

Prenatal Exposure to Metronidazole and Risk of Childhood Cancer

A Retrospective Cohort Study of Children Younger than 5 Years

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BACKGROUND. To evaluate the role of in utero exposure to metronidazole (a carcinogen in some animal models) and the risk of subsequent cancer, the authors conducted a retrospective cohort study of childhood cancer.

METHODS. The cohort included 328,846 children younger than 5 years born to women enrolled in Tennessee Medicaid at any time between the last menstrual period (LMP) and the date of delivery. The cohort was identified by linking files of Tennessee Medicaid mothers ages 15–44 years and children and the children's birth and death certificates for the period January 1, 1975 through December 31, 1992. Exposure data were obtained from Medicaid pharmacy records and exposure was defined as filling a metronidazole prescription that had at least a day's supply between the 30 days prior to the LMP and the date of delivery. Study cases were cohort children diagnosed with a first primary cancer before age 5 years, identified by linking the cohort with a statewide childhood cancer database for the study period.

RESULTS. Cohort members contributed 1,172,696 person-years of follow-up for analysis, with children exposed (8.1%) and not exposed (91.9%) in utero to metronidazole contributing 79,716 and 1,092,980 person-years, respectively. Of 952 children younger than 5 years in the statewide cancer database, 175 met study eligibility criteria. Of these, 42 had leukemia, 30 had central nervous system (CNS) tumors, 28 had neuroblastoma, and 75 had other cancers. Using Poisson regression modeling, children exposed to metronidazole in utero had no significant increase in adjusted relative risk (RR) for all cancers (RR: 0.81; 95% confidence interval [95% CI], 0.41–1.59), leukemia (no exposed case), CNS tumors (RR: 1.23; 95% CI, 0.29–5.21), neuroblastomas (RR: 2.60; 95% CI, 0.89–7.59), and other cancers (RR: 0.57; 95% CI, 0.18–1.82).

CONCLUSIONS. The authors conclude that although there was no increase in risk for all cancers associated with in utero exposure to metronidazole, the observed increased risk for neuroblastomas, although not significant, requires further evaluation. *Cancer* 1998;83:1461–8. © 1998 American Cancer Society.

KEYWORDS: childhood cancer, metronidazole, fetal exposure, neuroblastoma.

Metronidazole, a synthetic nitroimidazole introduced in the early 1960s, has a broad spectrum of activity against parasitic and bacterial infections. Currently, its primary clinical indications are the protozoal infections in trichomoniasis, amebiasis, and giardiasis and infections due to susceptible anaerobic bacteria. It has come to be known as a relatively safe drug in therapeutic doses with few serious acute toxicities.¹ The antimicrobial activity of metronidazole is believed to depend on its nitroreduction to form short-lived cytotoxic metabolites that produce structural DNA damage leading to impairment of cell function and ultimate cell death. These cytotoxic properties have led to concern that metronidazole may increase the risk of

cancer and to evaluation of its carcinogenic and mutagenic properties in both animal models and humans.²

Studies of Swiss mice³⁻⁵ and rats^{6,7} administered different regimens of metronidazole have reported subsequent development of tumors but studies of hamsters have not.² Although these data do not establish that metronidazole is a potent carcinogen, they do suggest that in some animal models it may be carcinogenic. In humans, three cohort studies⁸⁻¹⁰ of adults who were treated with metronidazole did not find an increased risk of subsequent cancer. One study⁸ did report an increased risk of bronchogenic carcinoma and another an excess risk of cervical carcinoma.⁹ However, inadequate control for smoking in the former⁸ and confounding by sexual activity and detection bias in the latter⁹ make the findings of these studies inconclusive. Three cases of cancer in young persons with Crohn's disease who were treated with prolonged, heavy doses of metronidazole were reported; however, no analysis of risk relative to unexposed controls was performed.¹¹

Although the extant data do not suggest that metronidazole is a potent carcinogen in adults, there is an additional basis for concern that metronidazole is a fetal carcinogen. Metronidazole readily crosses the placental barrier and enters the fetal circulation. An animal study suggests that metronidazole may have some carcinogenicity. Chacko and Bhide⁵ noted that the male progeny of Swiss mice given relatively low doses of metronidazole during gestation had a significant increase in tumor incidence. Eighty percent of the tumors were lung tumors. The Food and Drug Administration has received 2 case reports of infants diagnosed with neonatal leukemia at 2 and 5 weeks of age, respectively, whose mothers received metronidazole during pregnancy (unpublished data). A case of adrenal neuroblastoma in a 2-week-old infant whose mother had been treated with oral metronidazole during pregnancy recently was reported.¹² However, we are not aware of any published controlled studies of this question in humans. Given that metronidazole now is among the ten most common drugs¹³ used during pregnancy and that its use during pregnancy has been increasing over the past decade,¹⁴ there is an urgent need to address this question.

We conducted a retrospective cohort study of cancer in children younger than 5 years in a cohort of 328,846 children, born to women enrolled in Tennessee Medicaid during their pregnancy, who were exposed and unexposed to metronidazole in utero.

MATERIALS AND METHODS

Study Design

Identification of Cohort

As described previously,¹⁵ we established a linkage between Tennessee Medicaid mothers ages 15-44 years and children and the children's birth and death certificates for the period beginning January 1, 1975 through December 31, 1992. This linkage utilized information in the Medicaid enrollment file for the woman and child, Medicaid inpatient and outpatient records of medical care provided during labor and delivery, and birth and death certificates. The linkage utilization used conservative match criteria to keep the false-positive rate < 1%.¹⁵ Of the women enrolled in Medicaid who either had a Medicaid delivery or were linked with a child enrolled in Medicaid at birth, 94.2% were linked to a child's birth or fetal death certificate. The final cohort comprised 328,846 children who were linked with women who were enrolled in Tennessee Medicaid at any time between the last menstrual period (LMP) and the child's date of birth.

The information available in this file includes the mother and child's Medicaid enrollment records, maternal delivery claims, child's birth/fetal death certificate, and, when applicable, the child's death certificate (obtained from a separate link between the birth and death certificates). The birth certificate data include birth weight, gender, race, estimated date of LMP, maternal gravida and parity, age at birth, education, marital status, and county of residence at birth.

Tennessee Childhood Cancer Database

We developed a childhood cancer database for Tennessee for the period of 1975-1992 using multiple sources for case ascertainment. The primary source included the four major tertiary care medical centers in Tennessee (Vanderbilt University Medical Center in Nashville, St. Jude Children's Research Hospital in Memphis, East Tennessee Children's Hospital in Knoxville, and Erlanger Medical Center in Chattanooga) that managed the majority of childhood cancer cases.

We reviewed records of the medical centers from 1975-1992 to identify children 15 years or younger diagnosed with cancer. Records reviewed included tumor registry logs, hospital discharge logs, hospital pathology logs, hematology-oncology clinic records, and/or computerized printouts/tape of children with a discharge diagnosis of cancer. The latter cases were identified using the 8th (1975-1988) and 9th revisions of the *International Classification of Diseases* and the Standard Nomenclature of Disease and Operation discharge codes for cancer. The availability of these records and the time period for which they were avail-

able varied by hospital. A standard data collection form was used to collect pertinent data including the name of child, date of birth, race, gender, address, patients' names if available, cancer diagnosis, date of cancer diagnosis, method by which cancer was confirmed, and institution the patient was seen at.

We supplemented the database with cases identified from two secondary sources: the Tennessee Cancer Reporting System (TCRS) and additional hospitals treating childhood cancer cases. The TCRS was established in 1987 and is maintained by the Tennessee State Department of Health. Institutions in Tennessee are mandated by state law to report cases of cancer in a standard format to the TCRS in a timely manner. Cases not already identified from the four tertiary centers were included in the childhood cancer database. We also reviewed the records of nine additional hospitals to ascertain cases not seen by the major tertiary centers. These hospitals were identified from Medicaid hospitalization of cohort children with a cancer diagnosis, cancer deaths from death certificates, and the TCRS.

Study Cases

Potential cases were identified by linking the Tennessee childhood cancer database with the study cohort, using our standard computer software.¹⁵ Study cases had to have been diagnosed with a first primary cancer before age 5 years, a Tennessee resident and seen at a Tennessee hospital at the time of diagnosis. Cases were limited to the < 5 years age group to minimize the potential bias that may be introduced by loss of cases due to out-of-state migration. No cases had a history of therapeutic radiation or chemotherapy prior to diagnosis of cancer ($n = 0$).

For each potential case, a trained nurse-abstractor, unaware of drug exposure status, reviewed medical records to confirm diagnosis and eligibility and completed a structured study abstract form. Data collected included date of diagnosis, type, cancer stage and histologic grade at diagnosis, and method of diagnosis; any congenital or hereditary diseases; family history of cancer (particularly for siblings); and history of exposure to radiation, cytotoxic or immunosuppressive drugs, or other toxic substances. In addition, copies of histopathologic and/or radiologic reports or surgical reports also were reviewed by one of the investigators (P.B.T.) to verify the diagnosis of cancer and classification type. Borderline cases were reviewed by a pediatric oncologist (J.W.). Six percent of potential cases identified from the database were excluded after records review.

Medication Exposure

Medicaid pharmacy files were used to identify prescriptions of metronidazole filled by mothers of the cohort children. The daily dose, number of days' supply, delivery method, and date for each metronidazole prescription filled were available in these files. Exposure was characterized as metronidazole use at any time during the pregnancy, defined as any filled prescription for metronidazole for at least 1 day's supply between 30 days before the LMP and the date of delivery. The 30 days prior to the LMP was included so that the exposure status of subjects who were filled a metronidazole prescription prior to the LMP and were still taking it up to and beyond the LMP could be classified correctly. Exposure was characterized further by trimester of use, defined as a filled metronidazole prescription 1) between 30 days before and 90 days after the LMP (first trimester); 2) between 91 and 180 days after the LMP (second trimester); and 3) between 181 days after the LMP and the date of delivery.

Statistical Analysis

Cohort members contributed person-time from date of birth to the date of cancer diagnosis, death regardless of cause, fifth birthday, or end of the study (December 31, 1992), whichever came first. Because outcomes were ascertained from sources independent of Medicaid, follow-up was not terminated if Medicaid enrollment ceased.

Factors that potentially could confound the relation between cancer and metronidazole exposure were obtained primarily from birth certificate data and dichotomized into maternal age ≤ 24 years, rural county of residence, male gender, white race, not married, maternal education < 12 years, and being first born. Age at diagnosis was defined as birth–1 year, 2–3 years, and > 3 years and calendar year of birth as 1975–1979, 1980–1984, 1985–1989, and 1990–1992.

Univariate and multivariate analyses were conducted with Poisson regression models using PROC GENMOD of PC-SAS to estimate relative risks and 95% confidence intervals (95% CI).¹⁶ Variables with a P value of 0.10 in the univariate analysis were included in the initial model for the multivariate analysis. Inclusion of variables in the final model was determined by backward elimination. In addition to metronidazole exposure status, the final model included terms for maternal age ≤ 24 years, rural county of residence, white race, not married, maternal education < 12 years, and being first born. All P values were two-sided and a P value of 0.05 was the cutoff for determining statistical significance.

TABLE 1
Comparison of Cancer Rates between the Tennessee Childhood Cancer Database (per 100,000 Person-Years) and the Surveillance, Epidemiology, and End Results Program Registry (per 100,000 population) in Children Younger Than 5 Years

Cancer type	SEER ^a (1983–1987)	TNCCD (1975–1992)
All cancers	18.8	18.3
Leukemias	6.6	5.5
CNS tumors ^b	3.5	3.2
Neuroblastomas	2.8 ^c	2.2
Retinoblastomas ^d	1.3	0.9
Wilms' tumor ^e	1.9	2.0
Soft tissue sarcomas	1.4	1.0
Germ cell tumors	0.8 ^f	0.6
Hepatomas	0.5	0.4
Lymphomas	0.5	0.5
Other cancers	—	2.0

SEER: Surveillance, Epidemiology, and End Results program; TNCCD: Tennessee Childhood Cancer Database; CNS: central nervous system.

^a Source: Ries LAG, Hankey BF, Edwards BK. SEER cancer statistics review 1973–1987. Bethesda (MD): National Cancer Institute; 1990 NIH Pub. No. 90-2789.

^b Surveillance, Epidemiology, and End Results program registry rate grouped as brain and central nervous system (includes neuroblastomas).

^c Source: Bernstein ML, Leclerc JM, Bunin G, Brisson L, Robison L, Shuster J, et al. A population-based study of neuroblastoma incidence, survival, and mortality in North America. *J Clin Oncol* 1992; 10:323–9.

^d Surveillance, Epidemiology, and End Results program registry rate grouped as eye and orbit.

^e Surveillance, Epidemiology, and End Results program registry rate grouped as kidney and renal pelvis.

^f 0.8 for white males, 0.3 for white females, 0.7 for black males, and 0.4 for black females. Source: Austin DF, Flannery J, Greenberg R, Isaacson P, Key C, Kolonel LN, et al. The SEER program, 1973–1982. In: International incidence of childhood cancer. IARC Scientific Pub. No. 87 Lyon, France: International Agency for Research on Cancer, 1988.

RESULTS

The Tennessee childhood cancer database (TNCCD) identified 952 children younger than 5 years at the time of initial cancer diagnosis who also linked with Tennessee birth certificates. The rate of all cancers was 18.3 per 10⁵ person-years, which is comparable to the Surveillance, Epidemiology, and End Results program (SEER) rate of 18.8 per 10⁵ person-years (Table 1).¹⁷ Rates by cancer type in the TNCCD were comparable to those published by the SEER program (Table 1).¹⁷

The 328,846 children in the study cohort contributed 1,172,696 person-years of follow-up for analysis, with children exposed (8.1%) and not exposed (91.9%) to metronidazole contributing 79,716 and 1,092,980 person-years, respectively. Table 2 compares the characteristics of the cohort by metronidazole exposure status. Child and maternal characteristics in cohort members exposed and unexposed to prenatal metronidazole were similar, although exposed children tended to have mothers who were younger, unmarried, less likely to have been enrolled in Medicaid

TABLE 2
Characteristics of Study Cohort by Metronidazole Exposure Status (Tennessee Medicaid Children 1975–1992)

Factors	Metronidazole	
	Exposed ^a (N = 79,716 person-years)	Not exposed (N = 1,092,980 person-years)
Child factors		
Male	49.3	50.2
White	54.2	52.2
Birthweight < 2500 g	29.9	32.8
First born	39.0	42.8
Age at diagnosis (yrs) ^b		
< 2	38.7	34.7
2–3	33.3	33.7
> 3	28.0	31.6
Maternal factors		
Age ≤ 24 yrs	54.6	44.2
< 12 yrs of education	52.3	50.5
Not married	57.5	51.4
Rural county of residence	41.2	48.2
Trimester of Enrollment in Medicaid		
1st	58.0	84.0
2nd	22.1	13.9
3rd	19.9	2.1

^a Any exposure to metronidazole during pregnancy.

^b Limited to children diagnosed with cancer.

during or before the first trimester of pregnancy, and with a rural county of residence.

The current study included 175 TNCCD cases who also were members of the study cohort and met study eligibility criteria. Cancer diagnosis in these cases was based on histology in 89.1%, direct visualization in 5.7%, radiologic imaging in 1.7%, and unspecified methods in 3.4%. The 175 cases corresponded to a childhood cancer rate of 14.9 cancers per 100,000 person-years in the cohort. Of these cancers, 42 were leukemia, 30 were central nervous system (CNS) tumors, 28 were neuroblastomas, and 75 were other cancers (retinoblastoma [n = 14], Wilms' tumor [n = 22], soft tissue sarcomas [n = 10], germ cell tumors [n = 8], hepatoma [n = 3], lymphoma [n = 1], and other miscellaneous cancers [n = 17]).

Children exposed in utero to metronidazole had no significant increase in relative risk (RR) for all cancers (RR 0.81; 95% CI, 0.41–1.59), leukemia (no exposed cases), CNS tumors (RR 1.23; 95% CI, 0.29–5.21), and other cancers (RR 0.57; 95% CI, 0.18–1.82) (Table 3). However, although based on 4 exposed cases, these children had a 2.5-fold increase in the risk of neuroblastoma (RR 2.60; 95% CI, 0.89–7.59) but it did not achieve statistical significance.

Because women were enrolled in Medicaid at dif-

TABLE 3
Relative Risk of Cancer and Prenatal Use of Metronidazole, by Cancer Type in Tennessee Medicaid Children, 1975–1992

Cancer	Metronidazole exposed (n = 79,716 person-years)		Metronidazole not exposed (n = 1,092,980 person-years)		Adjusted relative risk ^a	95% CI
	Cases	Rate per 10 ⁵	Cases	Rate per 10 ⁵		
All cancers	9	11.3	166	15.2	0.81	0.41–1.59
Leukemias	0	0.0	42	3.8	—	—
CNS tumors	2	2.5	28	2.6	1.23	0.29–5.21
Neuroblastoma	4	5.0	24	2.2	2.60	0.89–7.59
Other cancers ^b	3	3.8	72	6.6	0.57	0.18–1.82

95% CI: 95% confidence interval; CNS: central nervous system.

^a Adjusted for maternal age ≤ 24 years, rural county of residence, white race, unwed status, maternal education < 12 years, and being first born.

^b Includes retinoblastoma (n = 14), Wilm's tumor (n = 22), soft tissue sarcomas (n = 10), germ cell tumors (n = 8), hepatoma (n = 3), lymphoma (n = 1), and other miscellaneous cancers (n = 17).

ferent stages of their pregnancy, use of metronidazole during the phase of pregnancy prior to enrollment would not be documented. This could have led to some subjects being classified as unexposed when in fact they may have been exposed. To minimize the potential for exposure misclassification, we redefined the unexposed group in two ways. First, we restricted the unexposed group to children of women who were enrolled in Medicaid 30 days prior to the LMP (n = 429,290 person-years) and remained enrolled throughout the pregnancy. There would be no misclassification in this group. Second, we restricted the unexposed group to those who were enrolled in Medicaid on or at any time after the LMP (n = 663,596 person-years). We repeated the analysis comparing these 2 unexposed groups, separately, with the exposed group (n = 79,716 person-years). The results were similar.

Because the growing fetus may be more sensitive to the effects of a potential carcinogenic insult during the early stages of pregnancy, we attempted to repeat the analysis by trimester of metronidazole exposure by restricting the cohort to those who were enrolled in Medicaid during the entire pregnancy (n = 450,937 person-years). None of the 57 cases was exposed in the first and second trimesters and only 2 were exposed in the third trimester. No further analysis was performed.

The number of cases exposed to metronidazole was too small to permit any dose or duration of use related analysis. We repeated the analysis for all cancers stratified by levels of child and maternal characteristics and found no evidence of effect modification.

The prevalence of metronidazole use in this cohort of Medicaid women rose from 29 users per 1000 pregnant women in 1975 to 123 users per 1000 pregnant women by 1992 (Fig. 1).

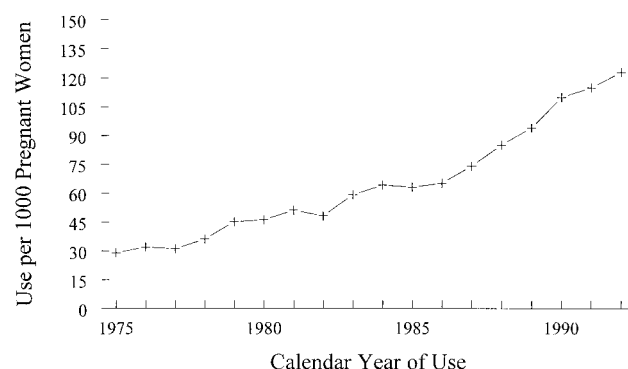


FIGURE 1. Prevalence of metronidazole use in pregnant women enrolled in Tennessee Medicaid between 1975–1992.

DISCUSSION

Metronidazole is the only effective treatment of vaginal infections due to *Trichomonas vaginalis*. Although metronidazole use is not recommended in pregnancy, especially during the first trimester,¹⁸ its use among pregnant Medicaid women has been increasing over the past two decades, with 8.1% of the current cohort having received prescriptions for the drug. Recently, it has been recommended, in conjunction with a broad-spectrum antibiotic and an antisecretory agent, for the treatment of duodenal ulcers associated with *Helicobacter pylori*.¹⁹ This possibly may result in an increase in the prevalence of metronidazole use in pregnant women. Given its cytotoxic properties, its ability to easily cross the placental barrier, and its role as a perinatal carcinogen in an animal model, a potential increase in risk of cancer in the offspring of women exposed to metronidazole during pregnancy is of public health concern. It thus is reassuring that we did not find an increased risk of childhood cancer associated with in utero exposure to metronidazole in this retrospective study of a cohort of 328,846 children with approximately 1.2 million person-years of follow-up. However, this finding must be interpreted in light of

the fact that despite the large cohort, our study had adequate power (80%) to detect a 2-fold increase in risk of all childhood cancer among those exposed to metronidazole.

In the current study, none of the leukemia patients was exposed to metronidazole. We found two patients with CNS tumors who were exposed to metronidazole with a modest but statistically insignificant increase in risk. However, although statistically not significant, we did find a nearly three-fold increase in the risk of neuroblastomas among children exposed to metronidazole in utero. Four of the nine cancer patients exposed to metronidazole had neuroblastomas. Again the major limitation of these results is small sample size. Our study has adequate power to detect an RR of 3.1, 3.7, and 4.0 for leukemia, CNS tumors, and neuroblastomas, respectively.

Maternal use of diethylstilbestrol during pregnancy and the subsequent development of vaginal adenocarcinoma in the female offspring is the only well established transplacental carcinogen.²⁰ Since then, there have been several studies of maternal use of medication in pregnancy and subsequent risk of cancer in the offspring.²¹⁻³⁴ Although several medications and medication categories have been suggested to be transplacental carcinogens, the results from these studies have not been consistent. Because childhood cancers comprise a histologically heterogeneous group of cancers with differing demographics and etiology, the majority of these studies have been limited to individual groups of cancer. Of four studies²¹⁻²⁴ that evaluated the risk for childhood cancers overall with maternal medication use during pregnancy, one²¹ found an association between analgesics and antipyretics use and risk of cancer whereas another²² found an association with use of pethidine during labor. Although a variety of medications were evaluated, none of these studies specifically mentioned the role of metronidazole.

To our knowledge there are few studies of maternal use of medication and childhood leukemias.³⁵ A case-control study specifically evaluating this issue reported maternal use of antiemetic medication for > 11 weeks and marijuana use to be associated significantly with the development of leukemia.²⁵ A second case-control study evaluating prenatal and postnatal risk factors for childhood leukemia did not find any association with prenatal maternal use of medication, but did find a significant association with postnatal use of chloramphenicol by the children.³⁶

Several case-control studies of brain tumors have examined maternal use of medication as a risk factor.²⁶⁻³² Drugs used during pregnancy that were found to be associated with brain tumors included barbiturates,²⁶ diuretics, and antihistamines.²⁷ The latter two

drugs contain nitrosaturable amines, which are precursors of *N*-nitroso compounds, which have been found to cause brain tumors in experimental studies. However, several studies examining the use of nitrosatable drugs during pregnancy did not find any association with brain tumors.^{29,31,32} Only one of these studies is relevant to the role of metronidazole.³² Bunin et al.³² examined the effects of medication taken for vaginal infection, although not specifically those of metronidazole, and found a protective effect (odds ratio 0.4; 95% CI, 0.2-0.9) in a study of astrocytoma when adjusted for income level. However, several kinds of medication, including metronidazole, could be used for the treatment of vaginal infection.

Neuroblastomas are embryonal tumors of the sympathetic nervous system, and usually manifest before age 2 years.³⁷ Thus, the prenatal environment has been postulated to play an important role in their development. Two case-control studies^{33,34} specifically evaluated the association between prenatal medication exposure and the subsequent development of neuroblastomas. Both studies found prenatal exposure to diuretics and neurally active drugs (tranquilizers,³⁴; tranquilizers, amphetamines, barbiturates, narcotic analgesics, and muscle relaxants³³) to be associated significantly with neuroblastomas. Medications found to be associated with the development of neuroblastomas in at least one of the studies included sex hormones, nonprescription analgesics, and antiemetic medications.^{33,34} Although a number of other medications were evaluated, metronidazole was not. It is not clear whether the mothers of the study subjects did not report taking metronidazole or whether they were not specifically asked about it. There have been several independent case reports of neuroblastomas in the children of women who used diphenylhydantoin during pregnancy.³⁸

Although, to the best of our knowledge, no controlled studies have reported this association, a recent case report¹² documented an infant with neuroblastoma whose mother was treated with metronidazole during pregnancy. Nevertheless, given the potential importance of prenatal environmental factors in the development of neuroblastomas, its increasing incidence (especially in developed countries)³⁹⁻⁴¹ and that it is the most common tumor in children age < 1 year,⁴² this issue needs to be studied further.

Because of the low incidence rate of childhood cancer, nearly all the analytic epidemiologic studies have been retrospective, interview-based, case-control studies.^{21,25-34,43-45} However, use of this design to study fetal drug exposures has major potential problems of selection bias, recall bias, and information bias. Investigators have recognized these potential bi-

ases and attempted to minimize them. Nevertheless, in these studies, 30–50% of childhood cancer cases and potential controls were not included,^{26,27,33,34,43} primarily because they were not suitable candidates for interview or refused participation. Mothers were asked about their use of medications that may have occurred several years prior to the interview, which may be subject to considerable recall error.⁴⁶ If recall accuracy were differential (mothers of children with cancer are more likely to recall drug use during pregnancy than mothers of controls), then the resulting bias would create spurious associations.⁴⁷

To minimize the potential for biases inherent in case–control studies, we conducted a retrospective cohort study. We utilized a methodology developed for pharmacoepidemiologic studies that Medicaid enrollment files to identify a cohort of pregnant mothers and utilized computerized records of prescriptions filled at the pharmacy to define drug exposure groups.⁴⁸ These records were available for persons enrolled in the Medicaid program.⁴⁸ The advantages of this definition of drug exposure are 1) it is relatively complete and accurate for prescription drugs; 2) there is detailed data regarding the specific drug and dose received and on timing and duration of use; and 3) the information is collected before the outcome develops and thus there is no potential for information bias. However, we define drug exposure as a Medicaid claim for the filling of a prescription. The accuracy of this surrogate measure is affected by the accuracy and completeness of the Medicaid files, patient compliance, and use of drugs from other sources. Pharmacies are audited periodically by Medicaid to assure that submitted claims are accurate. The Health Care Financing Administration also audits state Medicaid claims files. Thus, Medicaid files accurately reflect filling of qualifying prescriptions.⁴⁸ Noncompliance would result in exposure misclassification; however, this effect should be of small magnitude and would tend to introduce a small conservative bias.

However, a limitation of this approach is that we could have missed cohort members with cancer because of the incompleteness of our case ascertainment methods in establishing the TNCCD and because of loss of follow-up of members who migrated out of state and subsequently developed cancer. This could decrease our chances for detecting a true association only if the cancer in the missed cases was related to metronidazole exposure. We made extensive efforts to ascertain cancer cases in Tennessee using multiple sources and methods. We reviewed records from one or more sources from the four major tertiary centers in the state that managed cancer in children. Furthermore, we used Medicaid and death certificate files to identify nine additional hospitals at which we re-

viewed records to ascertain additional cases. We also limited the current study to children younger than 5 years to minimize loss of follow-up due to out-of-state migration. In addition, a recent study⁴⁹ evaluating bias caused by migration in case–control studies of prenatal risk factors for childhood and adult diseases estimated that if < 25% of the controls migrated or died before the end of the study, migration bias would be unlikely to affect the finding materially. Census data suggest this out-of-state migration would be > 60% over the years of the study.⁵⁰

A major impediment to researching the effects of fetal drug exposure to the risk of childhood cancer is its low incidence. Investigators traditionally have had to rely on the case–control study design to evaluate such exposures. We have used a methodology utilizing linked, computerized records to conduct a retrospective cohort study of fetal exposure to metronidazole with precise definitions of medication exposure. This approach has the advantage of being able to conduct similar studies evaluating the transplacental carcinogenic effects of other medications used during pregnancy.

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