# Diagnostic Procedures for biliary intraepithelial neoplasm

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## Introduction

Neoplasms of biliary epithelium are relatively rare but form an important category. Recent advances in diagnostic imaging have led to an increase in the clinical recognition of biliary neoplastic lesions, which allows surgical pathologists to be more frequently exposed to them. Pathological diagnosis of biliary neoplasms, especially intraepithelial neoplasia of flat type, can be challenging, because the biliary tract is often affected by inflammatory conditions, and the resultant changes of the biliary epithelium make it difficult to differentiate them from neoplasia. At present, flat epithelial lesions of the biliary tract cannot be detected by the image analysis, and the diagnosis entirely depends on pathological examination. Therefore, it is important to recognize the histological features of biliary flat epithelial lesions for an accurate pathological diagnosis.

The pathological features of flat epithelial lesions, particularly biliary intraepithelial neoplasia (BilIN), were reviewed in this article. The diagnostic criteria and the differential diagnosis of BilIN were presented and discussed to provide help to advance clinical and research applications of the BilIN system.

## BilIN

**Concept:** BilIN is defined as a precursor lesion of invasive adenocarcinoma in the biliary tract, and represents a multistep carcinogenesis process (1,2). BilIN is applicable to the epithelial lesions of the intrahepatic and extrahepatic bile ducts, and also peribiliary glands and the gallbladder. Although the World Health Organization (WHO) classification has applied the BilIN system to the atypical epithelial lesions of the gallbladder, their features in the gallbladder have been far less studied compared with those in the intrahepatic and extrahepatic bile ducts. Therefore, in this article, BilIN of the intrahepatic and extrahepatic bile ducts including peribiliary glands was discussed hereafter.

BilIN is the term to describe flat or micropapillary dysplastic epithelium in the bile duct, and is used instead of the traditional term, biliary dysplasia. Based on atypia, it is classified in three grades; BilIN-1 (low-grade lesion), BilIN-2 (intermediate-grade lesion), and BilIN-3 (high-grade lesion). The terminology and diagnostic criteria of BilIN were proposed in 2005,

and consensus studies confirmed the validity of the criteria in 2007 (3,4). The three-grade classification system is based on the concept that BilIN is a biliary counterpart of pancreatic intraepithelial neoplasia (PanIN) (5).

According to the WHO classification, BilIN-3 corresponds to a premalignant lesion (1). Carcinoma in situ is considered as part of the spectrum of high-grade lesion, and both of them can be histologically identical. It seems somewhat problematic that carcinoma in situ is classified as pTis in the American Joint Committee on Cancer TNM staging system, whereas high-grade lesion is generally not staged (pT0).

***Associated diseases and conditions:*** BilIN is not infrequently associated with chronic biliary diseases such as primary sclerosing cholangitis (PSC), choledochal cyst, and hepatolithiasis (6,7). Recently, the occurrence of BilIN in association with autoimmune pancreatitis has been reported (8), and we have encountered BilIN in a portion of cases of IgG4-related sclerosing cholangitis. BilIN also arises in the livers with non-biliary diseases such as chronic hepatitis C, and alcoholic cirrhosis (9-11), and can be incidentally found in the livers that are otherwise normal. It has been shown that BilIN was observed in approximately 70% of hilar cholangiocarcinoma cases (12).

***Pathological features*** BilIN is macroscopically and radiolodically unidentifiable, and grossly presents flat lesions. Microscopic appearances of the lesions are classified in flat, pseudopapillary (papillary projection without fibrovascular stalk), and micropapillary (papillary projection with fibrovascular stalk). These appearances are invariably admixed in one case, and multifocal lesions can be observed. As for the height of papillary projection, there has been no definition that determines whether the lesion is papillary or flat, and the determination is arbitrary.

Morphological features of each grade of BilIN are summarized in Table 1. The grading is based on the histological appearance of H&E-stained sections, and is determined according to degree of atypia such as loss of cellular/nuclear polarity, increased nucleus-to-cytoplasmic ratio, and nuclear hyperchromasia. Detailed diagnostic criteria of BilIN are described in the consensus paper (4). For the distinction between BilIN-1 and BilIN-2, protruding of the nuclei from the basal lamina up to the apical surface of the bile duct is a useful morphological feature, while diffuse disturbance of cellular/nuclear polarity is useful for the distinction between BilIN-2 and BilIN-3. BilIN-3 is usually seen in association with invasive adenocarcinoma.

Metaplastic changes are occasionally encountered in BilIN, and these include gastric (foveolar, pseudopyloric gland) and intestinal (goblet cell) metaplasia. The metaplastic changes are more frequently observed in BilIN-2/3 than BilIN-1, and foveolar metaplasia is the most

frequently observed change (6). Abundant mucin expression can be noted in these metaplastic lesions.

***Proposed diagnostic algorithm of BilIN:*** Our proposal of diagnostic algorithm of histological grading of BilIN is shown in Figure. The diagnostic algorithm is applied as follows: On H&E-stained section at a high-power field (x400), the presence of protruding of the nuclei from the basal lamina up to the ductal apical surface in at least one part within the field is graded as score 1+, while the presence of diffuse disturbance of the cellular/nuclear polarity within the field is graded as score 1+. When the sum of the two scoring factors corresponds to 0, the lesion is classified in BilIN-1 or reactive change. Score 2+ corresponds to the lesion of BilIN-2 or BilIN-3. When the sum corresponded to score 1+, immunostaining of S100P is additionally performed, in which the cytoplasmic expression of S100P is graded as negative, mild to moderate, and marked. According to the results of S100P immunostaining, the lesion is classified in either of the categories shown in Figure A. Representative histological images for the grading are shown in Figure B.

In the application of the algorithm, there are several biliary epithelial lesions requiring consideration. First, the immunohistochemical expression of S100P is observed not only in BilIN and cholangiocarcinoma, but also in several foci of non-neoplastic biliary epithelium. However, misdiagnosis will be prevented because most of such reactive lesions are graded as score 0 on H&E-stained section.

Next, this algorithm is not intent for the application to the diagnosis of BilIN with metaplastic changes. The metaplastic BilIN lesions, especially with gastric metaplasia, tend to be classified in lower grade than they really are. In fact, the focus of BilIN-2 shown in Figure 6C is graded as score 0 on H&E-stained section, which corresponds to BilIN-1 or reactive change. Such lesions should be graded individually.

Because the atypia of BilIN increases gradually from BilIN-1 to BilIN-3, the distinct grading of BilIN cannot be achieved using the algorithm, and it may be also true for the distinction of reactive epithelia and BilIN-1. We consider that the significance of the algorithm is that it may allow non-neoplastic reactive epithelium to be distinguished from BilIN-2/3.

The diagnostic algorithm was made mainly based on the histological findings of surgically resected livers with hepatolithiasis. The validation including the applicability to the bile duct biopsy samples is to be further tested prior to practical use.

## Differential diagnosis of BilIN

There are several flat epithelial biliary lesions that need to be differentiated from BilIN, or that exhibit similar histological appearances to BilIN.

***Reactive atypia:*** Mild nuclear atypia and hyperchromasia are common in inflamed biliary epithelium, which makes distinguishing BilIN and reactive atypia very difficult. The presence of acute inflammation, especially intraepithelial neutrophils, favors a reactive process. Thus, cautions are required in diagnosing BilIN in the setting of acute inflammation or ulceration. The history of instrumentation also favors the diagnosis of reactive atypia. Criteria to differentiate dysplasia from reactive atypia shown in the gallbladder are also applicable to the differentiation of BilIN and reactive atypia, and they are summarized in Table 2 (28). The diagnostic algorithm (Figure) can also be helpful for this purpose.

***Intraepithelial spreading of cholangiocarcinoma:*** It is well recognized that cholangiocarcinoma of the hilar and extrahepatic bile ducts has a tendency to show a growth pattern characterized by the intraepithelial spreading of carcinoma cells. On a conceptual basis, intraepithelial carcinoma of the bile duct may include at least three different conditions; (1) BilIN-3, (2) de novo development of cholangiocarcinoma, and (3) intraepithelial invasion of periductal invasive carcinoma through the basement membrane of the bile duct.

In the pancreas, the histological lesions of intraepithelial carcinoma are conceptually divided into PanIN-3 and cancerization of the ducts. BilIN-3 can be regarded as a biliary counterpart of PanIN-3, while intraepithelial spread of cholangiocarcinoma resembles cancerization of the ducts of pancreatic ductal adenocarcinoma. The histological characteristics of intraepithelial carcinoma in the ducts have been studied in the pancreas (13). Cancerization of the ducts is recognized as the lesion showing an abrupt transition from markedly atypical to normal-appearing epithelium and continuity of the involved duct with invasive carcinoma (14). Recently, however, it has been described that cancerization of the ducts and PanIN-3 can be virtually indistinguishable (15).

Several, but not a distinct, differences of the histological features have been observed between BilIN-3 and the intraepithelial spreading of cholangiocarcinoma, and these include less frequent appearance of micropapillary pattern, more frequent involvement of the septal and small bile ducts, and increased immunohistochemical expression of p53 in the latter (unpublished data).

Recent studies have shown that the presence or absence of intraductal spreading at a resection margin is not a prognostic factor in cases of cholangiocarcinoma, but the remnant intraepithelial carcinoma is likely to develop late local recurrence (16-19). In the pancreas, the presence of PanIN at a resection margin has been shown not to affect survival in patients who undergo R0 resection for ductal adenocarcinoma (20). The impact of the presence of BilIN at the resection margin has not been studied so far, and further studies are required.

In the previous literatures as well as the pathologic practice, these different conditions of intraepithelial carcinoma in the biliary tract seem to be occasionally confused. We emphasize that it is important to recognize BilIN-3 and intraepithelial carcinoma spreading as a separate lesion to better understand the biology of the disease. Further studies based on such classification may reveal its clinical significance in the biliary tract pathology.

## Conclusions

The diagnostic criteria of BilIN were presented with respect to the current problematic issues. There are several flat epithelial lesions that require to be differentiated from BilIN, and in some of them, the histological characteristics have not been fully characterized. For generalized clinical application of the BilIN system, further studies are warranted.

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Figure

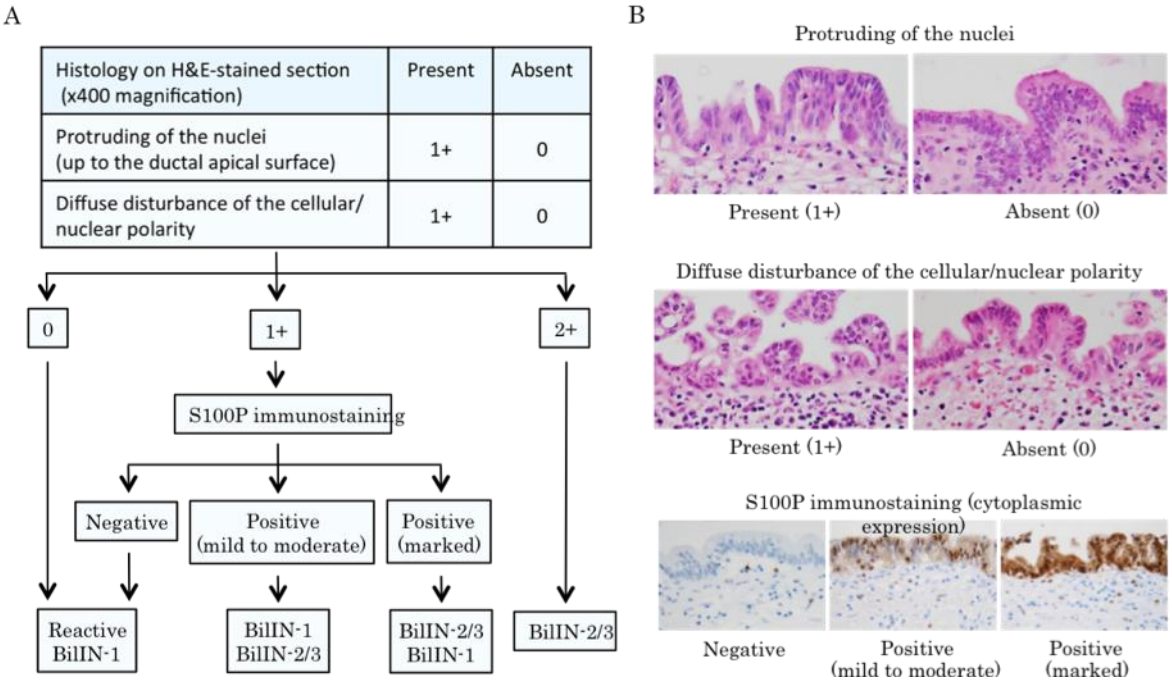


**Figure legend: Proposed diagnostic algorithm of histological grading of BilIN**

The epithelial lesion ia observed on H&E-stained section under the light microscopy at a high-power field (x400). The presence of protruding of the nuclei from the basal lamina up to the ductal apical surface in at least one part within the field is graded as score 1+, while the presence of diffuse disturbance of the cellular/nuclear polarity is graded as score 1+. When the sum of these two scoring factors corresponds to 0, the lesion is classified in BilIN-1 or reactive change. Score 2+ corresponds to the lesion of BilIN-2 or BilIN-3. When the sum corresponds to score 1+, immunostaining of S100P is additionally performed. According to degree of cytoplasmic expression of S100P, the lesion is classified in either of the categories (A). Representative histological images of H&E-stained sections and S100P immunostained-sections for the grading are shown (B). Note that this algorithm is not intent for the diagnosis of BilIN with metaplastic changes.

Table 1. Histological features of BilIN

| Histology | BilIN-1 | BilIN-2 | BilIN-3 |
|---|---|---|---|
| Cellular/nuclear atypia | + | + | ++ |
| Nuclear pseudostratification | + | + | + |
| Protruding of the nuclei (up to the ductal apical surface) | - | + | + |
| Loss of cellular/nuclear polarity | - | + | ++ |

BilIN, biliary intraepithelial neoplasia. -, likely absent; +, present; ++, prominent.

Table 2. Histological features to distinguish BilIN from reactive atypia

| Histology | BilIN | Reactive atypia |
|---|---|---|
| Acute inflammation and/or ulceration | - | + |
| Intraepithelial neutrophils | - | + |
| Abrupt transition between normal and atypical epithelium | + | - |
| Fine nuclear chromatin | + | - |
| Prominent nucleoli | -/+ | + |
| Loss of cellular/nuclear polarity | + | - |

BilIN, biliary intraepithelial neoplasia. -, absent; -/+, can be present or absent; +, present.