Associations between Three Types of Maternal Bacterial Infection and Risk of Leukemia in the Offspring

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A case-control study was nested within two maternity cohorts with a total of 7 million years of follow-up for assessment of the role of bacterial infections in childhood leukemia. Offspring of 550,000 mothers in Finland and Iceland were combined to form a joint cohort that was followed for cancer up to age 15 years during 1975–1997 through national cancer registries. For each index mother-case pair, three or four matched control mother-control pairs were identified from population registers. First-trimester serum samples were retrieved from mothers of 341 acute lymphoblastic leukemia cases and 61 other leukemia cases and from 1,212 control mothers. Sera were tested for antibodies to the genus Chlamydia, Helicobacter pylori, and Mycoplasma pneumoniae. Odds ratios and 95% confidence intervals, adjusted for sibship size, were calculated as estimates of relative risk. M. pneumoniae immunoglobulin M appeared to be associated with increased risk (odds ratio (OR) = 1.6), but the association lost statistical significance when the specificity of the immunoglobulin M was considered (OR = 1.5, 95% confidence interval: 0.9, 2.4). In Iceland, H. pylori immunoglobulin G was associated with increased risk of childhood leukemia (OR = 2.8, 95% confidence interval: 1.1, 6.9). Since H. pylori immunoglobulin G indicates chronic carriage of the microorganism, early colonization of the offspring probably differs between Iceland and Finland, two affluent countries.

antibodies; case-control studies; child; Chlamydia; Helicobacter pylori; leukemia, lymphocytic, acute; Mycoplasma pneumoniae

Abbreviations: ALL, acute lymphoblastic leukemia; CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G; IgM, immunoglobulin M; OR, odds ratio.

Leukemia accounts for 25 percent of childhood cancers worldwide (1). Acute lymphoblastic leukemia (ALL) comprises approximately 80 percent of all childhood leukemias (1, 2). Clustering of ALL cases, the association of increased ALL incidence with population mixing, and the association of increased ALL risk with birth order and sibship size suggest a link between infection and ALL (3–5), especially for cases diagnosed at less than 6 years of age. Recent studies support congenital infection or postnatal infection and microbial antigen stimulation as possible causes of ALL (5–8). However, most studies do not attempt to identify the causal agent.

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Infection with *Mycoplasma pneumoniae* has been suggested to be associated with childhood ALL (8, 9). Two other ubiquitous human bacterial pathogens, *Helicobacter pylori* and the genus *Chlamydia*, have been associated with lymphoproliferative disorders in adults (10–13). *H. pylori* is especially well recognized as an etiologic agent in mucosa-associated lymphoid tissue lymphoma (11). The evidence for associations of *H. pylori* and *Chlamydia* with leukemia is lacking.

To study the role of these three bacterial infections in childhood leukemia, we conducted a case-control study nested within a joint cohort of 550,000 mothers and their offspring. Acute maternal infection was defined by the presence of specific immunoglobulin M (IgM) antibodies to the genus *Chlamydia*, *H. pylori*, and *M. pneumoniae*. The presence of immunoglobulin G (IgG) antibodies to *H. pylori* was interpreted as evidence of persistent infection (14), with an increased predisposition of the offspring.

**MATERIALS AND METHODS**

**Serum banks and cancer registries**

The Finnish Maternity Cohort comprises 1,200,000 serum samples that have been collected from 650,000 pregnant women by the Finnish National Public Health Institute (Helsinki, Finland) since 1983 (6, 15). The samples are taken from practically all (>98 percent) pregnant Finnish women in municipal maternity care units at 12–14 weeks’ gestation to screen for intrauterine infection. The samples are stored at −25°C. In addition, data on reproductive history (numbers of previous pregnancies and deliveries) and place of residence at the time of serum sampling are available in the Finnish Maternity Cohort data files.

The Rubella Screening Serum Bank at the Department of Virology, University of Iceland (Reykjavik, Iceland), comprises 75,000 serum samples collected during 1975–1997 from practically all (>95 percent; i.e., 50,000) pregnant women in Iceland at 12–14 weeks’ gestation. The samples are stored at −20°C. Pertinent data on reproductive history were retrieved from the Icelandic Maternity Registry (6).

The Finnish and Icelandic cancer registries, which are population-based and country-wide, were established in 1952 and 1954, respectively. Various checks have shown that they receive notifications of virtually all histologically confirmed new cases of cancer (16, 17).

**Identification of cases and controls**

For the present study, cases of childhood leukemia among young persons registered in the Finnish and Icelandic cancer registries were classified into two categories: ALL and other leukemia (non-ALL). Stratification into four categories by age at diagnosis (<1, 1, 2–6, and >6 years) was applied to distinguish between infant leukemia cases, cases occurring in the ALL peak period, and other childhood leukemia cases.

Mothers of all children who developed leukemia before 15 years of age were identified through the Finnish and Icelandic national population registries. Final index mothers were those who had serum samples in the Finnish and Icelandic maternity cohorts (402 women altogether). For matching, we applied incidence density sampling; that is, three control mothers in Finland and four control mothers in Iceland with totally cancer-free offspring at the time of childhood leukemia diagnosis were matched with the index mother according to age at serum sampling (±2 years), date of specimen collection (±2 months), and offspring characteristics: date of birth (±2 months) and sex of the child. The matching was performed by country to ensure that differences between the national cohorts did not affect the validity of the study. If three or four control mothers were not found, the matching criteria for age and storage time were expanded stepwise by 1 month until control mothers were found.

The control group comprised 1,212 women altogether. The median and maximum differences in age between the index mothers and the control mothers were 0.3 years and 6.6 years, respectively.

Permissions for linkage between the population, cancer, and maternity cohort data files for identification of index and control pairs and use of the joint cohort data file were obtained from the national data protection authorities, the Finnish Ministry of Health, population registry centers, and the national ethical review boards.

**Laboratory methods**

To identify maternal infection or offspring susceptibility to perinatal infection, the presence of IgM and IgG antibodies to three human bacterial pathogens—*Chlamydia trachomatis*, *H. pylori*, and *M. pneumoniae*—was determined according to manufacturers’ instructions by means of standard enzyme-linked immunosorbent assays (ELISAs) that used the same batches of purified elementary body (*C. trachomatis*), bacterial lysate (*H. pylori*), and P1-adhesin-enriched (*M. pneumoniae*) antigens. For *C. trachomatis*, we used IgG and IgM ELISAs (Thermo Labsystems, Helsinki, Finland) with reported sensitivity and specificity of 100 percent. For *M. pneumoniae*, we used IgG and IgM ELISAs (Thermo Labsystems) with reported sensitivity of 75.7 percent and reported specificity of 98.9 percent. For *H. pylori*, we used an IgG ELISA (Orion Diagnostica, Espoo, Finland) with reported 100 percent sensitivity and 94.3 percent specificity, as well as an IgM ELISA (Immunobiological Laboratories GmbH, Hamburg, Germany).

The cutoff levels were preassigned following the manufacturers’ recommendations relative to internal positive and negative reference sera used on all plates. We further controlled for the specificity of the IgM antibody response by separately considering only IgM-positive mothers who were negative for IgM antibodies to the other two bacteria.

The laboratory analyses were carried out with masked samples, whereafter the data were submitted to the Finnish Cancer Registry for decoding and statistical analysis.

**Statistical analyses**

Relative risks, expressed as matched odds ratios and their 95 percent confidence intervals, were estimated by
RESULTS

We found 378 Finnish cases (203 girls and 175 boys) and 24 Icelandic cases (13 girls and 11 boys) for whom an archival serum sample taken from the index mother during the pregnancy was available. The 402 cases comprised almost all cases of leukemia in children born to Finnish and Icelandic mothers in 1983–1997 and 1975–1997, respectively, and their median ages were 3.1 years and 3.2 years. The median ages of the index mothers at the time of serum sampling were 28.4 years and 27.0 years, respectively. A total of 187 girls and 154 boys had ALL, and 29 girls and 32 boys had other forms of leukemia (non-ALL) (table 1). For both the Finnish ALL cases and the Icelandic ALL cases, the median age was 3.2 years. For the Finnish and Icelandic non-ALL cases, the median ages were 2.0 years and 3.2 years.

Chlamydia seroprevalence, as defined by the presence of IgG antibodies among the controls, was two times higher in Iceland (31 percent) than in Finland (16 percent) (table 1). H. pylori IgG antibodies were equally common in Finland (32 percent) and Iceland (33 percent), but there was a clear predominance of both IgG and IgM antibodies in Icelandic index mothers (58 percent and 8 percent) compared with Finnish index mothers (30 percent and 2 percent). The frequencies of M. pneumoniae IgG and IgM antibodies were high in both countries: 81 percent and 5 percent, respectively, in Finland and 90 percent and 7 percent, respectively, in Iceland (table 1).

In the matched analyses, acute maternal M. pneumoniae infection, as defined by specific IgM positivity, appeared to be associated with an increased risk of childhood leukemia (OR = 1.6; 95 percent confidence interval (CI): 1.0, 2.5; table 2). However, the statistical significance was lost when specific M. pneumoniae IgM positivity, in the absence of C. trachomatis and H. pylori IgM antibodies, was used for the definition of acute infection (OR = 1.5; 95 percent CI: 0.9, 2.4). Adjusting for sibship size also dropped the lower
DISCUSSION

In this study, the presence of maternal *H. pylori* IgG antibodies was associated with increased risk of childhood leukemia in both ALL and non-ALL in the offspring (OR = 2.8, 95 percent CI: 1.1, 6.9; table 2). Moreover, a considerably increased point estimate was observed when the analysis was restricted to those Icelandic cases who were under 6 years of age at diagnosis (OR = 3.7, 95 percent CI: 1.4, 9.9). In Finland, *H. pylori* IgG antibodies were not associated with an increased risk of childhood leukemia (table 2), even if the case-index mother pairs were stratified by the cases’ age at diagnosis or calendar time of serum sampling (data not shown).

Maternal Bacterial Infection and Offspring Leukemia Risk

TABLE 2. Odds ratios for acute lymphoblastic leukemia and other leukemia according to maternal seropositivity for immunoglobulin G and immunoglobulin M antibodies to *Mycoplasma pneumoniae*, *Chlamydia trachomatis*, and *Helicobacter pylori* in a joint cohort study of 550,000 Finnish and Icelandic women and their offspring, 1975–1997

<table>
<thead>
<tr>
<th>Category</th>
<th>Acute lymphoblastic leukemia (n = 341)</th>
<th>Other (non-acute lymphoblastic) leukemia (n = 61)</th>
<th>Total (all leukemia) (n = 402)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgG*</td>
<td>IgM*</td>
<td>OR</td>
</tr>
<tr>
<td><em>Mycoplasma pneumoniae</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iceland</td>
<td>8</td>
<td>NA*</td>
<td>0.0</td>
</tr>
<tr>
<td>Finland</td>
<td>1.2</td>
<td>1.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Both countries</td>
<td>1.2</td>
<td>1.6</td>
<td>0.9</td>
</tr>
<tr>
<td><em>Chlamydia trachomatis</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iceland</td>
<td>2.2</td>
<td>8.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Finland</td>
<td>1.0</td>
<td>0.4</td>
<td>1.0</td>
</tr>
<tr>
<td>Both countries</td>
<td>1.0</td>
<td>0.7</td>
<td>1.0</td>
</tr>
<tr>
<td><em>Helicobacter pylori</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iceland</td>
<td>2.2</td>
<td>0.8</td>
<td>7.6</td>
</tr>
<tr>
<td>Finland</td>
<td>0.9</td>
<td>0.9</td>
<td>0.6</td>
</tr>
<tr>
<td>Both countries</td>
<td>1.0</td>
<td>1.0</td>
<td>0.8</td>
</tr>
</tbody>
</table>

* IgG, immunoglobulin G; IgM, immunoglobulin M; OR, odds ratio; CI, confidence interval; NA, not available (no positive index mothers).

 bound of the 95 percent confidence interval below 1 (OR = 1.6, 95 percent CI: 0.9, 2.6). Moreover, there was no significant interaction between *M. pneumoniae* and birth order per se, and the observed joint odds ratio (OR_{obs} = 1.1) for *M. pneumoniae* IgM positivity (solitary OR = 1.6) and birth order (solitary OR = 1.3) did not differ from that expected on the basis of a multiplicative model (OR_{exp} = 2.0; p = 0.8, likelihood ratio statistic).

In Iceland, maternal IgG antibody positivity to *H. pylori* was associated with a significantly increased total risk of childhood leukemia (both ALL and non-ALL) in the offspring (OR = 2.8, 95 percent CI: 1.1, 6.9; table 2). Moreover, a considerably increased point estimate was observed when the analysis was restricted to those Icelandic cases who were under 6 years of age at diagnosis (OR = 3.7, 95 percent CI: 1.4, 9.9). In Finland, *H. pylori* IgG antibodies were not associated with an increased risk of childhood leukemia (table 2), even if the case-index mother pairs were stratified by the cases’ age at diagnosis or calendar time of serum sampling (data not shown).
Independent confirmatory studies are needed. The possibility of an association between maternal H. pylori antibody findings, including cases and controls with multiple positive IgM titration was considered further using a robust method of exclusion. Generally poor specificity of the IgM antibody determination was considered further using a robust method of excluding cases and controls with multiple positive IgM antibody findings.

To our knowledge, we have documented for the first time the possibility of an association between maternal H. pylori infection and risk of childhood leukemia in the offspring. Independent confirmatory studies are needed.

ACKNOWLEDGMENTS

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REFERENCES


