Cholangiocarcinoma: Epidemiology, Mechanisms of Carcinogenesis and Prevention

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Abstract
Cholangiocarcinoma is a relatively rare cancer; worldwide it accounts for an estimated 15% of liver cancers. In most areas, the etiology is rather obscure, and identified risk factors such as hepatolithiasis, inflammatory bowel disease, and exposure to Thorotrast can account for only a small proportion of cases. In certain areas of southeast and eastern Asia, however, incidence rates are very high, and here there is a strong association with infection with the liver flukes Clonorchis sinensis and Opisthorchis viverrini. The mechanisms of carcinogenesis in O. viverrini infection have been the subject of considerable research; it seems that the presence of parasites induces DNA damage and mutations as a consequence of the formation of carcinogens/free radicals and of cellular proliferation of the intrahepatic bile duct epithelium. Preventive strategies in areas endemic for liver flukes appear straightforward, but breaking the cycle of infection has proved difficult in practice.

Introduction
CCA is one of the major histological types of primary cancer of the liver, which usually occurs rather less frequently than HCC. The tissue of origin is the epithelium of the bile ducts. Strictly speaking, only tumors of the intrahepatic bile ducts are considered to be cholangiocarcinomas (1) although clinically it is often difficult to distinguish between intra- and extrahepatic cancers of the hilar region. Although cholangiocarcinoma is considered a specific morphological entity for classification purposes (with a specific ICD-0 code, 8160), it is histologically identical to adenocarcinoma, and diagnoses such as duct carcinoma and adenocarcinoma are considered to be synonymous (2).

Epidemiology
Frequency
In published studies, the frequency of CCA is almost always reported from clinical series, as a percentage of all liver cancers (on biopsy, or at autopsy). Frequencies reported vary from 5 to 30% of all liver cancers (3). The case-selection inherent in clinical series can be overcome by studying population-based material (which includes all cases of liver cancer arising in a defined population). Table 1 shows relative frequency of intrahepatic carcinoma of glandular epithelium, stated to be primary, in 15 large population-based series for time periods around 1985. The tumors include adenocarcinomas (ICD-0 codes 8140 + 8141) and duct carcinomas (ICD-0 8500) as well as cholangiocarcinomas, and the denominator comprises all primary liver cancer of known histological type. Apart from the very high frequency of cholangiocarcinoma in Khon Kaen, Thailand, the percentage of such cancers lies between 5 and 30% in men and between 15 and 45% in women.

These figures have only limited utility. Apart from any bias which may arise from differential biopsy rates for hepatocellular and cholangiocarcinomas, the frequency of cholangiocarcinoma will vary inversely with that of hepatocellular carcinoma, which is known to vary widely in different parts of the world (4).

Incidence
Figs. 1 and 2 show incidence rates for liver cancer in 26 populations around 1985, from data published in Ref. 5. Based upon the distribution of the different histological subtypes, within age groups (0–14, 15–49, 50–89, 60–89, and 70+ years), estimated incidence rates for the major histological types of liver cancer have been prepared. Hepatocellular carcinoma (ICD-0 8170) is distinguished from intrahepatic bile duct carcinoma, or cholangiocarcinoma (ICD-0 8140 + 8141, 8160 + 8161, and 8500), hepatoblastoma (ICD-0 8970), and other types. Cases with unknown or unspecified histology were reallocated prorata within each age/sex grouping to these four categories, before calculation of rates.

Despite a rather wide range in incidence of liver cancer as a whole, the incidence of cholangiocarcinoma shows rather little variation, with rates in males mainly ranging 0.5–2.0 and a little lower in females. A very high incidence is estimated for Khon Kaen in northeast Thailand (84.6/100000 in men; 36.8/100000 in women), with moderately high rates in Chiang Mai (northern Thailand) and Hong Kong. There is a suggestion that elsewhere in Asia (Japan, Philippines, and Singapore) the incidence is slightly higher than in Europe or the Americas.

The sex ratio for CCA in the series in Table 1 is relatively close to unity (range, 0.7–1.6). This is much lower than for
Cholangiocarcinoma

Table 1. Cholangiocarcinoma as a percentage of all liver cancer

<table>
<thead>
<tr>
<th>Cancer registry</th>
<th>Total No. (liver cancer)</th>
<th>% of cholangiocarcinoma Male</th>
<th>% of cholangiocarcinoma Female</th>
<th>Ratio: FT (Female/FT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thailand, Khon Kaen</td>
<td>1235</td>
<td>87</td>
<td>92</td>
<td>1.8</td>
</tr>
<tr>
<td>Thailand, Chiang Mai</td>
<td>633</td>
<td>29</td>
<td>43</td>
<td>1.2</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>6765</td>
<td>11</td>
<td>32</td>
<td>1.6</td>
</tr>
<tr>
<td>Japan, Osaka</td>
<td>12,260</td>
<td>6</td>
<td>18</td>
<td>1.4</td>
</tr>
<tr>
<td>Philippines, Manila, and Rizal</td>
<td>2,610</td>
<td>5</td>
<td>22</td>
<td>0.7</td>
</tr>
<tr>
<td>Singapore: Chinese</td>
<td>1,295</td>
<td>5</td>
<td>19</td>
<td>0.6</td>
</tr>
<tr>
<td>United States (SEER)</td>
<td>21,36</td>
<td>19</td>
<td>30</td>
<td>1.1</td>
</tr>
<tr>
<td>white</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canada</td>
<td>2,749</td>
<td>16</td>
<td>30</td>
<td>1.1</td>
</tr>
<tr>
<td>Slovakia</td>
<td>585</td>
<td>26</td>
<td>40</td>
<td>1.3</td>
</tr>
<tr>
<td>Denmark</td>
<td>1,317</td>
<td>30</td>
<td>50</td>
<td>1.6</td>
</tr>
<tr>
<td>Italy (six registries</td>
<td>1,290</td>
<td>7</td>
<td>20</td>
<td>0.9</td>
</tr>
<tr>
<td>Spain (six registries)</td>
<td>1,049</td>
<td>9</td>
<td>23</td>
<td>0.8</td>
</tr>
<tr>
<td>United Kingdom, Scotland</td>
<td>847</td>
<td>24</td>
<td>39</td>
<td>1.1</td>
</tr>
<tr>
<td>Australia (4 states)</td>
<td>963</td>
<td>9</td>
<td>19</td>
<td>1.1</td>
</tr>
<tr>
<td>Puerto Rico</td>
<td>449</td>
<td>13</td>
<td>22</td>
<td>1.6</td>
</tr>
</tbody>
</table>

*Excluding cases of unknown or ill-defined histology. Calculated from data published in Ref. 5.
* Registries of the Surveillance Epidemiology and End Results Programme.
* Florence: Genoa; Lombardy Region, Varese Province; Parma Province; Romagna; Trieste.
* Basque Country; Catalonia, Tarragona; Granada; Murcia; Navarra; Zaragoza.
* New South Wales, South Australia, Tasmania, Victoria.

This is probably related to the different baseline (non-Thorotrast-associated) risk of hepatocellular carcinoma in different populations and to the fact that cases of CCA seem to occur rather earlier after Thorotrast than do cases of angiosarcoma or HCC (11).

In a cohort study of 241 males exposed to Thorotrast around 1945 (12), follow-up between 1980 and 1985 revealed 51 deaths from liver cancer, a relative risk, compared with the general Japanese population, of 46.9. Based on autopsy findings of 30 cases and the results of autopsies in Japan (1980–1984), relative risks for the different histological types vary considerably between the different series.

Etiological Factors

There have been relatively few epidemiological studies of cholangiocarcinoma. Most of the information on factors which are likely to be important derives from clinical series, in which association with other diseases or exposures appears to be, a priori, higher than expected or higher than observed in the cases of HCC in the same clinical series.

Congenital Cystic and Dysplastic Lesions. These are associated with a proportion of cases (2).

Intrahepatic Calculi. Carcinoma of the gall bladder is well known to be a complication of cholelithiasis. By analogy, the presence of intrahepatic stones (hepatolithiasis) is observed in a fairly impressive proportion of CCA cases, from 5.7 to 17.5% of cases in four Japanese series (7). The bile duct epithelium in hepatolithiasis shows chronic proliferative cholangitis and epithelial hyperplasia (8).

Inflammatory Bowel Disease. Carcinoma of the bile duct has been observed as a complication of ulcerative colitis, although it is much less frequent than cancers of the large bowel itself. Ritchie et al. (9) report the frequency of occurrence as 1 in 246 ulcerative colitis patients, with one-fifth of cases occurring in the intra-hepatic bile ducts. In a series of cases from the Mayo Clinic, the average age of diagnosis of bile duct cancer in ulcerative colitis patients was 38 years, after a mean follow-up period after ulcerative colitis diagnosis of 19 years (10). Using these data, and the age-specific incidence rates of intrahepatic bile duct cancer in the United States estimated as described in "Incidence", the expected frequency of cancers between ages 20 and 39 years is 1 in 95,000 (compared to about 1 in 1,250 observed), a relative risk of about 75 in ulcerative colitis patients compared with the general United States population.

Thorotrast. Thorotrast (a colloidal preparation of thorium dioxide) is a radioactive α-particle emitter widely used as a radio-opaque contrast medium between 1930 and 1955. It was administered by many different routes. When given i.v., 70% of the dose is taken up in the liver. Liver tumors develop some 10–12 years later, but the proportions of different histological types vary considerably between the different series.

Parasites. The association between the occurrence of liver cancer and the presence of liver flukes has been known for almost a century. Hou (15) in Hong Kong was the first to point out that, although 15% of liver cancers were associated with flukes (in this case, Clonorchis sinensis), the cancers concerned were mainly adenocarcinomas. Gibson (16), reporting on an autopsy series (1,484 subjects) from Hong Kong, found that while 29% of 89 HCC cases had clonorchiasis (35.4% expected, based on age-sex-specific prevalence of the whole series), 65% of 17 CCA cases (37.6% expected) were infected. Belsmar (17), in the same population, identified flukes in 18 of 19 autopsies of patients with cholangiocarcinoma, but only one-third of "control" patients showed cellular change suggestive of clonorchiasis. In animals infected with Clonorchis, intrahepatic bile duct cancers were observed (18).

C. sinensis was originally endemic in Korea, Japan, China, and Vietnam. However, it is much less prevalent than it was, and cholangiocarcinoma from this cause appears to be relatively infrequent in recent years.

This is not the case for OV, the fluke found in northeast Thailand. Recent population surveys suggest a continuing...
high prevalence of infection. The evidence for the role of OV in induction of CCA is compelling. The first reports were essentially case series, drawing attention to the coincidence of two diseases, normally rare, in the same geographic area and in the same individuals. Thus, cholangiocarcinoma was observed to comprise a high percentage of biopsied livers cancers in northeast Thailand (19, 20) and prevalence of OV infection is higher in northeast Thailand than elsewhere (21, 22).

More formal correlations have been performed. The incidence of cholangiocarcinoma in the five regions of Thailand varies at least 1 5-fold and correlates strongly with prevalence of OV infection, as measured by anti-OV antibody titer in the general population (23). HCC shows no such relationship. The association with fecal egg count was less strong, presumably because this may be more affected by recent therapy and a poorer indicator of duration/intensity of infection. A similar association between the intensity of OV infection and the risk of CCA has been observed (27). One hundred three cases that were inhabitants of northeast Thailand were compared with a similar number of age- and sex-matched controls. Infection with OV (past or present) was estimated in terms of an increase in titer of anti-OV antibodies in serum, since this is known to correlate with intensity of infection (28, 29) while the count of OV eggs in feces may be low or zero in cases of CCA with biliary obstruction. A relative risk of 5.0 for an antibody titer greater than 1 40 was found; this implies that at least two-thirds of CCA cases were attributable to OV. These estimates are conservative, since the antibody test used a crude extract of parasite as antigen and is known to be rather nonspecific (30) with some consequent misclassification of exposure status. The study suggested that the risk associated with OV infection was higher in males than in females, a finding consistent with the higher incidence of CCA in men in OV-endemic areas in the face of a similar prevalence of infection in the two sexes, but if it is true, the responsible mechanism is obscure.

Other Factors. The case-control study in Thailand (27) found no association with chronic carriage of hepatitis B, nor with recent aflatoxin intake. No dietary constituents increased or decreased risk and there was no association with tobacco use; there was, however, a strong association with the regular use of betel nut (odds ratio, 6.4).

In a case-control study of primary liver cancer in Sweden (31), 15 cases of cholangiocarcinoma were included; no significant association was found with exposure to organic solvents or with alcohol intake (the odds ratio for one-half of a bottle of spirits per week was 3.3 (not significant) compared with teetotallers).

A case-control study of liver cancer in women aged 20–44 years in England (32) which included 11 cases of CCA
LIVER CANCER: Hepatocellular & Cholangiocarcinoma

Fig. 2. Age-standardized incidence of liver cancer, per 100,000, in females 15). Rates for cholangiocarcinoma and hepatocellular carcinoma are estimates (see text). Poland: registries of Cracow and Nowy Sacz. Spain: registries of Basque Country, Tarragona, Granada, Murcia, Navarra, and Zaragoza. Australia: registries of New South Wales, South Australia, Tasmania, and Victoria.

found no association with past use of oral contraceptives, as recorded in general practitioner notes. A similar study in the United States included 22 deaths from cancer of the intrahepatic bile ducts in women aged 25–49 years; no excess risk of past oral contraceptive use, ascertained from questionnaires to relatives, was found (33).

Mechanisms of Liver Fluke-induced Carcinogenesis

Human Studies. Srianuajita et al. (34) observed that humans infested with liver fluke had higher urinary excretion of nitrate and N-nitrosopropline than the uninfected. A study in five different areas of Thailand (35) found excretion of nitrate and nitrosamine, and endogenous nitration potential did not correlate with the incidence of CCA. However, within the two high-risk areas of northeast Thailand, subjects who were positive for OV (by antibody level) showed considerably enhanced endogenous nitration, and this was reduced to the same level as control (noninfected) subjects by administration of vitamin C, which inhibits this process.

These results suggest that, in common with other situations where cancer is a sequel of long-term infectious processes, there is a role of nitrosation/nitrosamines consequent upon fluke infestation in the etiology of CCA.

The possibility that immunological reactions are involved in pathogenesis has been postulated, based on a correlation between OV-specific IgG antibody and ultrasound-diagnosed changes in the biliary tract in an endemic area (36). It seems equally likely that this association is indirect; the antibody levels simply reflect duration/intensity or some other relevant parameter of OV infection.

There is currently a great interest in the relationship between specific mutation spectra in protooncogenes (such as ras) and tumor suppressor genes (such as p53) and exposures to different etiological factors (37). For example, hepatocellular carcinomas in subjects probably exposed to dietary aflatoxin B1 contain frequent G to T transversions occurring at codon 249 of p53 gene (38, 39). This type of mutation, however, is seldom observed in the tumors of subjects from areas in which aflatoxin B1 exposure is low. Recently, Tada et al. (40) observed that 9 of 18 intrahepatic cholangiocarcinomas from Japan had point mutations at codon 12 (2 GGT → GAT; 1 GGT → GAT; 1 GGT → GTT), but none of the 12 tumors from northeast Thailand contained these mutations (41). It seems equally likely that this association is indirect;
associated carcinogenesis, although other genetic alterations resulting from infection and mechanical irritation by the flukes could be important. It would be interesting to compare mutation spectra in the p53 tumor suppressor gene in cholangiocarcinomas from O. viverrini-endemic and non-endemic areas.

**Animal Studies.** Studies on experimental OV infestations in Syrian golden hamsters have demonstrated that liver fluke infestation alone rarely induces cholangiocarcinoma, but if infested hamsters are treated with hepatocarcinogens such as N-nitrosodimethylamine (NDMA) and N-nitroso-di-iso-propanolamine, they can develop bile duct tumors resembling those seen in humans (42, 43). The effect of OV infestation and NDMA dose on the development of cholangiocarcinoma was synergistic (44). These results suggest that the development of cholangiocarcinoma in the OV-infested host is a multifactorial process.

There are several possible mechanisms underlying the enhancement of neoplasia by opisthorchiasis to be considered. First, the presence of the parasite in the bile duct mechanically damages tissues, resulting in increased cell proliferation, which converts and fixes DNA-carcinogen adducts to mutations, and also results in an elevated "spontaneous" mutation at a later modulation stage of carcinogenesis (45, 46). Repeated treatment of the infested hamsters with Praziquantel at levels sufficient for removal of parasite infestation resulted in reduction in chronic proliferative cholangitis, considered to be a precancerous stage of cholangiocarcinoma (47).

Second, reactive oxygen species released by activated macrophages and neutrophils induce DNA damage and subsequent mutations leading to cancer (48). A third possibility is that presence of parasites may stimulate release by macrophages and other cell types of cytokines such as interleukin 1 and tumor necrosis factor, which may be involved in tumor enhancement by stimulating angiogenes (49).

A fourth possibility is involvement of NO synthase. Our recent studies show that NO synthase is induced in macrophages, eosinophils, and mast cells which are present in
Fig. 4. Age-specific incidence rates of liver cancer: Osaka (5). Median ages of hepatocellular carcinoma were 60.4 (male) and 67.9 (female) years. Median ages of patients with cholangiocarcinoma were 65.2 (males) and 71.8 (female) years.

In summary, it seems that the presence of parasites could induce DNA damage and mutations as a consequence of the formation of carcinogens/free radicals and of cell proliferation in the intrahepatic bile ducts, which may play a crucial role in the development of cholangiocarcinoma.

**Prevention**

Early detection of liver cancer, essentially hepatocellular carcinoma, has been attempted in an effort to improve prognosis and reduce mortality, relying upon testing for α-fetoprotein or ultrasound examination of high-risk groups (54, 55). The prognosis of cholangiocarcinoma is equally poor (56) and the observation of better survival for small (less than 4 cm) lesions has induced similar attempts at early diagnosis. One possibility is the use of tumor markers such as CA19-9 and CEA to identify early cancers. Used in combination, they have high sensitivity for detecting clinical cases (97.5% according to Sripa et al. (57)), although experience with gallbladder cancer (58) suggests that this could only be achieved with such a low specificity that it would be useless for the purpose of screening. The feasibility of using anti-OV IgG antibodies to identify high-risk individuals who could be

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2. G. Kirby, manuscript in preparation.
followed up by ultrasound is also under study (59). As for screening will yield benefits commensurate with the costs involved, and a more rational approach to control is through primary prevention.

Primary prevention of cholangiocarcinoma in the high-risk areas where the tumors are associated with liver fluke infestation is apparently straightforward; control of the parasite should result in a fall in incidence of cholangiocarcinoma to that observed in nonendemic areas. Effective treatment for opisthorchiasis is available by use of the drug Praziquantel which, administered in a single dose, can successfully eliminate parasites from infested individuals (60, 61). Unfortunately, studies in Thailand have shown that reinfection occurs rapidly after successful treatment, particularly in individuals with high pretreatment intensities of infection (62). This is presumably related to persistence of the parasite in the environment (encysted in the fish intermediate hosts) and the lifestyle habits associated with infection (eating these raw fish). Successful control will require repeated treatment, concentrating upon individuals at highest risk, coupled with attempts to change traditional dietary patterns, although the latter have proved rather refractory to educational programs in the past. It is possible that, if endogenous nitrosation is truly important in the etiological process, administration of vitamin C may be effective in reducing the risk of cholangiocarcinoma in infected subjects.

It is unlikely that the effectiveness of programs to reduce parasitic infection in reducing incidence of cholangiocarcinoma will ever be demonstrable through a controlled trial. Leaving aside the ethical difficulties of maintaining an untreated control group, a trial to demonstrate an effect on cancer incidence would need to be very long and, in the face of noncompliance and reinfection, extremely large (63). An opisthorchiasis control program has been introduced by the Health Ministry in the 17 provinces of northeast Thailand, and it will be important to establish careful monitoring of the prevalence of infection via population surveys, and the incidence of cholangiocarcinoma via cancer registration.

North and northeast Thailand and Laos constitute areas of particularly high risk for cholangiocarcinoma, and it can be estimated from the incidence rates of liver cancer and proportion of cases which are cholangiocarcinomas (64) that currently some 9,000–10,000 new cases occur there each year. There are, however, some 315,000 new cases of liver cancer in the world each year (65). Using the appropriate proportions of cholangiocarcinomas from Table 1 (7.5% in men and 20% in women in high risk areas of Africa and Asia, and 20% of cases in men and 33% in women elsewhere), about 15% of liver cancer (46,000 cases a year) can be estimated to be cholangiocarcinoma. At least 80% of this world total is unrelated to Opisthorchis, therefore, and, based on current knowledge of the epidemiology, there are no clues to appropriate preventive measures.

References

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