Biomarkers for the Diagnosis of Cholangiocarcinoma: A Systematic Review

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Abstract. Cholangiocarcinoma (CCA), a malignant tumor of the bile duct, is a major public health problem in many Southeast Asian countries, particularly Thailand. The slow progression makes it difficult for early diagnosis and most patients are detected in advanced stages. This study aimed to review all relevant articles related to the biomarkers for the diagnosis of CCA and point out potential biomarkers. A thorough search was performed in PubMed and ScienceDirect for CCA biomarker articles. Required data were extracted. A total of 46 articles that fulfilled the inclusion and had none of the exclusion criteria were included in the analysis (17, 22, 3, 4, and 1 articles on blood, tissue, bile, both blood and tissue, and urine biomarkers, respectively). Carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic antigen (CEA), either alone or in combination with other biomarkers, are the most commonly studied biomarkers in the serum. Their sensitivity and specificity ranged from 47.2% to 98.2% and 89.7% to 100%, respectively. However, in the tissue, gene methylations and DNA-related markers were the most studied CCA biomarkers. Their sensitivity and specificity ranged from 58% to 87% and 98% to 100%, respectively. Some articles investigated biomarkers both in blood and tissues, particularly CA19-9 and CEA, with sensitivity and specificity ranging from 33% to 100% and 50% to 97.7%, respectively. Although quite a number of biomarkers with a potential role in the early detection of CCA have been established, it is difficult to single out any particular marker that could be used in the routine clinical settings.

INTRODUCTION

Rationale. Cholangiocarcinoma (CCA) is a highly malignant tumor found in the epithelial cells lining the bile duct.1–15 This type of cancer imposes a major public health concern in Southeast Asian countries, particularly Thailand.4 The highest incidence is found in the Khon kaen province, Northeastern Thailand, where the age-standardized annual incidence rates are 36.3 and 87.7 per 100,000 population in females and males, respectively.5 Infection with the liver fluke, Opisthorchis viverrini, which is present in Koi pla (a raw freshwater cyprioid fish meal of the people in the region) together with the consumption of nitrosoamine are considered the main risk factors that lead to the cancer development.6–9

The worldwide incidence of CCA has increased to 18% of all liver cancers during the past 30–40 years.10 As per the United States Vital Statistics databases, in the United States alone, the incidence of CCA increased markedly between 1973 and 1997 with an estimated annual percent change of 9.11% (95% confidence interval [CI]: 7.46–10.78).11 The trend in incidence of the two types of CCA, that is, intrahepatic CCA (ICC) and extrahepatic CCA (EHC) may differ. For instance, between 1990 and 2008, ICC increased from 0.43 to 1.84 per 100,000 population/year in males and from 0.27 to 1.51 in females in England and Wales, whereas EHC in males declined from 0.78 to 0.51 and from 0.62 to 0.39 in females;12 however, the overall incidence of CCA is still on the rise throughout the world.10 The prognosis of this malignancy is poor because of its silent clinical characteristics and limited therapeutic measures.

At present, the treatments available for CCA are surgical resection, liver transplantation, and adjuvant therapy (radiation, chemotherapy). Surgical resection is currently the only intervention with the possibility of cure. However, all the patients diagnosed with CCA cannot undergo surgery. They require full evaluation for resectability. Evaluations should be based on physiological suitability, tumor location, hepatic lobar atrophy, hepatic ductal extension, and many other factors.13 Another invasive method is liver transplantation. Despite high 5-year survival rates, liver transplantation has received contradictory views from many authors mainly because of high recurrence rates.14 It has been suggested that liver transplantation should be reserved only for early stage CCA.15 Chemotherapy is one of the adjuvant therapies to surgery. It has been reported to provide inconsistent results in improving survival, both in resected and unresected patients. Previously, fluorouracil either alone or in combination with methotrexate, leucovorin, cisplatin, or mitomycin were the most studied. However, survival rates were not as good as surgery.16 Use of gemcitabine-based combination regimens showed increased responses of about 22–50%.17 Studies using gemcitabine in combination with cisplatin showed increased progression free survival and overall survival.18 Radiation is another adjuvant therapy that is recommended post-surgery. It is most commonly used as combination of external beam irradiation and brachytherapy with Iridium-192. Retrospective studies have demonstrated that radiation significantly increased 5-year survival rates.19,20

However, radiotherapy and chemotherapy provide inconsistent cure rates, and surgical resection at early stage is the only curative treatment at present.21 Nevertheless, the disease has a slow progression which makes it difficult for early diagnosis and hence most patients are detected only in advanced or metastatic stages.22

One possible solution for the poor prognosis and delayed treatment would be to establish reliable biomarkers that are specific to the early stages of the disease. If such specific biomarkers are recognized and standardized, then the disease can be detected at an early stage and necessary treatments can be implemented. Several studies have been conducted to explain the pathogenesis of CCA and also to discover and establish the biomarkers that can be used in

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diagnosing CCA. A number of genes have been reported to be overexpressed, whereas some were under-expressed in biological samples obtained from CCA patients. Some of the cell surface proteins such as selectins, integrins (ITGA6 and ITGAV), and cadherins (E-Cadherin) have also been widely investigated in the pathophysiology of several cancers. The protein sets identified provide a rich resource of potential circulating markers and targets for imaging and therapeutics for cancers because they play a pivotal role in cancer invasion and metastasis.

The protein sets identified provide a rich resource of potential circulating markers and targets for imaging and therapeutics for cancers. TFN-α, one of the most important pro-inflammatory cytokines, has been shown to be increased in chronic infections and reinfections of O. viverrini. It is reported to promote the invasiveness of CCA via the TFN-α receptor 2.

Serum biomarkers such as carbohydrate antigen 19-9 (CA19-9) and cancer antigen 125 (CA125) are being applied in most routine laboratory tests for CCA diagnosis. Nevertheless, their wide ranges of sensitivity (50–90%) and specificity (54–98%) have been reported. Overexpression of oxysterol binding protein-like 8 (OSBPL-8) was reported in O. viverrini and N-nitrosodimethylamine-induced CCA hamster model.

There have also been extensive studies on the differential expression of proteins in CCA cells compared with normal cells. This included studies of secreted proteins that are directly accessible in the biological fluids of CCA patients. Another type of molecule that is directly accessible in biological fluids are the autoantibodies (AAbs). Their presence in the serum samples from patients of various malignancies has been reported. Because circulating AAbs are produced in large quantities against even small amounts of antigens, they could be of significant diagnostic values. However, so far, there has been limited information on the application of AAbs as biomarkers especially with regard to primary liver tumors such as CCA. Despite the fact that there are quite a large number of studies both in vitro and ex vivo with attempts to establish biomarkers that can be used for the diagnosis of CCA and/or differentiation of CCA from other resembling tumors, the results from these studies cannot be applied to clinical practice. This is mainly because of their insufficient sensitivity and/or specificity, or inconsistency of results in subsequent studies. Considering all the lapses, relevant articles on the investigation of the biomarkers for the diagnosis of CCA were reviewed and analyzed in the present study to point out the potential biomarkers that can be applied in clinical settings. Information on the sensitivity and specificity of the proposed biomarkers were included in the analysis because the diagnosis accuracy is determined by these two parameters.

METHODS

A literature search was performed on PubMed and ScienceDirect on March 9, 2017. The search terms used were “cholangiocarcinoma,” “bile duct cancer,” “Klatskin tumor,” “diagnosis,” and “markers.” No other search conditions were applied. All articles obtained from the two databases were checked for duplication. The remaining articles were initially screened as per the inclusion criteria based on the content of the abstract section. The criteria for inclusion of the articles in this systematic review were as follows: 1) studies of which CCA are the main subject, 2) studies on biomarkers related to CCA diagnosis, 3) prospective or retrospective studies, 4) in vitro, in vivo in animal models, or clinical studies, and 5) full published research articles. The exclusion criteria included at least one of the following criteria: 1) articles in languages other than English, 2) articles on other cancers with inclusion of CCA, 3) case reports, 4) review articles, or 5) unavailability of information on sensitivity and specificity.

Data extraction from all articles was performed by two independent researchers. When conflicting opinions arose, the decision was sought from a higher professional level personnel and the decision was considered final. The information extracted included the following: name of first author, year of publication, study type, study objective, sample size, sample characteristics, type of biomarkers measured, and results. For the evaluation of performance of the diagnostic biomarkers, the sensitivity and specificity and/or receiver operating characteristic (ROC) values (if available) were also extracted.

RESULTS

Study selection. A total of 652 articles related to CCA diagnosis published up to March 9 were retrieved from PubMed and ScienceDirect, of which five were duplicates. Through initial screening of the abstract, 561 articles were excluded from the analysis as they did not fulfill the inclusion criteria. The remaining 86 articles were downloaded for full text assessment. Forty-four articles were excluded from the analysis; 14 articles did not study CCA as the main subject, nine articles did not provide clear results and interpretations, and 21 articles did not provide information on the test performance (sensitivity and specificity). Finally, a total of 41 articles fulfilled all the inclusion criteria and had none of the exclusion criteria for the review (Figure 1). We also did a manual search of relevant articles in the references of the included articles but did not get any additional relevant ones.

Study characteristics. The characteristics of the articles included in the systematic review are summarized in Table 1 with detailed information in Supplemental Table 1. Of the 46 articles, 17, 22, 3, 4, and 1 articles, respectively, were related to the investigation of biomarkers in the blood (serum or plasma), tissue, bile, both blood and tissue, and urine samples.

Biomarkers in the blood. A number of potential blood biomarkers for diagnosis and differentiation of CCA from other similar types of tumors were reported. These included carbohydrate antigen (CA), carcinoembryonic antigen (CEA), micro-RNA (miRNA), exostosin1 (EXT1), cathepsin B to cystatin C ratio, heat shock protein (HSP70) related, and angiopoietin-2 (Angpt-2).

Carbohydrate antigen and CEA were two of the most commonly studied biomarkers in the serum. Carbohydrate antigen, particularly CA19-9, is an antigen found in the normal epithelial cells of many organs including biliary ducts. Elevated levels of CA19-9 are found to correlate with not only the tumor mass but also with the degree of tumor differentiation. Carcinoembryonic antigen is a glycoprotein produced by the gastrointestinal tract. Carcinoembryonic antigen level has been shown to correlate with tumor metastasis. Several studies investigated the application of the two biomarkers, either alone or a combination of the two, and even in combination with other relevant biomarkers for the
diagnosis of CCA or differentiation of CCA from other similar tumors. Carbohydrate antigen-S27, studied as a diagnostic marker, was found to be significantly higher in CCA cases compared with controls \((P < 0.001)\).\(^7\) In a study that investigated the accuracy of the combination of CA19-9 and CEA in the diagnosis of CCA in patients with primary sclerosing cholangitis (PSC), median levels of the biomarkers were found to be higher in the histologically proven CCA patients than that in other patients.\(^6\) In terms of sensitivity and specificity, however, CA and CEA provided wide ranges of sensitivity (47.2–98.2%) and specificity (89.7–100%).

Micro-RNA is a small noncoding RNA that plays an important role in regulating protein expression.\(^8\) It has been shown to be involved in the development and metastasis of many kinds of human cancers.\(^9\)–\(^9\) Because of its presence in the early stages of many cancers, it has often been used as diagnostic indicators of those cancers.\(^9\)–\(^9\) A study that tried to characterize miRNA in the serum of ICC patients found out that miRNAs (miR-21, miR-34c, miR-200b, and miR-221) showed a 2.18–3.79 fold increase in the tumor cells compared with non-tumor cells.\(^4\) In another study that evaluated the diagnostic utility of miRNA in differentiating PSC and CCA, upregulation of miR-222 and miR-483-Sp was demonstrated in CCA compared with PSC \((P < 0.0011\) and \(P = 0.0013)\).\(^5\) Yet, in another study that determined the diagnostic value of miR-26a in CCA, it was found to be highly expressed in CCA patients compared with healthy controls \((P < 0.01)\). The sensitivity and specificity were 84.8% and 81.8%, respectively.\(^8\)

Exostosin1 is one of the five genes encoding the exostosin family, and its mutations are believed to be responsible for inherited heredity disorders.\(^9\) The plasma level of EXT1 was shown to be significantly higher in the CCA patients than healthy subjects \((P < 0.05)\). Its sensitivity and specificity were 91.7% and 50%, respectively.\(^5\)

Cathepsin B is a lysosomal cysteine protease that is detected in many cancers.\(^9\) It is regulated by cysteine protease inhibitors, namely cystatin C and steﬁn A.\(^9\) Measurement of cathepsin B to cystatin C ratio has been suggested to be a beneficial tool for diagnosis of cancers because an imbalance between the proteinases and their inhibitors is correlated with tumor progressions.\(^9\) The ratio was reported to be significantly higher in patients with CCA than healthy subjects \((P = 0.005)\). Its sensitivity and specificity were 63% and 100%, respectively.\(^6\)

HSP70 is a stress response protein,\(^1\) and its overproduction can lead to increased resistance of the cancer cells against apoptosis-inducing agents such as tumor necrosis factor-α.\(^1\) The highest titers of plasma antibodies against HSP70 were found in CCA patients compared with the cholangitis group \((P < 0.05)\) and the healthy controls \((P < 0.001)\). Sensitivity and specificity were 70.97% and 82.61%, respectively.\(^6\)

Angpt-2 is an antagonistic ligand to the tyrosine kinase Tie2 of the Angpt/Tie2 system.\(^1\) Because Angpt-2 is strongly expressed in the vasculature of many tumors, it is hypothesized to promote tumor progressions along with other cytokines.\(^1\) Evaluation of serum Angpt-2 as potential biomarkers for diagnosis of CCA provided a satisfactory result. Median Angpt-2 levels were found to be highest in CCA patients compared with PSC and patients with bile duct stones \((P = 0.01)\). Sensitivity and specificity were 74% and 94%, respectively.\(^7\)
### Table 1

Summary of sensitivity and specificity of biomarkers for the diagnosis of cholangiocarcinoma (CCA) based on the research articles included in the study

<table>
<thead>
<tr>
<th>Biomarkers in the blood</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>ROC</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-21</td>
<td>–</td>
<td>–</td>
<td>0.94</td>
<td>47</td>
</tr>
<tr>
<td>miR</td>
<td>84.8</td>
<td>81.8</td>
<td>0.723</td>
<td>92</td>
</tr>
<tr>
<td>miR (miR-1281, miR-126, miR-26a, miR-30b, and miR-122)</td>
<td>32–68</td>
<td>88–93</td>
<td>–</td>
<td>84</td>
</tr>
<tr>
<td>miR200c</td>
<td>–</td>
<td>–</td>
<td>0.74</td>
<td>91</td>
</tr>
<tr>
<td>miR-483-5p</td>
<td>–</td>
<td>–</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>miR-194</td>
<td>–</td>
<td>–</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>EXT1</td>
<td>91.7</td>
<td>50</td>
<td>0.74</td>
<td>50</td>
</tr>
<tr>
<td>Tu M2-PK</td>
<td>84.2</td>
<td>90</td>
<td>0.924</td>
<td>53</td>
</tr>
<tr>
<td>CA19-9</td>
<td>68.4</td>
<td>75</td>
<td>0.693</td>
<td>54</td>
</tr>
<tr>
<td>Serum AFP, CA19.9, CA125, and CEA</td>
<td>90</td>
<td>90</td>
<td>0.94</td>
<td>55</td>
</tr>
<tr>
<td>CEA, CA19-9, CYFRA, and MMP7</td>
<td>92</td>
<td>95.8</td>
<td>0.64</td>
<td>56</td>
</tr>
<tr>
<td>Serum cathepsin B to cystatin C ratio</td>
<td>63</td>
<td>100</td>
<td>≥ 0.06</td>
<td>60</td>
</tr>
<tr>
<td>CCA-CA and ALP combined</td>
<td>92.36</td>
<td>91.67</td>
<td>–</td>
<td>66</td>
</tr>
<tr>
<td>CEA</td>
<td>53.3</td>
<td>86.3</td>
<td>–</td>
<td>67</td>
</tr>
<tr>
<td>CA19-9*</td>
<td>60</td>
<td>91</td>
<td>–</td>
<td>68</td>
</tr>
<tr>
<td>Plasma autoantibodies against HSP70, ENO1, and RNH1</td>
<td>70.97</td>
<td>82.61</td>
<td>–</td>
<td>69</td>
</tr>
<tr>
<td>Serum CA-S27</td>
<td>87.5</td>
<td>58.8</td>
<td>0.622</td>
<td>72</td>
</tr>
<tr>
<td>Combined AFP-, CA242-, and CA19-9+</td>
<td>47.2</td>
<td>89.7</td>
<td>–</td>
<td>75</td>
</tr>
<tr>
<td>A1BG/AFM ratio</td>
<td>84</td>
<td>87.5</td>
<td>0.886</td>
<td>76</td>
</tr>
<tr>
<td>Serum Angpt-2</td>
<td>74</td>
<td>94</td>
<td>0.85</td>
<td>77</td>
</tr>
</tbody>
</table>

Biomarkers in tissues/bile

| miR                     | 67              | 96              | –   | 107        |
| miR                    | 95              | 100             | 0.995| 108        |
| miR(miR-412, miR-640, miR-1537, and miR-3188) | 50–67 | 89–92 | – | 84 |
| CD01, SFRP1             | 87              | 100             | 0.924| 39         |
| ZSCAN18 and DCLK1       | 85              | 98              | 0.944| 40         |
| STIP9 and VIM           | 8/85.3/36       | 100/44.2/80.2   | 0.496/0.69/0.611| 41 |
| SerpinH1                | 64/93.3/68      | 93.9/90.9/94.8  | 0.819/0.945/0.891| 44 |
| 14–3–3Sigma†           | 71.1/86.8/71.1  | 84.8/97.5/93.1  | 0.75/0.967/0.869| 44 |
| AGR3                   | –               | –               | 0.801| 43         |
| HAAH and HoxB7          | 73              | 80              | –   | 46         |
| Human pancreatic cancer 2 (HPC2) (a mAb) | 80 | 68 | – | 48 |
| N-cadherin              | 58              | 73              | –   |            |
| p53                    | 47              | 90              | –   | 49         |
| Ki-67                   | 42              | 80              | –   |            |
| CEA                    | 63              | 73              | –   |            |
| CA19-9                 | 16              | 82              | –   |            |
| CAM5.2                 | 16              | 100             | –   |            |
| ICL                    | 42              | 98              | –   |            |
| BMP3                   | 58              | 100             | 0.83| 51         |
| CLDN4, HOXB7, TMSB4, and TTR | 90 | 100 | 0.98 | 52 |
| WFA                    | 87.4            | 92.1            | 0.93| 57         |
| L1CAM                  | 66              | 93              | 0.82| 58         |
| Bile M2-PK             | 50              | 100             | 0.73| 61         |
| IGFBP3, HOXB7, and NEK2 | 77.8 | 87.2 | – | 62 |
| BGN, IGFBP3, and CLDN4 | –               | –               | 0.987| 63 |
| Claudin-4              | 96.6            | 92.3            | 0.962| 64         |
| Claudin-7 genes         | 98.2            | 100             | 0.981| 65         |
| αvβ6 integrin           | 86              | 100             | –   | 66         |
| Bile HSP27 and HSP70   | 89.1            | 90.7            | –   | 74         |
| Villin                 | 100             | –               | –   | 78         |
| Mammaglobin            | –               | –               | –   |            |
| TUBB3 (both cases)‡    | 50              | 100             | –   | 79         |

Biomarkers in both blood and tissues/bile

| CEA                    | 33              | 85              | –   | 42         |
| CA19-9                 | 63              | 50              | –   |            |
| CA19-9                 | 78              | 67              | 0.79| 44         |

(continued)
Biomarkers in the tissues/bile. The potential biomarkers in the body tissues and in the bile reported from various studies included gene methylations and DNA-related markers, miRNAs, human aspartyl beta-hydroxylase (HAAH) and homeobox B7 (HoxB7) mRNA, CA19-9 and CEA, and p38δ mitogen-activated protein kinase (MAPK). Alterations at the molecular level, either genetic or epigenetic aberrations, can play important roles in cancer development and progression. In a study applying an epigenome-wide approach to identify suitable DNA methylation-based biomarkers, the proportions of samples with expression of CD10, SFRP1, ZSCAN18, and DCLK1 genes were higher in primary tumors (77%, 59%, 54%, and 44%, respectively) compared with the nonmalignant controls (0%, 19%, 33%, and 23%, respectively) in tumor samples compared with non-malignant tissues. Its sensitivity and specificity were 89.1% and 90.7%, respectively.74

Histologic evaluation alone provided a sensitivity and specificity of 58% to 87% and 98% to 100%, respectively. Other than in the blood, miRNA has also been detected in the tissues and bile. A study quantified the miRNA present in the extracellular vesicle of bile to develop a diagnostic panel for CCA. Its results showed that 54 miRNA species had amplifications beyond the specified cutoff value. Applying different mathematical approaches to assess predictive values provided reliable results. The sensitivity and specificity were 67% and 96%, respectively.82

In another study that assessed miRNA dysregulation in CCA patients, the CCA patient was found to be higher in CCA tissues compared with nonmalignant samples (<0.0001). The sensitivity and specificity of this study were 89.1% and 90.7%, respectively.83

Human aspartyl beta-hydroxylase (AshP) is an enzyme in humans encoded by the AshP gene. An elevated level of HAAH has been correlated with several types of human carcinomas, and as such it has been investigated for use as biomarkers for those carcinomas.111,112 Homeobox constitutes a large family of genes that usually regulates the formation of cell and organ structures during embryonic development. However, there are some homeobox genes that function as tumor suppressors.114 Therefore, changes in the activities of such genes have been associated with cancer formations. In a study to establish and validate a semiquantitative reverse transcriptase-polymerase chain reaction assay for detection of HAAH and HoxB7 mRNA, the detection limits of these proteins were 10 ng/mL and 10 ng/mL, respectively. The intra-assay variability was between 0.6% and 10.3% and the inter-assay variability was between 3.3% and 11.8%. The sensitivity and specificity were 73% and 80%, respectively.46

Besides being the most commonly investigated biomarkers in the serum, CA and CEA were also found in several tissues. The diagnostic utility of markers such as CA19-9 and CEA along with other markers such as tumor protein p53 (p53), antigen Ki-67 (Ki-67), anticytokeratin CAM5.2 (CAM5.2), and intracytoplasmic lumina (ICL) improved the sensitivity of histologic evaluations without compromising the specificity. Histologic evaluation alone provided a sensitivity and specificity of 53% and 100%, respectively. Combining the histologic evaluation with the markers significantly increased the sensitivity. The highest improvement in diagnostic sensitivity was found in the combination of histologic evaluation with CEA (from 53% to 83%), and the lowest improvement was in the combination with CA19-9 (from 53% to 60%). The other markers (p53, Ki-67, CAM5.2, and ICL) improved the sensitivity to 75%, 73%, 60%, and 73%, respectively.59

Mitogen-activated protein kinase 13 (MAPK13) or stress-activated protein kinase 4 (SAPK4) is a member of the p38 subfamily of the MAP kinases. It is an enzyme that, in humans, is encoded by the MAPK13 gene.5 The p38δ MAPK is believed to be activated by an inflammatory cytokine that is associated with biliary tract inflammation named interleukin-6. Thus, p38δ MAPK can be detected in tumors of the biliary tract. In a study aiming to identify p38δ MAPK as a novel marker for distinguishing CCA from hepatocellular carcinoma (HCC), p38δ MAPK positive staining was detected in 92.6% of CCA cases but with only 9.3% of HCC cases (<0.00001). The sensitivity and specificity were 89.1% and 90.7%, respectively.

**Biomarkers in both blood and tissue/bile.** There are relatively limited articles related to the investigation of the biomarkers.
that are present in both the blood (plasma/serum) and the tissue/bile samples. The markers studied included CA, CEA, and slgG4.

Carbohydrate antigen 19-9 and CEA are two of the most commonly studied markers. In a study to evaluate the diagnostic accuracy of serum and bile levels of CA19-9 and CEA in differentiating PSC patients with or without CCA, serum CA19-9 levels in 33.3% of PSC patients with CCA were found to be greater than the upper reference value (200 ng/mL). In PSC patients without CCA, only 15.6% patients had values greater than the same reference value. The CEA level on the other hand, was similar in both the groups. In the bile samples, however, the individual distribution values of both CA19-9 and CEA were heterogenous and as such no clear estimations could be made. It was concluded that there was no relationship between serum and bile levels of CA19-9 and CEA. The sensitivity and specificity of CA19-9 were 33% and 81%, respectively, at the cutoff value of 200 ng/mL.42 A retrospective study evaluated the diagnostic utility of serum CA19-9 when used in combination with other cytological techniques (ultrasoundography, computed tomography, or magnetic resonance imaging) in detecting CCA in patients with PSC. Individually, CA19-9 was found to exhibit low accuracy in detecting the cancer (78% sensitivity and 67% specificity) at a cutoff value of 20 U/mL. The sensitivity significantly improved when CA19-9 was combined with the cytologic techniques. Carbohydrate antigen 19-9 combined with either ultrasonography, computed tomography, or magnetic resonance imaging increased the sensitivity to 91%, 100%, and 96%, respectively however, the specificity decreased to 62%, 38%, and 37%, respectively.44 In another retrospective study, the efficiencies of brush cytology, CA19-9, and CEA for the diagnosis of CCA were evaluated. The brush cytology was shown to accurately diagnose 46.4% of CCA.61 This was further supported by significantly higher mean serum levels of CA19-9 and CEA in the CCA patients compared with non-CCA patients (68.4 versus 3.5 ng/mL and 5,994.1 versus 66.7 U/mL respectively; P < 0.01). Combination of brush cytology and serum CA19-9 provided the most accurate diagnosis (95.6%), whereas the most sensitive strategy for detecting CCA was the combination of CA19-9 with CEA (100%). The overall sensitivity and specificity were 66% and 97.7%, respectively.73

Serum IgG4 elevation is characteristic of immunoglobulin G4-associated cholangitis (IAC). Immunoglobulin G4-associated cholangitis has many symptoms similar to CCA.118 Therefore, use of biomarkers to differentiate the two diseases could be of clinical benefit. Identification of the key factors that would assist in differential diagnosis of CCA from IAC was reported in a retrospective study using serum IgG4 (slgG4), CA19-9, CA242, and CEA as markers. The average level of slgG4 was as high as 16,028 mg/L in about 16.1% of the CCA patients, whereas the level was 896 mg/L in 100% of the IAC patients. The slgG4 provided a sensitivity and specificity of 100% and 87.1%, respectively.45

Biomarkers in urine. Availability of biomarkers in the urine samples would be of great advantage in the clinical settings as it will reduce the use of invasive methods that need to be applied to obtain other biomarkers. Urine contains proteins and peptides that originate as the ultrafiltrate of plasma. The urinary proteomes are highly sensitive toward changes that are of renal origin and also other nonrenal disorders. Urine proteomic analysis (UPA) of a urine peptide marker model was shown to be able to differentiate CCA from other biliary diseases with an area under curve of 0.87 (95% CI: 0.80–0.92); P = 0.0001. The sensitivity and specificity were 83% and 79%, respectively.59

DISCUSSION

Summary of evidence. Considering its malignancy and silent clinical characteristics, detecting CCA at the early stages is of particular clinical interest. Early detection will not only benefit the patients in receiving broader treatment methods, but will finally lessen the financial burden on the public health system that occurs with more intensive treatments at metastatic stages. The availability of biomarkers specific to the early stages of CCA appears to be one of the most promising approaches for early diagnosis.

Various potential biomarkers in serum as well as tissue and other body fluid samples have been reported for the diagnosis and disease differentiation for CCA. Because of the use of different equipment at different places and also personnel variations, results from different studies are conflicting even with the same biomarkers. Because there is such a large variation in the diagnostic accuracy of different biomarkers obtained by different studies, systematic analysis of the relevant studies to point out the most accurate biomarkers that can be used in routine clinical laboratories is important. This study reviewed all relevant articles that were related to the biomarkers for diagnosis of CCA. Several potential biomarkers that were useful in the diagnosis of CCA were reported. The biological fluids under analysis of the biomarkers included blood (plasma/serum), tissue from the bile duct, bile, and urine samples (Supplemental Table 1). Carbohydrate antigen 19-9 and CEA were the most commonly studied serum biomarkers. These two biomarkers, either alone or in combination provided satisfactory results with regard to specificity (89.7–100%), although with questionable sensitivity (47.2–98.2%). These results were in agreement with those reported in a previous review.119 The main tissue biomarkers studied were gene methylation and DNA related. Both were found to provide favorable specificity (98–100%) but inconsistent sensitivity (58–87%) in diagnosing CCA. The efficiency of combination of the markers that are found in the serum and in the tissue samples has also been reported. Although the combined biomarkers improved the accuracy of diagnosing CCA, none was considered adequate for clinical application.

Because all of the previously mentioned sample collection procedures for the biomarker analysis are invasive methods, other samples including urine have been investigated. This UPA method provided a number of advantages over other methods; it is noninvasive, urine can be obtained in large quantities, and it is more stable and is sensitive to the renal-related changes as well as other non-renal disorders. However, the accuracy of the UPA is questionable with sensitivity and specificity of 83% and 79%, respectively. It is noted however that, despite the availability of several candidate biomarkers for CCA, yet at present, no single biomarker can be used for clinical settings. One limitation of the current review is that articles in languages other than English were not included in the analysis. This might have left out some important articles that contain information that could lead to alterations in the conclusions. The serum CA19-9 has been
proposed as the gold standard biomarker for the diagnosis of CCA. However, its wide variation of serum levels and diagnostic sensitivity limit its clinical application. In addition, CA19-9 cannot be used in patients who are negative for the Lewis antigen. Keeping in mind that histopathological examination which is the current universally accepted gold standard method for diagnosis of CCA is an invasive method involving a lot of risks to the patient, application of biomarkers in routine laboratory tests would provide more of benefit than harm. Although no single biomarker can be used to ensure accurate diagnosis of CCA, combining these potential biomarkers with other laboratory tests would be expected to greatly improve the accuracy of diagnosis. This is useful, in particular as a screening tool for those people who are at high risk of O. viverrini infections in endemic areas.

CONCLUSION
Cholangiocarcinoma is becoming a burdensome public health issue in many countries, especially in the Southeast Asian countries. Current management strategies provide inconsistent results mainly because of the silent features of the disorder. Most of the cases are diagnosed only at the metastatic stages that are late for any invasive treatments. Early diagnosis might be possible if biomarkers that are specific to the early stages are identified and standardized. After reviewing the articles that investigated the biomarkers for diagnosis of CCA, we found out that there are many biomarkers which could diagnose and differentiate CCA against other similar disorders and healthy controls with many of them showing significant results. However, there is no single marker that can be used alone for diagnosis of CCA with satisfactory accuracy. Nevertheless, certain markers could be used as a screening tool for those people in risky areas.

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