methods are susceptible to all the conventional biases inherent in conventional epidemiologic studies. The potential for misclassification is great because of substantial under-representation in the interview data of the women sterilized in Santiago. On the other hand, there may be some basis for sus-

Table 7 Cancer Cases by Year, Geographic Location, and Anatomic Site, Quinacrine Cohort 1977 to 1991

| Year | Cancers by anatomic site* | P value as more than expected | O:E ratio† |
|------|---|-------------------------------|------------|
| 1977 | | | |
| 1978 | | | |
| 1979 | Breast | 0.202 | 4.42 |
| 1980 | | | |
| 1981 | Cervix | 0.202 | 4.42 |
| 1982 | | | |
| 1983 | Thyroid | 0.501 | 1.44 |
| 1984 | <u>Gallbladder</u> | 0.501 | 1.44 |
| 1985 | Breast | 0.501 | 1.44 |
| 1986 | Cervix, bile duct, <u>colon, brain, breast, uterine sarcoma</u> | 0.001 | 8.62 |
| 1987 | Cervix, <u>cervix</u> | 0.422 | 1.39 |
| 1988 | | | |
| 1989 | | | |
| 1990 | Breast, <u>gallbladder</u> | 0.422 | 1.39 |
| 1991 | Breast, <u>cervix</u> | 0.422 | 1.39 |

* The cases from Valdivia are underlined.

† O:E ratio, observed cases:expected cases ratio.

pecting a level of detection bias associated with the 91% follow-up in Valdivia.

Although the rate of follow-up interviews was far from complete in Santiago, this potential misclassification may not invalidate the study. Clinical experience in Santiago (J.Z.) suggests that the women in this cohort would have come to the study clinic for any gynecologic problems, including cancer. The issue of a selection bias operating between the two study locations evidenced by the difference in follow-up is explained by local characteristics of the populations. Santiago, the capital of Chile, is a large city and the clients of the study clinic in Santiago include many poor women from urban slums. Women in these areas move about more frequently than women in Valdivia and their homes do not have street addresses, which seriously impairs active follow-up efforts, but would not prevent the women from seeking medical attention at the Santiago clinic. By comparison, Valdivia is a much smaller city (population approximately 150,000), with a stable population and much less migration.

Although follow-up studies of occupational cohorts are often able to achieve high rates of long-term follow-up, such follow-up generally is more difficult for studies of sterilization. We are aware of only two similar studies of long-term follow-up of women after sterilization: one in the United Kingdom and one in the United States. They have had widely differing rates of follow-up. The U.K. study by Vessey et al. (18) was limited to married "white British subjects" with a relatively high proportion of middle and upper class participants recruited from two family planning clinics. They reported a very low loss-to-follow-up for "relevant reasons" of 0.3% per year, or approximately 3% after 10 years. The U.S. study, done by the Centers for Disease Control and Prevention (CDC) is known as the CREST (Collaborative Review of Sterilization) study. It includes the population of all women undergoing sterilization at a sample of university and community hospitals, 63.5% of whom were white and 36.5% were black and other ethnic groups (19). The CDC's preliminary report of the 10-year results of the U.S. study showed a follow-up rate of only 58%, similar to the follow-up rate of the Santiago women (Wilcox LS, Jamison P, Peterson HB, Hughes J, abstract).

In the best form for cluster studies, the findings from the originating observation were followed by an evaluation of a larger, similarly exposed group. In this case, the original observations were complemented by a more active follow-up of the women from the original studies and of all other women who had been sterilized with quinacrine. The cancer experience for over four times as many quinacrine treated women was examined and nine additional

cancers were found, but the final results did not corroborate the initial observations: when the total number of observed cancers was compared with the expected, there was no significant excess. The excess that was observed can be attributed to the cluster of cancers in Valdivia, the observation that prompted the study. However, the study population size is not sufficient to reject the possibility of a small or moderate increase in cancer risk.

Regarding the distribution of the sites of cancers observed in the cohort, the absence of endometrial cancers may be intriguing for North American or European readers. In fact, only 0.2 to 0.3 cases of all types of uterine corpus cancer were expected. It is important to note that the pattern of gynecologic cancers in Latin America differs substantially from the pattern in North America or Europe (7). For example, among white women in San Francisco, the crude ratio of cancers of the cervix to cancers of the uterine corpus was approximately 1:3. On the other hand, among women in Cali, Colombia the ratio was approximately 9:1. Data from other South American countries and Chile show a pattern similar to the Cali, Colombia data (7) (Dabancens A, personal communication).

An ideal study design would have involved comparison with local cancer registry data, but there is no suitable registry in Chile. The Cali registry was chosen because it appeared to have the most comparable data, but it may under-register cases by 10% to 15%. From the point of view of biologic plausibility, the gynecologic cancers are of most concern. Regarding the suitability of the Cali data as a basis for comparison, both Colombia and Chile have high rates of cervical cancer. Based on International Agency for Research on Cancer (IARC) data analyzed by Cuzick (20), Chile had the highest reported mortality rate from cervical cancer among a diverse group of 39 countries, which included 6 other Latin American countries, but not Colombia. Although we observed a small absolute excess of cervical cancers, the distribution of cancers by site was virtually identical. Cervical cancers represented 29.4% of the observed cancers in Chile and 33.5% of expected cancers from the Cali registry. Uterine sarcomas are uncommon cancers and no convincing hypotheses have been proposed for their causation (21).

We discussed the choice of cancer registries with Dr. Sharon Whelan at IARC, and she advised that the choice of Cali was reasonable, and that other possible comparison registries were those of Asuncion, Paraguay, or Quito, Ecuador. The estimates from those two registries bracket our estimates based on the Cali registry, with total numbers of expected cancers of 13.85 and 11.43, respectively, compared with 11.86. For breast cancers the esti-

mates are 3.19 and 2.03, respectively, compared with 3.04, and for cervical cancers the estimates are 6.16 and 3.56, respectively, compared with 3.96. Dr. Whelan cautioned that any comparison across countries is by its very nature very crude, and one should use great caution in drawing any conclusions from such comparisons.

In addition, rates of cervical cancer may vary considerably by socioeconomic groups within a given city. Because many of the women who received quinacrine in Chile are from an area of relatively low socioeconomic status in Santiago, there may be bias toward a higher rate of cervical cancer in our cohort compared with the rate from a cancer registry that would include a more representative mixture of socioeconomic strata.

In any case, the inferences that can be drawn from this study are limited. It was performed in response to the observation of a cluster of cancer cases. After an analysis of all the cancer cases that we could identify, we conclude that the observed cluster was probably a random event. However, the study is not sufficiently large or powerful enough to permit one to conclude that transcervically administered quinacrine is not carcinogenic.

Quinacrine has been an approved drug for many years and its oral use has been advocated in the United States as a "steroid-sparing" anti-inflammatory agent in the treatment of rheumatic diseases (6). The question of whether transcervical quinacrine might be carcinogenic cannot be answered by this study, but must rely on other data. It should be noted that women receiving quinacrine for sterilization are exposed to total doses of only approximately 500 mg (two insertions) or 750 mg (three insertions), whereas quinacrine was used in much higher dosages for the prophylaxis and treatment of malaria. During World War II, soldiers took oral doses of 100 mg/d (and sometimes 200 mg/d), i.e., ≥ 36 g/yr (5). Because quinacrine is absorbed rapidly and distributed throughout the body; it would seem that a systemic carcinogenic effect would have been more likely to appear among World War II veterans taking prophylactic quinacrine than among women receiving quinacrine for sterilization. Although studies of exposed servicemen at the time did reveal the rare occurrence of a dose-related marrow aplasia (22), we have been unable to find any long-term follow-up studies that specifically addressed the issue of carcinogenicity.

A panel of reviewers commissioned by FHI to evaluate quinacrine's toxicology concluded that quinacrine was unlikely to be a human carcinogen (Tice RR, Griffith J, Recio L, unpublished observations). However, because quinacrine was marketed before the use of modern screening methods, the reviewers

recommended that quinacrine be evaluated with modern mutagenicity tests and that any other available human exposures be reviewed.

Since the completion of this analysis, FHI is considering a renewal of its efforts to obtain FDA approval for the transcervical administration of quinacrine. That process would include a careful assessment of the mutagenicity and potential carcinogenicity of quinacrine using modern toxicologic techniques.

The biologic plausibility of transcervical quinacrine being implicated as a factor in human carcinogenesis is difficult to assess. On the one hand, the low dose and brief exposure would suggest there is little likelihood of any significant risk. On the other hand, the unique route of administration, high local exposure and associated cytotoxicity are reasons for possible concern. In the mouse it is possible to produce cervical cancers with the vaginal instillation of chemical carcinogens (23). Bladder leiomyosarcomas have occurred in humans after exposure to cyclophosphamide, a drug that forms covalent bonds with DNA (24). Concerning the relevance of these observations to quinacrine, Nasim and Brychcy (4) noted that "Up until now, only those chemicals that interact covalently with DNA have been shown to be both mutagens and carcinogens. As acridines [including quinacrine] belong to a class of compounds which intercalate DNA and do not bind covalently, it is therefore of major importance that further investigations be carried out."

Although past testing has shown that quinacrine is mutagenic in some systems, it has not been evaluated according to current FDA standards. A study of the chronic toxicity of quinacrine in rats was done in the 1940s by the FDA itself, and the authors concluded that quinacrine was not a carcinogen (25), but that study was not conducted according to current standards and the drug was administered orally rather than transcervically.

We have extended our study of this cohort and plan to gather at least another 5 years of follow-up data. In addition, FHI, in collaboration with Vietnamese authorities, is planning to initiate a long-term follow-up study of a group of women in Vietnam who were sterilized with quinacrine (2).

The occurrence of the single case of uterine sarcoma may make it difficult to license quinacrine for female sterilization in the United States because of the possibility of a cancer risk compared with the low-risk surgical alternatives already available. If it were approved by the U.S. FDA, quinacrine sterilization would probably not become widely used in the United States because of its relatively low effectiveness compared with surgery. However, quinacrine sterilization could be useful for women with a contra-

indication or fear of surgery or in developing countries where the availability and safety of surgical sterilization are often less than in developed countries.

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