Teratogen Update: Methotrexate

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Methotrexate and aminopterin are folic acid antagonists that inhibit dihydrofolate reductase, resulting in a block in the synthesis of thymidine and inhibition of DNA synthesis. Methotrexate has been used for the treatment of malignancy, rheumatic disorders, and psoriasis and termination of intrauterine pregnancy. Recently, methotrexate has become a standard treatment for ectopic pregnancy. The misdiagnosis of an intrauterine pregnancy as an ectopic pregnancy can result in exposure of a continuing pregnancy to dose levels of methotrexate of 50 mg/m^2 (maternal body surface area). Experimental animal studies have associated methotrexate therapy with embryo death in mice, rats, rabbits, and monkeys. Structural malformations have been most consistently produced in rabbits at a maternal dose level of 19.2 mg/kg. Abnormalities in rabbits include hydrocephalus, microphthalmia, cleft lip and palate, micrognathia, dysplastic sacral and caudal vertebrate, phocomelia, hemimelia, syndactyly, and ectrodactyly. Based on human case reports of methotrexate exposure during pregnancy, a methotrexate embryopathy has been described that includes growth deficiency, microcephaly, hypoplasia of skull bones, wide fontanels, coronal or lambdoidal craniosynostosis, upswept frontal scalp hair, broad nasal bridge, shallow supraorbital ridges, prominent eyes, low-set ears, maxillary hypoplasia, epicanthal folds, short limbs, talipes, hypodactyly, and syndactyly. This syndrome may be associated with exposures between 6 and 8 weeks after conception and dose levels of 10 mg/week or greater. More recent case reports of methotrexate exposure for the misdiagnosis of ectopic pregnancy involve treatment before 6 weeks after conception and have raised the suggestion of a distinct syndrome due to such early exposures. Tetralogy of Fallot and perhaps other neural crest cell-related abnormalities may be features of this early syndrome. A disproportionality analysis of methotrexate and aminopterin case reports and series provides support for pulmonary atresia, craniosynostosis, and limb deficiencies as reported more often than expected in methotrexate-exposed children. Denominator-based data will be welcome to better define elements of a methotrexate embryopathy and possibly to distinguish an early exposure syndrome from anomalies traditionally associated with methotrexate exposure. *Birth Defects Research (Part A) 00:000–000, 2012.* © 2012 Wiley Periodicals, Inc.

Key words: methotrexate; aminopterin; dihydrofolate reductase; malformations; teratology; ectopic pregnancy; abortion

INTRODUCTION

Methotrexate is a folic acid analog that inhibits dihydrofolate reductase, thereby decreasing the availability of tetrahydrofolate (Fig. 1). Because tetrahydrofolate is an important cofactor in thymidylate synthesis and de novo purine synthesis, methotrexate inhibits the synthesis of DNA and has antiproliferative activity.

Methotrexate has been used clinically in the treatment of malignancy, psoriasis, rheumatoid arthritis, and other autoimmune and inflammatory disorders. Methotrexate has also been used with misoprostol for voluntary abortion (Moreno-Ruiz et al., 2007) and in the treatment of ectopic pregnancy (American College of Obstetricians and Gynecologists, 2008; Barnhart, 2009). Typical doses for the treatment of psoriasis or rheumatoid arthritis are 7.5 to 20 mg/week. The methotrexate dose used in the treat-

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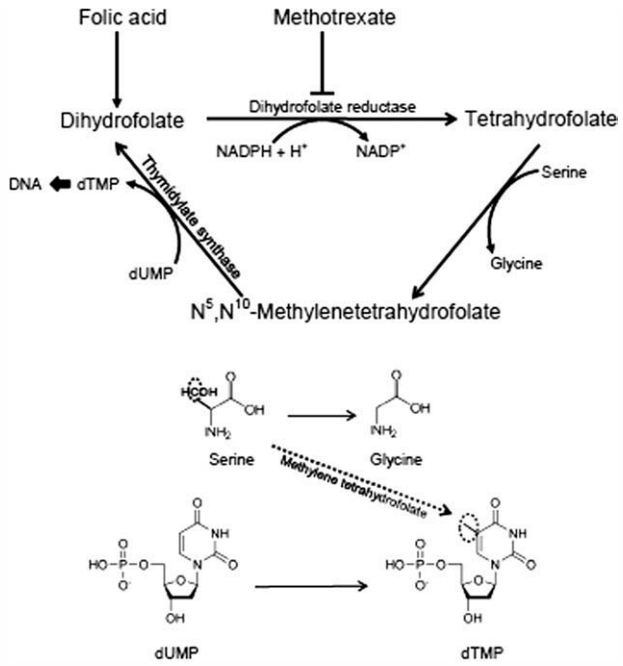

Figure 1. Methotrexate blocks the reduction of dihydrofolate to tetrahydrofolate, a step in the cycle that results in transfer of a methyl group from serine to deoxyuridine monophosphate (dUMP) to produce deoxythymidine monophosphate (dTMP), which is necessary for DNA synthesis. The bottom panel shows that N^5, N^{10}-methylene tetrahydrofolate serves as a shuttle for the transfer of a methyl group (dashed circle) from serine to dUMP to produce dTMP

ment of ectopic pregnancy is 50 mg/m^2, often given intramuscularly and repeated at intervals depending on the protocol used and the patient response. A 60-kg (132-lb) woman who is 168 cm (5 feet, 6 inches) tall has a body surface area of 1.67 m^2. Her methotrexate dose for ectopic pregnancy will be 83.5 mg or 1.4 mg/kg.

Methotrexate therapy is associated with nausea, vomiting, mucositis, immunosuppression, alopecia, pneumonitis, and liver injury. Cotreatment with leucovorin (also called citrovorum factor and 5-formyltetrahydrofolate) is used in some treatment regimens to reduce the toxicity of methotrexate. One of the accepted protocols for ectopic pregnancy treatment includes the use of methotrexate (1 mg/kg) on days 1, 3, 5, and 7 with administration of leucovorin on days 2, 4, 6, and 8 (American College of Obstetricians and Gynecologists, 2008).

The therapy of ectopic pregnancy with methotrexate results in the administration of this medication to a woman known to be pregnant. The misdiagnosis of ectopic pregnancy in a woman with an intrauterine pregnancy or treatment of a woman with a coexisting ectopic and intrauterine pregnancy can result in methotrexate exposure of a continuing pregnancy.

EXPERIMENTAL ANIMAL STUDIES

Embryotoxicity has been described after methotrexate treatment in mice, rats, rabbits, cats, and monkeys; however, the studies reporting these effects predate modern embryofetal toxicity study design. These older studies typically reported results on a per fetus basis and did not report statistical analyses. Despite these limitations, it appears that in the rat and monkey, embryolethality was more common than teratogenesis, which was relatively unusual. Mouse and rabbit produced clear malformation syndromes, albeit at exposure levels much higher on a milligram per kilogram basis than dose levels used in human therapy.

Mouse

Skalko and Gold (1974) administered methotrexate intraperitoneal (ip) to ICR mice on gestation day (GD) 10 (plug = GD 0) at dose levels of 0.3 to 50 mg/kg. There was an increase in resorptions at 10 mg/kg, with no reported adverse effect at 5 mg/kg. Malformations were increased at 25 and 50 mg/kg and consisted predominantly of ectrodactyly and cleft palate. Darab et al. (1987) also produced median facial clefts in fetuses after treatment of pregnant C57Bl/6J mice with methotrexate 20 mg/kg ip on GD 9 (plug = GD 0). Elmazar and Nau (1992) treated Han:NMRI mice with ip methotrexate on GD 8 (24 hours after detection of vaginal plug = GD 0) at dose levels of 1.25 to 20 mg/kg. There was increased embryolethality at 2.5 mg/kg, but no increase in exencephaly or any other external malformation at any dose level. Skeletal and visceral malformations were not evaluated.

Sutton et al. (1998) used a transgenic mouse with a mutation in dihydrofolate reductase that did not bind to methotrexate and was, therefore, not inhibited by it. This dihydrofolate reductase mutation resulted in protection from methotrexate teratogenicity. Methotrexate was administered at 30 mg/kg ip on GD 10 (plug = day 0 or day 0.5, depending on time of mating) to pregnant mice after hemizygous mating. Some fetuses in each litter carried resistant dihydrofolate reductase, whereas other fetuses had the wild type dihydrofolate reductase. Fetuses were evaluated on GD 14, with the exception of two litters that were permitted to go to term. Facial clefts and limb defects, the only malformations evaluated, occurred in 37% of transgenic fetuses compared with 77% of nontransgenic siblings. Resorptions were not genotyped.

None of the mouse studies showed an increase in malformations at ip methotrexate dose levels lower than 20 mg/kg.

Rat

Wilson (1971) reported that methotrexate given ip to an unspecified rat strain on GD 9 (GD 0 not defined) caused 64% resorptions and 30% malformations. Additional details were not provided. In a subsequent publication from Jordan et al. (1977), Wistar rats treated on GD 9 (positive smear = GD 0) with ip methotrexate were described as showing an increase in resorptions at a dose level as low as 0.1 mg/kg, although effects were not evaluated statistically. Treatment with methotrexate (2.5 mg/kg) before implantation on GD 4 had little effect on implantation failure or resorption, but embryo death or resorption increased with treatment on each subsequent day until GD 6, when 100% resorptions occurred after treatment. Malformations occurred in 75% of surviving fetuses after treatment with methotrexate (0.3 mg/kg on GD 9). The nature of the malformations was not discussed, but in another publication (Wilson et al., 1979) methotrexate 0.3 mg intravenously (iv) on GD 11 in Wis-

tar rats produced malformations confined largely to the caudal vertebrae. Peak embryo concentration after this treatment was 7.7 ng/g.

Berry (1971) administered methotrexate ip to Wistar rats on GD 12 (positive smear = GD 1) and evaluated uterine contents on GD 14. There was an increase in resorptions with increasing dose of methotrexate, beginning at 1 mg/kg, and with increasing delay before the administration of folinic acid. Administration of methotrexate on GD 17 or 18 produced delayed ossification and bipartite vertebral bodies when fetuses were examined 1 day later, but no abnormalities when fetuses were examined on GD 21. One fetus treated on GD 18 and evaluated on GD 19 had an encephalocele, and an additional fetus in this group had a bifid rib. It is unlikely that treatment as late as GD 18, a week or more after anterior neuropore closure, could have been responsible for the encephalocele noted in this study.

Tshibangu et al. (1975) cohabited Sprague-Dawley rats for 36 hours. On day 14 ''after coupling'' (not otherwise defined), one group of dams was treated with methotrexate ip at dose levels of 1.6 mg/kg (single dose). Another group was treated with methotrexate 2 mg/kg daily on days 18 to 20. A third group was treated on days 14 to 18 with 0.5, 1.5, or 2 mg/kg/day. Similar dosing schedules were used in experiments with Wistar rats. The authors reported maternal weight reduction, maternal mortality, and decreases in fetal weight with methotrexate treatment at any of the dose levels on GD 14 to 18. They concluded that decreased body weight is the main adverse effect of methotrexate treatment during pregnancy.

Woo et al. (1978) treated Charles River CD rats with ip methotrexate on GD 9 (plug = GD 0) at 0.1, 0.2, or 0.3 mg/kg. Fetuses were evaluated on GD 20. There was an increase in resorptions and a decrease in fetal body weight beginning at a methotrexate dose level of 0.2 mg/kg. There were no statistically significant effects on resorptions or fetal body weight with methotrexate treatment up to 3 mg/kg on GD 12. There were few external, visceral, or skeletal malformations with methotrexate. This study evaluated cotreatment with aspirin, and it is not possible to tell from the report whether any malformations could be attributed to methotrexate treatment alone.

The rat studies suggest embryotoxicity at methotrexate dose levels lower than those used in mice. A convincing malformation syndrome in rats has not been described.

Rabbit

New Zealand White rabbits were treated ip or iv on GD 10 (copulation = GD 0) with methotrexate at 0.3 to 19.2 mg/kg (Jordan et al., 1977). Embryo death or resorption appeared to be increased at iv dose levels of 0.3 mg/kg and higher; the author conclusions were not supported by statistical analysis, which was not described. The percent surviving fetuses that were malformed at evaluation on GD 30 increased with methotrexate dose and reached 80% after the highest dose of 19.2 mg/kg. When the day of treatment was varied, malformations occurred in 100% of survivors after treatment with a methotrexate dose of 19.2 mg/kg iv on GD 12, 13, and 14. Treatment on any of GDs 10, 11, or 12 resulted in hydrocephalus, microphthalmia, cleft lip and palate, micrognathia, dysplastic sacral and caudal vertebrae, and forelimb phocomelia, hemimelia, and ectrodactyly. The later treatments resulted in defects primarily confined to the paws. Treatment on GD 15 produced hind limb syndactyly in approximately one quarter of surviving offspring.

Another publication from this laboratory described treatment with methotrexate 19.2 mg/kg iv on GD 12 as producing cleft palate, micrognathia, hydrocephalus, short tail, ectrodactyly, syndactyly, and pedunculated thumb (DeSesso and Jordan, 1977). This study was the doctoral dissertation of future Teratology Society president John DeSesso, who went on in subsequent papers to describe the ultrastructural features of methotrexate damage in the fetal rabbit limb (DeSesso, 1981) and the amelioration of methotrexate teratogenicity by leucovorin and 1-(*p*-tosyl)-3,4,4-trimethylimidazolidine, both of which can provide 1-carbon transfer in the presence of dihydrofolate reductase inhibition (DeSesso and Goeringer, 1991, 1992). These papers confirm that inhibition of dihydrofolate reductase is likely to be responsible for much of the teratogenicity of methotrexate in the rabbit.

Cat

Khera (1976) treated shorthaired European and Persian cats with methotrexate 0.5 mg/kg/day by gavage on GD 11–14, 14–17, or 17–20 (first day of mating = GD 1; mated for 2 days). Fetuses were evaluated on GD 44 (term = GD 63 or 64). Maternal toxicity including death occurred in 8 of 55 methotrexate-treated females. There were no statistically significant effects of treatment on live fetuses or litters, fetal weight, or viability. There was an increase in malformations in litters exposed to methotrexate on GD 11 to 14 and 14 to 17. Malformations consisted primarily of umbilical hernia, reported in 12 of 114 fetuses in the study. Other malformations reported in one or two fetuses each across all dose groups included hydrocephalus, spina bifida, and malformed limbs.

Monkey

Wilson (1971) reported that 13 rhesus monkeys treated with iv methotrexate at various doses between GD 17 and 45 had three abortions, nine normal outcomes, and one infant with gut malrotation when evaluated on GD 100. The highest methotrexate dose used was 3 mg/kg/day for 4 days. In a study reported in more detail (Wilson et al., 1979), 20 pregnant rhesus monkeys were given methotrexate 30 mg/kg/day iv on GD 29 to 32 (positive smear = GD 0). Hysterotomy was performed within 24 hours of the last treatment. There were three dead and seven growth-restricted embryos. None of the remaining embryos were malformed. The peak embryo methotrexate concentration was 199 ng/g. The authors concluded that methotrexate was more likely to cause embryolethality than teratogenicity in the monkey. The authors cited a prior study that appeared in a book chapter and showed that methotrexate treatment of pregnant rhesus monkeys on GD 17–45 with iv dose levels as high as 4 mg/kg/day produced embryolethality and ossification impairment but not malformations.

HUMAN REPORTS

Aminopterin

Aminopterin is a folic acid antagonist that is a close structural analog of methotrexate. Farber and Diamond (1948) described the use of what they called ''the most

powerful folic antagonist we have yet encountered'' in the treatment of 16 children with leukemia. Ten of the children showed some improvement after therapy. Aminopterin was marketed thereafter for the treatment of malignancy. Aminopterin was subsequently abandoned because methotrexate was considered to have a more favorable therapeutic index. Aminopterin and methotrexate are viewed as nearly identical in their effects, although aminopterin is more potent.

Aminopterin is an important part of the methotrexate story, because aminopterin gave rise to the first report of human malformations after use during pregnancy of a folic acid antagonist (Thiersch, 1952). This report described 12 pregnancies in which abortion was considered indicated for maternal tuberculosis, mental illness, cancer, muscular dystrophy, rheumatic mitral valve disease, or Rh sensitization. The women were given 8 to 15 mg aminopterin orally depending on maternal body weight and gestational age in an attempt to cause fetal death. Three women received a second course of treatment because the urine pregnancy tests in use at the time remained positive. Treatment was given in small doses every 12 hours until the desired dose was achieved over 2 to 5 days. Ten of the 12 women aborted within 5 to 17 days of the last dose of aminopterin, and two women underwent surgical evacuation of the uterus because fetal expulsion did not occur. In most instances, the pregnancy tissue could not be examined for malformations, presumably because of autolytic changes; however, in three cases, including the two surgical abortions, malformations were identified, including one instance each of hydrocephalus, meningomyelocele, and cleft lip with cleft palate.

The use of aminopterin in the 1950s for therapeutic abortion and the use of methotrexate today for the termination of ectopic pregnancies are similar scenarios. There is also a less apparent similarity in the experiences with these two antifolate agents, namely the tendency to attribute all birth defects in exposed embryos or fetuses to the drug. In the Thiersch (1952) report, the hydrocephalus, meningomyelocele, and facial clefts were believed to have been due to aminopterin exposure; however, the meningomyelocele case was not exposed until an estimated 49 days after conception—3 weeks after neural tube closure was complete. Although pregnancy dating was inexact at the time, when the fetus was expelled, it was estimated to be 2 to 3 weeks older than anticipated. It is likely, then, that the meningomyelocele in this fetus was not due to the aminopterin treatment but was coincidental, leading us to wonder which of the malformations described subsequently in methotrexate-exposed children may have been coincidental and which were due to the medication exposure.

Other case reports involving aminopterin and congenital malformations during the years after the Thiersch (1952) report led to the specification of an aminopterin syndrome of malformations. According to one reviewer, these malformations include meningoencephalocele, anencephaly, brachycephaly, hydrocephaly, incomplete skull ossification, mental retardation, cleft lip and palate, low-set ears, micrognathia or retrognathia, syndactyly, short forearms, hypoplasia of the thumb and fibula, and positional abnormalities of the extremities, including talipes (Briggs et al., 2011).

Methotrexate

Case reports and case series of methotrexate exposure during pregnancy began appearing in the 1960s. Eight such publications were reviewed by Feldkamp and Carey (1993), who presented the methotrexate and aminopterin literature as part of their analysis of the potential risk to a patient who consulted them about exposure during early pregnancy. Based on the reports of six malformed infants, Feldkamp and Carey (1993) proposed that the sensitive period for the production of malformations by methotrexate is 6 to 8 weeks after conception. They further suggested that the methotrexate dose necessary to produce malformations is 10 mg/week or greater.

Additional studies and studies published since Feldkamp and Carey (1993) are summarized in Table 1. Some of these publications can be characterized as denominator-based samples in which exposure appears to have been ascertained before pregnancy outcome was known. These papers will be presented in more detail here.

Avilés et al. (1991) reported pregnancy outcome in women treated during gestation for acute leukemia. Nine of the women received methotrexate as part of their regimens. There were no reported malformations, but only five of the women were treated during the first trimester. Details of dosing and gestational weeks of exposure were not reported.

Donnenfeld et al. (1994) surveyed 63 teratology information services and identified 21 prospectively ascertained cases of methotrexate exposure before or during pregnancy. Five exposures occurred during pregnancy, four of which occurred between 0 and 6 weeks after conception and one of which occurred in the third trimester. All babies exposed during pregnancy were normal, although the child with third-trimester exposure was diagnosed with pneumonia at 1 month of age. Four of nine women with methotrexate exposure during the year preceding pregnancy had spontaneous abortions and one child had a cavernous hemangioma. The authors concluded that the normal outcomes in the four children with first-trimester exposure were consistent with the report by Feldkamp and Carey (1993), because the exposures were less than 10 mg/week or because the treatment was discontinued by 6 weeks after conception.

Østensen et al. (2000) presented four women referred to their rheumatology center for methotrexate exposure during early pregnancy. One woman spontaneously aborted and three women had normal infants. One of these children was born at 36 weeks' gestation. All the exposures had been discontinued before 6 weeks after conception.

Chakravarty et al. (2003) used a questionnaire sent to rheumatologists to solicit information about pregnancies exposed to disease-modifying antirheumatic drugs. Among 38 methotrexate-exposed pregnancies with known outcomes, there were two children and one abortus with unspecified malformations. There was only a 29% response rate to the questionnaire, and it is not clear how and by whom the pregnancy outcomes were ascertained.

Lewden et al. (2004) sent a questionnaire to 31 French pharmacovigilance centers and two teratology information services. Only 10 centers recorded a pregnancy exposed to methotrexate during the 9-year study period; methotrexate was given in these instances for the treatment of rheumatologic or chronic inflammatory disorders. There were 28

Table 1
Human Reports of Methotrexate Exposure during Pregnancy Since Feldkamp and Carey (1993)

Reference	Disease	Gestational age at exposure	Weekly methorexate dose	Gestational age (weeks)	Weight at birth (g)	Outcome	Comments
Adam et al., 2003	Abortion	7-5/7 weeks postconception	50 mg/m^2, once	34 weeks induction of labor due to low amniotic fluid index	1619	Narrow bifrontal diameter, upslanting palpebral fissures, small eyes, flat nasal bridge, pectus excavatum, hypospadias, partial 4-5 syndactyly, absent fourth and fifth digits on the feet bilaterally, nail hypoplasia	Mother had hypertension; also treated with misoprostol 800 μg
	Abortion	6 weeks postconception	50 mg/m^2, once	40-3/7	1951	Large fontanels, bicoronal craniosynostosis, right cleft lip/palate, folding of the right ear helix, undescended right testis, mesomelic shortening of the forearms with single bone, bilateral antecubital pterygia, three-digit hands with absent fourth and fifth rays, talipes equinovarus, hypoplastic nails, tetralogy of Fallot with pulmonary atresia, atrioventricular canal	Also treated with misoprostol 800 μg
	Abortion	4 weeks postconception	50 mg/m^2, once	26	574	Low-set ears; short, downslanting palpebral fissures; broad nasal root; small, downturned mouth with smooth philtrum; micrognathia; radial deviations of the wrist; absent fifth digits bilaterally; single leg bone; talipes equinovarus; mild 3-4 syndactyly; hypoplastic toes	Placental abruption, foul-smelling amniotic fluid, infant expired at 5 hours of life; mother also treated with misoprostol 800 μg

Table 1
(Continued)

Reference	Disease	Gestational age at exposure	Weekly methorexate dose	Gestational age (weeks)	Weight at birth (g)	Outcome	Comments
	Suspected ectopic pregnancy	8 and 10 weeks (6 and 8 weeks postconception)	50 mg/m^2, twice	33	1508	Narrow flattened cranium, with occipital plagiocephaly; patent sagittal suture; three fontanels; ocular hypertelorism; epicanthal folds; low nasal bridge; small mandible; grade I germinal matrix hemorrhage	
Addar, 2004	Suspected ectopic pregnancy	5 weeks[a]	95 mg, once	—	—	Ambiguous genitalia, bifid scrotum, micropenis	
Avilés et al., 1991	Leukemia	1st trimester	Not given	36	3200	Healthy child	Also exposed to vincristine, prednisone, doxorubicin, 6-mercaptopurine, cyclophosphamide
	Leukemia	1st trimester	Not given	36	2500	Healthy child	Also exposed to cytosine arabinoside, doxorubicin, prednisone, 6-mercaptopurine, cyclophosphamide
	Leukemia	2nd trimester	Not given	38	3100	Healthy child	Also exposed to vincristine, prednisone, doxorubicin, 6-mercaptopurine, cyclophosphamide
	Leukemia	1st trimester	Not given	37	3000	Healthy child	Also exposed to vincristine, doxorubicin, 6-mercaptopurine, cyclophosphamide
	Leukemia	2nd trimester	Not given	40	3200	Healthy child	Also exposed to cyclophosphamide, doxorubicin, vincristine, prednisone, etoposide

Table 1
(Continued)

Reference	Disease	Gestational age at exposure	Weekly methorexate dose	Gestational age (weeks)	Weight at birth (g)	Outcome	Comments
	Leukemia	1st trimester	Not given	37	2850	Healthy child	Also exposed to cyclophosphamide, epidoxorubicin, vincristine, prednisone, bleomycin, cytosine arabinoside, etoposide
	Leukemia	3rd trimester	Not given	39	3100	Healthy child	Also exposed to cyclophosphamide, nflammato, vincristine, prednisone, cytosine arabinoside
	Leukemia	2nd trimester	Not given	40	4000	Healthy child	Also exposed to cyclophosphamide, nflammato, vincristine, prednisone, bleomycin, cytosine arabinoside, etoposide
	Leukemia	1st trimester	Not given	40	2800	Healthy child	Also exposed to cyclophosphamide, epidoxorubicin, vincristine, prednisone, bleomycin, cytosine arabinoside, etoposide, dexamethasone
Bawle et al., 1998	Abortion	6 weeks postconception or after last menstrual period	Not given	Not given	—	Hypertelorism, ptosis, short palpebral fissures, prominent nose, low-set ears, widow's peak, short stature, subluxation of radial heads, severe hypoplasia of the phalanges of the toes, only four metacarpals, gynecomastia at puberty	No mental impairment

Table 1
(Continued)

Reference	Disease	Gestational age at exposure	Weekly methorexate dose	Gestational age (weeks)	Weight at birth (g)	Outcome	Comments
	Breast cancer	9.5 weeks (7.5 weeks post conception)	80 mg on 6 occasions	29	820	Normal karyotype, short stature, underweight, small head circumference, hypertelorism, upswept frontal hairline, microcephaly, low set ears, micrognathia, simian crease, intelligence quotient of approximately 70	Lumpectomy for breast cancer, radiation (14 rad) to breast and chest wall area 15.5—25 weeks after conception; treated with fluorouracil from 7.5 weeks
	Abortion	11–25 weeks (9–23 weeks postconception)	50–100 mg	29	1160	Short stature, underweight, bulging forehead, bitemporal narrowing, upslanting palpebral fissures, sparse hair on temporal area, low-set ears, broad nasal tip, high arched palate, normal psychomotor development	Attempted medical abortion followed failed curettage
Buckley et al., 1997	Rheumatoid arthritis	Up to 8 weeks[a]	10–12.5 mg (100 mg over 8 weeks)	35	1790	Growth restriction, skeletal abnormalities, double outlet right ventricle, ventricular septal defect, transposition of the great vessels, pulmonic stenosis, atrial septal defect, brachycephaly, coronal ridging, small anterior fontanel, shallow orbits with hypertelorism, retrognathia, bifid ear lobule, folded helix, closed or stenotic canal, umbilical hernia, dorsal kyphosis, right thumb deformity, syndactyly of 4th and fifth toes, bilateral simian creases, hypoplastic nails	Mother took folic acid, but possibly inconsistently
Brudie et al., 2011	Choriocar-cinoma	24–28 weeks[a]	Not given	Labor induced at 32 weeks	1383	Healthy	Also treated with etoposide, actinomycin D, cyclophosphamide, vincristine

Table 1
(Continued)

Reference	Disease	Gestational age at exposure	Weekly methorexate dose	Gestational age (weeks)	Weight at birth (g)	Outcome	Comments
Chakravarty et al., 2003	Rheumatoid arthritis, n = 39	Not given	Not given	Not given	—	21 healthy term infants, 3 infants with congenital malformations (no details given), 8 elective abortions, 7 spontaneous abortions, 1 ongoing pregnancy	1 spontaneous abortus had unspecified malformations
Chapa et al., 2003	Abortion	6 weeks[a]	75 mg, once	27-week elective abortion	918	Asymmetric length of upper extremities, absent fibulae bilaterally, right foot polydactyly, small head, prominent eyes, low set ears, micrognathia, two-vessel umbilical cord	Also treated with misoprostol 400 μg
Corona-Rivera et al., 2010	Systemic lupus erythematosis	5–7 weeks postconception	35 mg	34	2200	Hydrocephalus, oxycephalus, large fontanels, wide forehead, deficient skull ossification, absent parietal and frontal bones, semilobar holoprosencephaly, sparse hair, absent lateral eyebrows, hypertelorism, blepharophimosis, prominent eyes, upslanting palpebral fissures, vertical nystagmus, epiblepharon, trichiasis, wide nasal bridge, broad and bifid nasal tip, microtia, absent earlobes, low-set posteriorly rotated ears, micrognathia, short thorax, hypoplastic nipples, umbilical hernia, lateral penile curvature, coronal hypospadias, cryptorchidism, mesomelic micromelia, brachydactyly, clinodactyly, absent palmar creases, hypoplastic nails, absent middle phalanx of 4th and fifth toes, hydronephrosis	Also exposed to ampicillin and acetaminophen in the first trimester and folic acid beginning in the fifth month, 46XY 16q karyotype

Table 1
(Continued)

Reference	Disease	Gestational age at exposure	Weekly methorexate dose	Gestational age (weeks)	Weight at birth (g)	Outcome	Comments
Del Campo et al., 1999	Severe psoriasis	First 8 weeks post conception	37.5 mg	40	1645	Widely separated sutures, large fontanels, bilateral epicanthal folds, sparse eyebrows, broad nasal bridge, anteverted nares, smooth long philtrum, hypoplastic nipples, small umbilical hernia, diastasis recti, shawl scrotum, decreased extension at the elbows, short proximal phalanges, hypoplastic fingernails, normal karyotype, delayed developmental milestones, flat affect, irritability	—
Donnenfeld et al., 1993	Intraductal breast carcinoma	37–38 weeks postconception	42 mg	41	3350	Healthy at birth; pneumonia at 1 month of age	—
	Bacterial infection	5–6 weeks postconception	Not given	42	3560	Healthy	—
	Rheumatoid arthritis	3–5 weeks postconception	Not given	36	4036	Healthy	—
	Not given	Up to 2 weeks postconception	7.5 mg	40	3885	Healthy	Also treated with aspirin and hydroxychloroquine
	Rheumatoid Arthritis	Up to 3 weeks postconception	7.5 mg	40	3220	Healthy	Also treated with aspirin and hydroxychloroquine
Georgiou et al., 2011	Heterotopic pregnancy with persistent ectopic trophoblast	13 weeks[a]	150 mg	27	830	Infant required resuscitation but no malformations were diagnosed	—
Giannakopoulou et al., 2000	Breast cancer	6–24 weeks (4–22 weeks postconception)	Not given	30	1000	Inguinal hernia, respiratory distress syndrome of prematurity, 3rd percentile, normal development at 22 months of age	Also treated with cyclophosphamide and 5-fluorouracil; infant karyotype 46 XY

Table 1
(Continued)

Reference	Disease	Gestational age at exposure	Weekly methorexate dose	Gestational age (weeks)	Weight at birth (g)	Outcome	Comments
Goffman et al., 2006	Abortion	6 weeks (4 weeks postconception)	70 mg during week 1, 30 mg during week 2	39 weeks: induction of labor for growth restriction	2370	Large fontanel, hypertelorism, broad nasal bridge, micrognathia, short torso, missing ribs, scoliosis, micropenis, shortened proximal long bones, bilateral fifth finger clinodactyly, bilateral second toe clinodactyly	Also treated with diclofenac 50 mg and misoprostol 200 μg daily for 4 days
Granzow et al., 2003	Molar pregnancy	8 weeks[a]	Unknown	Unknown	—	Cleft of the soft palate, agenesis of fifth toe on right foot with short second toe, left foot: fusion of third, fourth and fifth toes with clinodactyly of the second toe	
Hahn et al., 2001	Abortion attempt	''2 months pregnant''	Not known; estimated at 25–150 mg/m^2	Not given	—	Healthy	Leucovorin given every 6 hours for 6 doses
Kozma and Ramasethu, 2011	Abortion	7 weeks (5 weeks postconception)	75 mg	35-6/7	981	Growth restricted (<3rd percentile), wide anterior fontanel, shallow orbital ridges, depressed nasal bridge, micrognathia, posterior cleft palate, low-set ears, hypoplastic chest, syndactyly, clinodactyly, absent palmar creases, gap between first and second toes, ectrodactyly of toes, rib anomalies, brachycephaly, coronal and possibly sagittal craniosynostosis, arrhinencephaly	Also exposed to misoprostal 1600 μg vaginally; became positive for human immunodeficiency virus during the pregnancy
Krähenmann et al., 2002	Rheumatoid arthritis	4-6/7 and 5-6/7 weeks (probably 2-6/7 and 3-6/7 weeks postconception)	10 mg, twice	18-week elective abortion	170	Atrioventricular septal defect, diaphragmatic hernia	Folic acid 1 mg taken irregularly, increased nuchal translucency identified at 12-3/7 weeks

Table 1
(Continued)

Reference	Disease	Gestational age at exposure	Weekly methorexate dose	Gestational age (weeks)	Weight at birth (g)	Outcome	Comments
Lewden, et al., 2004	Rheumatic/ chronic inflammatory disorders; n = 28 pregnancies with follow-up	Various	Mean 30.7 ± 23.3 mg (SD)	16 were at full term (mean 39.2 ± 1.2 weeks [SD])	3179 ± 465 g (SD)	5 elective abortions, 4 spontaneous abortions, 19 live births, 1 child with bilateral metatarsus varus, right eyelid angioma	In the child with minor anomalies, mother was taking methotrexate 7.5 mg/week and sulfasalazine 3 g/day until 8.3 weeks; women also used nonsteroidal antiinflammatory drugs
Mulholland and Pollock, 2011	Rheumatoid arthritis	First 8 weeks[a]	15 mg	Not given	—	Peter's anomaly (dysgenesis of the anterior segment of the eye)	Folic acid 5 mg/week and hydroxychloroquine 400 mg/day also taken
Nguyen et al., 2002	Psoriasis	5.5 weeks (3.5 weeks postconception)	15 mg, twice	20	240	Two-vessel umbilical cord, choroid plexus cysts, low-set ears, micrognathia, abnormal position of the hands, absent left radius, occipital flattening, craniofacial abnormalities, absent auditory canals, micrognathia	Maternal depression, sertraline treatment until 3.5 weeks
Nurmohamed et al., 2011	Suspected ectopic pregnancy	5 weeks (3 weeks postconception)	50 mg	37	—	Tetralogy of Fallot with pulmonary atresia, scoliosis, abnormal number of ribs,	—
	Suspected ectopic pregnancy	6 weeks (4 weeks postconception)	50 mg, twice	30	—	Tetralogy of Fallot, horseshoe kidney, single umbilical artery	—
	Suspected ectopic pregnancy	5 weeks (3 weeks postconception)	83 mg	6	—	Spontaneous abortion	—
	Suspected ectopic pregnancy	4 weeks (2 weeks postconception)	80 mg	6	—	Spontaneous abortion	—
	Suspected ectopic pregnancy	6 weeks (4 weeks postconception)	50 mg	8	—	Spontaneous abortion	—
	Suspected ectopic pregnancy	4 weeks (2 weeks postconception)	50 mg	6	—	Elective abortion	—
	Suspected ectopic pregnancy	6 weeks (4 weeks postconception)	85 mg	9	—	Elective abortion	—
	Suspected ectopic pregnancy	8 weeks (6 weeks postconception)	92 mg	9	—	Elective abortion	—

Table 1
(Continued)

Reference	Disease	Gestational age at exposure	Weekly methorexate dose	Gestational age (weeks)	Weight at birth (g)	Outcome	Comments
Østensen et al., 2000	Psoriatic arthritis	Up to 5 weeks (3 weeks postconception)	7.5 mg	36	2600	Healthy	—
	Juvenile chronic arthritis	Up to 6 weeks (4 weeks postconception)	2.5 mg	8		Spontaneous abortion	Also exposed to naproxen
	Rheumatoid arthritis	Up to 3 weeks (1 week postconception)	15 mg	38	3230	Healthy	Folate supplementation
	Rheumatoid arthritis	Up to 3 weeks (1 week postconception)	7.5 mg	39	2960	Healthy	Also exposed to naproxen and folate supplementation
Østensen et al., 2007	Rheumatic diseases, n = 8	1st trimester	Not given	Not given	—	1 healthy infant, 2 elective abortions, 2 spontaneous abortions, 3 unknown outcomes	Cases ascertained from questionnaires given to patients and to rheumatologists
Piggott et al., 2011	Systemic lupus erythematosis	1st trimester	Not given	35	1700	Pulmonary atresia, ventricular septal defect, absent left auditory canal, micrognathia, dysplastic multicystic kidney, hydronephrosis, intestinal malrotation	—
	Suspected ectopic pregnancy	1st trimester	Not given	26	—	Tetralogy of Fallot, clubfoot, accessory digits, micrognathia, abnormal kidney position, renal parenchymal disease, ''aminopterin syndrome'' (apparently referring to craniofacial dysmorphology)	—
	Not given	1st trimester	Not given	Not given	—	Tetralogy of Fallot, pulmonary atresia, prominent renal pelvis	Absence of ''aminopterin syndrome''
Poggi and Ghidini, 2011	Suspected ectopic pregnancy	5-6/7 weeks (3-6/7 weeks postconception)	87 mg, once	30 (stillbirth)		Single umbilical artery, tetralogy of Fallot, horseshoe lung; normal karyotype, negative testing for 22q11 deletion or duplication.	Started on folic acid 4000 mg/day at 6-4/7 weeks

Table 1
(Continued)

Reference	Disease	Gestational age at exposure	Weekly methorexate dose	Gestational age (weeks)	Weight at birth (g)	Outcome	Comments
Seidahmed et al., 2006	Abortion	6th week postconception	52.5 mg	''Term''	1310	Prominent eyes, long eyelashes, epicanthic folds, shallow orbits, beaked nose, long philtrum, cleft palate, micrognathia, low-set malformed ears, narrow forehead, metopic and coronal craniosynostosis, wide posterior fontanel, low anterior hairline, mesomelia, hypoplastic first toes and nails, alobar holoprosencephaly	Parents were cousins, infant had normal female karyotype
Usta et al., 2007	Suspected ectopic pregnancy	5 weeks[a]	75 mg, once	38	2115	Lag in fetal growth after 29 weeks, hypertelorism, facial nerve palsy, right microtia, scoliosis, wide spaced nipples, perimembranous ventricular septal defect, patent foramen ovale, increased pulmonary artery pressure	
Wheeler et al., 2002	Abortion	8 weeks and 4 days from last menstrual period (6 weeks and 4 days postconception)	75 mg, once	39	2050	Mild dolichocephaly, tall forehead, prominent nose, lateral fullness, hypertelorism, small mouth, micrognathia, fifth finger and toe clinodactyly, hypoplastic toenails, hypotonia	Mother had severe preeclampsia, also treated with misoprostol 800 μg
Yedlinsky et al., 2005	Abortion	7 weeks[a]	50 mg/m^2, once	35	1681	No dysmorphic features; magnetic resonance imaging at 6 weeks of age: right lateral intraventricular cyst, dilation of lateral ventricle; at 5 months: anisocoria, slight bowing of the tibias, bilateral transverse palmar creases, third rib hypoplasia, shortening of the limbs, poor weight gain, linear growth <5%	Mother with preeclampsia

Table 1
(Continued)

Reference	Disease	Gestational age at exposure	Weekly methorexate dose	Gestational age (weeks)	Weight at birth (g)	Outcome	Comments
	Abortion	6 weeks[a]	50 mg/m^2, once	38 (twins)	2686 and 2570	Hyperextensible joints, overlapping second and third toes of both feet, bilateral fifth finger clinodactyly, bony foot abnormalities; both twins affected	Some features suggestive of Dubowitz syndrome (autosomal recessive); also treated with misoprostol 800 μg
Zand et al., 2003	Abortion	Not given	Not given	Not given	—	Elective abortion: shortened radius and ulna, absent femurs, hypertelorism, microcephaly, double outlet right ventricle	
	Abortion	Not given	Not given	Not given	—	Elective abortion: bilateral absence of radius and fibula, asymmetric frontal bones, large parietal foramina, absent thymic isthmus, accessory spleens, hypertelorism, bulbous nasal tip, high arched, prominent eyebrows, ectrodactyly	

[a]Dating could not confidently be determined to be based on last menstrual period or conception.

pregnancies with follow-up among 27 women. Only two women took methotrexate after 8 weeks' gestation (i.e., 6 weeks after conception). There were five elective and four spontaneous abortions. There were no major malformations among the 19 liveborn infants. One of the pregnancies exposed after 8 weeks' gestation was electively terminated, and the other pregnancy resulted in a child with an eyelid angioma and bilateral metatarsus varus. Although none of the exposed children had major malformations, this study does not contradict the conclusions of Feldkamp and Carey (1993), inasmuch as only one continuing pregnancy was exposed during the sensitive period proposed by them.

There have been suggestions that the embryopathy attributable to methotrexate should be expanded beyond the original aminopterin embryopathy based on the appearance of additional kinds of malformations with exposures outside the sensitive period proposed by Feldkamp and Carey (1993). Poggi and Ghidini (2011) reported a case of tetralogy of Fallot in a child after methotrexate was used for a misdiagnosis of ectopic pregnancy at less than 6 weeks' gestation (4 weeks after conception). Methotrexate treatment after misdiagnosis of ectopic pregnancy can involve higher doses during the 2 or 3 weeks after implantation than have been used historically for the treatment of rheumatic disorders, and the claim by Poggi and Ghidini (2011) is worth considering. Indeed, a case series of eight intrauterine pregnancies with methotrexate treatment for misdiagnosis of ectopic pregnancy reported tetralogy of Fallot in both pregnancies that continued to viability (Nurmohamed et al., 2011). These pregnancies had been exposed to methotrexate at 3 and 4 weeks after conception.

DISPROPORTIONALITY ANALYSIS

The Problem

Major congenital malformations have been associated with aminopterin and methotrexate in the literature for more than 60 years. The historical prominence of these dihydrofolate reductase inhibitors in the field of teratology and the compelling mechanism of action have resulted in the acceptance of the existence of a methotrexate embryopathy. Because the list of features of the methotrexate embryopathy was derived from case reports, we wonder whether any malformation in a methotrexate-exposed infant was necessarily caused by methotrexate. If, for example, congenital heart disease occurs in nearly 1% of newborns, it might reasonably be expected that congenital heart disease will occur in nearly 1% of methotrexate-exposed newborns, even if methotrexate does not cause congenital heart disease. This problem was articulated in a previous Teratogen Update in these pages by Dr. C.G.H. Newman, one of the physicians charged with determining what defects should be included in the thalidomide embryopathy. Newman (1985) wrote, ''When a drug is very widely used and fails to prevent other types of defect, sporadic defects will at times coincide, and infants who might have been affected but escaped the effects of the drug can continue to be affected by whatever other factors operate.''

Methotrexate is not as widely used as thalidomide was at its peak, but methotrexate may be more widely used than recognized by many teratologists. Before the advent of the so-called biologic agents (e.g., drugs acting to block tumor necrosis factor alpha), methotrexate was commonly recommended for the treatment of rheumatoid arthritis, a disorder that affects many women of childbearing potential. At present, methotrexate is routinely used for ectopic pregnancies, which occur in 1 to 2% of pregnancies and which are suspected in a larger number of pregnancies, representing a substantial opportunity for continuing intrauterine pregnancies to be exposed because of diagnostic error. We would expect some spontaneously occurring congenital malformations to be identified by chance in infants who have been coincidentally exposed to methotrexate.

Findings

We evaluated whether a malformation or closely related group of malformations occurred more often in case reports and case series than would be expected by chance. We compared the proportion of all malformations represented by each specific malformation with the same proportion derived from the Metropolitan Atlanta Congenital Defects Program (MACDP) (Correa et al., 2007). We used reports of malformations occurring after either aminopterin or methotrexate; removal of the aminopterin cases made little difference in our analysis.

The MACDP is an active surveillance program, and reporting of congenital malformations to this program can be expected to be different from reporting malformations in a published case report; therefore, these two sources of information are only roughly comparable. Moreover, we evaluated the proportion of total malformations represented by individual malformations, rather than the proportion of malformed children with a particular malformation, because the MACDP report does not permit identification of multiple malformations within the same child. Using a proportion of total malformations rather than a proportion of malformed children inflates the number of observations and may lead to spurious associations. We used chi-square or Fisher exact testing in making the comparison and reported defects for which methotrexate reports contained a disproportionate number of instances (Table 2). This analysis supported pulmonary atresia, craniosynostosis, and limb deficiencies as possibly associated with methotrexate exposure.

Our comparison was limited to malformations specifically reported by the MACDP. We were unable to evaluate the cranial and facial dysmorphology findings that have been reported with aminopterin and methotrexate, because of the lack of a counterpart in the MACDP report. We found it interesting that some malformations that have often been considered part of the aminopterin and methotrexate syndromes (e.g., neural tube defects, facial clefts) were not supported by our evaluation as being disproportionately associated with aminopterin or methotrexate. The limb malformations associated with methotrexate are likely to explain the description of short limbs reported with this exposure. The hypoplastic skull bones in the methotrexate case reports do not have a counterpart in the MACDP report and could not be evaluated in our analysis.

DISCUSSION

The question of what malformations are part of a methotrexate embryopathy is difficult to answer, in part because the case reports and case series that have been

Table 2
Disproportionality Analysis

Malformations	Methotrexate		General population
	n	%[a]	%[b]
TOTAL HEART	14	4.08	
Conotruncal defects	9	2.62	3.56
Tetralogy of Fallot	6	1.75	1.67
Double outlet right ventricle	2	0.58	
Transposition of great arteries	1	0.29	1.01
Two-vessel cord	3	0.87	
PULMONARY ATRESIA	3	0.87	0.17
Pulmonary stenosis	1	0.29	1.53
Increased pulmonary artery pressure	1	0.29	
Atrial septal defect	2	0.58	3.06
TOTAL VENTRAL SEPTAL DEFECT	2	0.58	10.44
Perimembranous ventral septal defect	1	0.29	2.96
Complete atrioventricular canal	1	0.29	0.85
Patent foramen ovale	1	0.29	
Dextrocardia	1	0.29	
TOTAL NEURAL TUBE	2	0.58	
Meningomyelocele	1	0.29	
Anencephaly	1	0.29	1.35
TOTAL BRAIN	7	2.04	
Hydrocephalus	5	1.46	1.74
Holoprosencephaly	2	0.58	
Hypoplastic cerebellum, prominent cisterna magna	1	0.29	
Absent corpus callosum	1	0.29	
Anterior horn of left lateral ventricle > right	1	0.29	
Forebrain malformation	1	0.29	
Missing olfactory bulbs	1	0.29	
TOTAL CRANIAL	27	7.87	
Large fontanels, separated sutures	11	3.21	
TOTAL CRANIOSYNOSTOSIS	10	2.92	1.46
Oxycephaly, coronal suture fusion	5	1.46	
Brachycephaly, coronal ridging	2	0.58	
Craniosynostosis	1	0.29	
Bicoronal synostosis	1	0.29	
Dolichocephaly (scaphocephaly)	1	0.29	
Narrow bifrontal diameter	2	0.58	
Narrow flattened cranium	1	0.29	
Absent parietal bone	2	0.58	
Parietal foramina	1	0.29	
Skull anomalies	2	0.58	
Absent sutures	1	0.29	
Delayed or minimal ossification of skull	3	0.87	
No ossification of parietal bone	1	0.29	
Ossification defect in frontal bone	1	0.29	
Absent or hypoplastic frontal bone	6	1.75	
Microcephaly	3	0.87	
Occipital flattening	1	0.29	
Bulging forehead, bitemporal narrowing	1	0.29	
Wide forehead	1	0.29	
TOTAL EAR	20	5.83	
Absent auditory canals	2	0.58	
Bifid ear lobule	1	0.29	
Stenotic auditory canal	1	0.29	
Low-set ears	15	4.37	
Abnormal ears	1	0.29	
Posteriorly rotated ears	1	0.29	
Folded helix	2	0.58	
Microtia	3	0.87	
Absent earlobes	1	0.29	
TOTAL FACE	29	8.45	
Cleft lip or palate	10	2.92	5.61
High arched palate	1	0.29	
Small mouth	2	0.58	
Long philtrum	1	0.29	
Short or smooth philtrum	3	0.87	

Table 2
(Continued)

Malformations	Methotrexate n	Methotrexate %[a]	General population %[b]
Micrognathia, retrognathia, small mandible	19	5.54	
Facial palsy	1	0.29	
Craniofacial abnormalities	1	0.29	
Sparse hair, lateral eyebrow absence	1	0.29	
High arched, prominent eyebrows	1	0.29	
Low hairline covering entire forehead	1	0.29	
Posteriorly combed hair appearance	2	0.58	
Unspecified dysmorphic facies	1	0.29	
TOTAL EYE	27	7.87	
Shallow orbits or supraorbital ridges	3	0.87	
Prominent eyes	3	0.87	
Hypertelorism	20	5.83	
Ptosis	1	0.29	
Blepharophimosis	1	0.29	
Upper and lower lid epiblepharon	1	0.29	
Trichiasis	1	0.29	
Short palpebral fissure	2	0.58	
Upslanting palpebral fissure	3	0.87	
Vertical nystagmus	1	0.29	
Microphthalmia	1	0.29	
Epicanthal folds	4	1.17	
Long eyelashes	1	0.29	
Anterior segment dysgenesis	1	0.29	
TOTAL NOSE	16	4.66	
Broad nasal tip	4	1.17	
Bifid nasal tip	1	0.29	
Prominent nose	2	0.58	
Beaked nose	1	0.29	
Flat or broad nasal bridge	11	3.21	
Anteverted nares	1	0.29	
TOTAL GENITAL	8	2.33	
Hypospadias	2	0.58	11.32
Undescended testicle	3	0.87	
Micropenis	2	0.58	
Lateral penile curvature	1	0.29	
Bifid scrotum	1	0.29	
Shawl scrotum	1	0.29	
Absent pubic bone	1	0.29	
Ambiguous genitalia	1	0.29	
TOTAL LIMB	27	7.87	
TOTAL INTERCALARY LIMB DEFICIENCIES	11	3.21	0.09
Absent radius	2	0.58	
Absent fibula	2	0.58	
Missing metacarpal	1	0.29	
Unspecified intercalary limb shortening	7	2.05	
TOTAL TRANSVERSE LIMB DEFICIENCIES	22	6.41	0.85
Missing middle or proximal phalanges on fingers or toes	2	0.58	
Short proximal phalanges	1	0.29	
Unspecified limb shortening	1	0.29	
Short or missing distal phalanges of fingers or toes	2	0.58	
Nail hypoplasia	9	2.63	
Hypoplastic toes	6	1.75	
Missing digits	5	1.46	
Total longitudinal limb deficiencies	2	0.58	0.53
Absent palmar proximal creases	2	0.58	
Wide gap between first and second toes	1	0.29	
Overlapping toes	1	0.29	
Polydactyly	3	0.87	4.30
Syndactyly	8	2.33	
Clinodactyly	6	1.75	
Bony foot abnormalities	1	0.29	
Antecubital pterygia	1	0.29	
Wrist deviation	2	0.58	
Bradydactyly (brachydactyly)	3	0.87	
Right thumb deformity	1	0.29	

Table 2
(Continued)

Malformations	Methotrexate n	Methotrexate %[a]	General population %[b]
Clubfoot	5	1.46	4.72
Metatarsus varus	1	0.29	
Subluxation of radial head	1	0.29	
Hyperextensible joints	1	0.29	
Decreased extension of elbow	1	0.29	
Absent knee ossification centers	1	0.29	
TOTAL TRUNK	11	3.21	
Scoliosis or kyphosis	4	1.17	
Pectus excavatum	1	0.29	
Skeletal abnormalities	1	0.29	0.51
Short torso	1	0.29	
Short thorax	2	0.58	
Partial or absent ribs	3	0.87	
Absent thymic isthmus	1	0.29	
Hypoplasia of scapulae	1	0.29	
Intestinal malrotation	1	0.29	
Inguinal hernia	1	0.29	
Umbilical hernia	3	0.87	
Diaphragmatic hernia	1	0.29	0.63
Diastasis recti	1	0.29	
Accessory spleens	1	0.29	
Hypoplastic nipples	2	0.58	
Wide-spaced nipples	1	0.29	
TOTAL RENAL	6	1.75	
Multicystic dysplastic kidney	1	0.29	1.14
Hydronephrotic kidney	2	0.58	
Abnormal kidney position	1	0.29	
Chronic renal parenchymal disease	1	0.29	
Left renal pelvis prominence	1	0.29	
TOTAL PULMONARY	3	0.87	
Anterior airway	2	0.58	
''Horseshoe'' lung	1	0.29	
Pulmonary hypoplasia	1	0.29	
OTHER	5	1.46	
Short stature	3	0.87	
Angioma	1	0.29	
Pancytopenia	1	0.29	
TOTAL DEFECTS	343		

[a](n/343) × 100.
[b]Derived from Correa et al. (2007) as (n/23855) × 100 for cardiac malformations and (n/32938) × 100 for other malformations.
Bolded rows indicate statistically significantly higher proportion of defect in methotrexate than control population, $p < 0.05$ by chi-square or Fisher exact test.

used to address the question involve different clinical settings, different methotrexate dose levels, and different times of administration. The description by Jones (2006) includes malformations that have arisen and continue to be reported in many children with methotrexate exposure and includes growth deficiency, microcephaly, hypoplasia of skull bones, wide fontanels, coronal or lambdoidal craniosynostosis, upswept frontal scalp hair, broad nasal bridge, shallow supraorbital ridges, prominent eyes, low-set ears, maxillary hypoplasia, epicanthal folds, short limbs, talipes, hypodactyly, and syndactyly. In their seminal paper, Feldkamp and Carey (1993) suggested that methotrexate embryopathy may be associated with exposure between 6 and 8 weeks after conception to dose levels of 10 mg/week or greater. Although most case reports conform to this formulation, occasional reports appear to present exceptions (e.g., Adam et al., 2003).

Poggi and Ghidini (2011) raised the intriguing possibility that treatment of misdiagnosed ectopic pregnancies produces a different spectrum of malformations. Ectopic pregnancies are commonly treated by 6 weeks after conception, and dose levels are much higher than those traditionally used for rheumatic disorders. We note that in the seven cases of suspected ectopic pregnancy in Table 1 in which malformations were evaluated, five included conotruncal heart defects, four of which were tetralogy of Fallot and one of which was perimembranous ventricular septal defect (Usta et al., 2007; Poggi and Ghidini, 2011; Nurmohamed et al., 2011; Piggott et al., 2011). The two cases without conotruncal heart defects included a case exposed at 6 to 8 weeks after conception in which micrognathia, another neural crest defect, may have occurred (Adam et al., 2003). Neural crest malformations, or at least conotruncal heart defects, may be a feature of pregnancy exposure to high dose levels of methotrexate before 6 postconception weeks. The findings of cleft palate and micrognathia in the rabbit (DeSesso and Jordan, 1977) are consistent with a neural crest mechanism; however, we can only speculate about whether this mechanism would apply to the reported human cases.

Case reports involving the use of methotrexate for abortion or for cancer therapy may be limited by cotreatment with misoprostol for abortion and other antimetabolites for cancer. Some of the malformations in these reports could be due to methotrexate. As a result, we included these malformations in our disproportionality analysis; however, we were unable to determine whether the other exposures contributed to the outcomes.

The experimental animal literature has not been concordant with reported human experience, with the exception of studies performed in the rabbit in which abnormalities are suggestive of those seen in the case reports and case series (DeSesso and Jordan, 1977; DeSesso and Goeringer, 1991, 1992). The dose level used to produce abnormalities in the rabbit (19.2 mg/kg) is considerably higher than dose levels used in human therapy. We are not aware of pharmacokinetic comparisons between rabbits and human beings, which might explain the large difference in apparently effective dose levels. Embryolethality is a common finding in all species. We note that conotruncal heart defects have not been identified in any experimental animal model.

Our disproportionality analysis supported pulmonary atresia, craniosynostosis, and limb deficiencies as associated with methotrexate exposure. This analysis did not distinguish early from later first trimester exposures. The lack of comparability of numbers of malformations in case reports and case series with the numbers reported from an active surveillance program is an important limitation of our analysis, and we welcome the reporting of denominator-based experience with prospectively ascertained pregnancies.

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