Fine Needle Aspiration in the Diagnosis and Classification of Hepatoblastoma
Analysis of 21 New Cases

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Background: Diagnosis of hepatoblastoma (HBL) is based on characteristic clinical and radiological presentation, young age and marked elevation of serum α-fetoprotein (aFP). Fine needle aspiration (FNA) technique is successfully used in the diagnosis of hepatoblastoma. To evaluate the value of FNA in the diagnosis and subtyping of HBL, we report our experience correlated to histological sections (core needle biopsy, CNB).

Methods: From 1991 to 2015, 21 cases from 20 patients were cytologically diagnosed as HBL. The patients were 15 males and 5 females, mean age being 3 years, and median age being 2 years and 4 months. Serum aFP levels ranged from negative to 1,285,000 ng/ml. We defined cytological criteria to diagnose fetal, embryonal, mesenchymal, and small cell undifferentiated components.

Results: The accurate cytological diagnosis of HBL was made in all cases; 8 cases exhibited a single component and 13 cases exhibited two or more components. Fetal and embryonal components were seen in 18 and 13 cases, respectively, and small cell undifferentiated component was seen in one case. Mesenchymal component was seen in 12 cases. Comparing cytology and histology, identical components were identified on both, FNA and CNB in 14 cases. When analyzing only the presence of epithelial components, 17 cases were concordant in both techniques.

Conclusion: FNA allows to accurately diagnose HBL and recognize its histological subtypes. On the basis of high concordance between cytological and histological diagnosis, FNA is validated as an alternative diagnostic method to CNB.

Key Words: fine needle aspiration; hepatoblastoma; cytology; core needle biopsy

Introduction
Although rare in general, hepatoblastoma (HBL) is the most common primary hepatic tumor of childhood accounting for up to 50% of all pediatric primary hepatic malignancies.1 Children usually present with an enlarging palpable abdominal mass accompanied by abdominal symptoms, such as nausea and pain, and weight loss. In most cases, the diagnosis is based on characteristic radiological presentation, young age and marked elevation of serum α-fetoprotein (aFP).2 It is reported that in around 10% of HBL, especially in small cell undifferentiated/anaplastic (SCUD) subtype, serum aFP levels can be normal.1,3

Histologically, HBL is classified into wholly epithelial or mixed epithelial and mesenchymal (MEM) type. Furthermore, the epithelial component is specified according to the predominant histological pattern into “well-differentiated” fetal, “poorly differentiated” embryonal, macrotrabecular, and SCUD subtype.4,5 Cells of the fetal subtype resemble mature hepatocytes whereas at the other end of the spectrum the SCUD subtype composed of primitive cells is the least differentiated form of HBL. Subtyping of HBL and recognition of poorly differentiated components is essential for therapeutic and prognostic
reasons. Treatment of some pure fetal HBLs is different than treatment of tumors with embryonal and/or SCUD elements. Furthermore, although the histological subtype alone does not influence the outcome, SCUD is considered an unfavorable prognostic factor even when present focally. HBL of exclusively SCUD subtype is uncommon, more often SCUD pattern is a focal component of other subtypes of HBL.

There is a variety of intra-abdominal benign and malignant tumors and tumor-like lesions occurring at this age group from which HBL should be differentiated. Since the treatment regimens are different and specific to each tumor entity, it is crucial to obtain an accurate and specific diagnosis. Fine needle aspiration (FNA) is a rapid and safe alternative to core or open biopsy for the diagnostic work-up of hepatic and other abdominal lesions in children. However, in many centers, the use of FNA alone is judged insufficient to make a final diagnosis and histological material using core needle biopsy (CNB) or excisional biopsy is needed for confirmation and especially correct determination of the histological tumor subtype.

There is only a limited number of reports in the literature regarding FNA in HBL, most of them being single case reports (Table I). The aim of the present study is to report our experience with cytological diagnosis of HBL. The cytological findings, clinical, and radiological presentation and histological correlations are discussed, and the literature reviewed.

**Materials and Methods**

This study received ethics committee approval.

**Patients’ Characteristics**

A consecutive series of 21 cases from 20 patients with HBL diagnosed using FNA from 1991 to 2015 constitute the subject of this study. The patient population consisted of 15 male and 5 female patients (male to female ratio 3:1), ranging in age from 1 month to 15 years and 7 months with a mean age of 3 years, and median age of 2 years and 4 months.

Serum aFP levels ranged from negative (0 ng/ml) to 1,285,000 ng/ml, with a mean level of 253,118 ng/ml. They were highly elevated in 16 cases (mean 316,341, range 4,000–1,285,000), whereas four cases were normal or only slightly elevated. Of these four cases, one was a local and metastatic recurrence after 9 months and was SCUD subtype on resected specimen; the other three cases were fetal subtype. In one case, aFP was elevated but the exact amount is not known.

Radiologically, hepatic tumors were detected and, in five patients were multifocal. Tumors ranged from 50 to 170 mm with a mean size of 100 mm. Three patients presented with pulmonary metastases at initial diagnosis. One patient had a relapse presenting as local recurrence and pulmonary metastasis. All patients received neoadjuvant chemotherapy prior to surgical resection according to international therapeutic regimens.

The clinical, radiological, and biological data are presented in Table II.

**Cytology**

Pretherapeutical FNA was performed using a 22-Gauge needle with ultrasound guidance under general anesthesia in all cases. Cytological smears were air-dried and stained according to May–Grünwald–Giemsa (May–Grünwald–Giemsa) method. In seven cases, additional smears were fixed in 96% ethanol and stained according to Papanicolaou (PAP). In three cases, cell blocks (CB) were prepared.

Cytological smears were evaluated for the presence of various epithelial subtypes, including fetal, embryonal,
SCUD, and mesenchymal elements. Additionally, presence of necrosis and mitotic figures was noted.

Fetal subtype was characterized by large, orderly sheets, three-dimensional clusters, acinar formations, and occasionally, papillary structures. Round to spindle-shaped cells, resembling hepatocytes, were usually monomorphic. They exhibited low nuclear/cytoplasmic ratio, round nuclei, fine chromatin and usually a single small nucleolus (more evident on Papanicolaou stain). Abundant cytoplasm with variable vacuolization depending on the amount of glycogen and lipids was also observed (Fig. 1).

In contrast to fetal subtype, embryonal subtype was characterized by large solid sheets, clusters and/or rosettes/glandular formations that appeared crowded and disorganized with overlapping of cells. Cells were pleomorphic, hyperchromatic and exhibited an elevated nuclear/cytoplasmic ratio. Cytoplasm was scant and basophilic using May–Grunwald–Giemsa, and the nucleoli were prominent. Furthermore, some mitotic figures and necrosis were detected (Figs. 2–4).

SCUD subtype was characterized by noncohesive small round cells with highly elevated nuclear/cytoplasmic

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**Table II. Clinical, Radiological and Biological Data**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex</th>
<th>Age at Diagnosis</th>
<th>Radiological Size of Hepatic Tumor (mm)</th>
<th>Metastasis</th>
<th>AFP (ng/ml)</th>
<th>FU (mo)</th>
<th>Status</th>
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<tr>
<td>1</td>
<td>F</td>
<td>2y 5mo</td>
<td>60</td>
<td></td>
<td>12</td>
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<td>M</td>
<td>1y 3mo</td>
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<tr>
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<td>M</td>
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<td></td>
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<td>ADF</td>
</tr>
<tr>
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<td>M</td>
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<td>145</td>
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<td>ADF</td>
</tr>
<tr>
<td>5a</td>
<td>M</td>
<td>2y 4mo</td>
<td>110</td>
<td></td>
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<tr>
<td>5b*</td>
<td>M</td>
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<td>M</td>
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<td>M</td>
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<td>1y 4mo</td>
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<td></td>
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<td>13</td>
<td>ADF</td>
</tr>
<tr>
<td>19</td>
<td>F</td>
<td>3y 5mo</td>
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<td>ADF</td>
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<tr>
<td>20</td>
<td>M</td>
<td>15y 7mo</td>
<td>150, MF</td>
<td>Costal</td>
<td>33,450</td>
<td>Recent Case</td>
<td>ADF</td>
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</tbody>
</table>

FU, follow-up; AFP, alpha-Fetoprotein; ADF, alive disease free; MF, multifocal; DOD, dead of disease; LTFU, lost to follow-up.

*Patient no. 5: 5a, first diagnosis; 5b, relapse

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**Fig. 1.** Fetal subtype. Sheets of monomorphic cells without prominent cytonuclear atypia. May–Grunwald–Giemsa stain. [Color figure can be viewed at wileyonlinelibrary.com]
ratios. The cells exhibited ovoid nuclei with irregular membranes, coarse chromatin, and scant or absent cytoplasm. Nucleoli were usually invisible (Fig. 5).

Mesenchymal components consisted either of osteoid (acellular hyaline matrix staining light pinkish-orange), or of fragments of nonspecific connective tissue (poorly-cel-

Histology and Immunohistochemistry

Histological sections from CB, CNB, and postchemotherapy surgical specimens were stained with hematoxylin, eosin, and safran (HES) stain. Immunohistochemistry with a selected panel of markers (aFP, keratin, CEA, EMA, vimentin, bHCG, NSE, Ki67, INI1) was performed on histological sections from CB or CNB in cases where necessary. Definitive classification of HBL was performed on preoperative histology according to the WHO classification.1

Since there are no specific molecular markers in HBL, no material was used for genetic analysis, but more recent cases were subject of tissue banking (tumoral and nontu-

Results

The accurate cytological diagnosis of HBL was made in all cases (Table III). All FNA smears were hypercellular and composed of malignant epithelial/primitive cells. Eight cases exhibited a single component and 13 cases exhibited two or more components. Fetal and embryonal components were seen in 18 and 13 cases, respectively, and SCUD component was seen in one case.

Fig. 2. Embryonal subtype. Crowded irregular cells with high nuclear/cytoplasmic ratio forming glandular structures. May–Grunwald–Giemsa stain. [Color figure can be viewed at wileyonlinelibrary.com]

Fig. 3. Corresponding cell block with embryonal subtype. HES stain. [Color figure can be viewed at wileyonlinelibrary.com]
Mesenchymal component was seen in 12 cases. Mitotic figures and necrosis were seen in seven and eight cases, respectively.

Analysis of CNB showed that 9 cases exhibited a single component and 12 cases two or more components. Fetal and embryonal components were seen in 19 and 12 cases, respectively, and SCUD component was seen in one case. Mesenchymal component was seen in eight cases.

Analysis of postchemotherapy surgical specimens showed that 9 cases exhibited a single component and 10 cases two or more components. In one case, there was no residual tumor in the surgical specimen. In another case, the information on the histological components was not available. Fetal and embryonal components were seen in 18 and three cases, respectively, and SCUD component was seen in one case. Mesenchymal component was seen in 9 cases. No macrotrabecular pattern was observed.

Comparing cytology and histology, identical components were identified on both FNA and histological material from CNB in 14 cases. When analyzing only the presence of epithelial components 17 cases were concordant by both methods.

Bearing in mind that the cellular components alone have no prognostic value, we noted that in six cases FNA was more accurate than histologic material from CNB, including four cases in which FNA detected a mesenchymal component, in one case fetal and in the other embryonal. In two cases, FNA was less accurate than histological material from CNB, where an additional fetal component was present only on CNB. Finally, with the caveat that some morphological modifications may be present after chemotherapy, the postchemotherapy resection specimens in two cases revealed fetal and mesenchymal components that were not detected in the FNA and CNB, respectively.

Follow-up was available in 18 patients; one patient was lost to follow-up and one patient was a recent case. Mean follow-up was 108 months (range 12–283 months). Three patients died of disease, all of which had pulmonary metastases at initial diagnosis.

Discussion
HBL is the most common primary malignant hepatic tumor in children, usually diagnosed before the age of 2 years. Young age, characteristic radiological and clinical presentation and elevated serum aFP levels have been considered characteristic diagnostic elements for many years. More recently, FNA and CNB have been used to
confirm the diagnosis prior to therapy.\textsuperscript{18–21} However, FNA was preferred to CNB because it was judged to be less invasive, minimizing side effects and providing excellent material for immunohistochemistry (on CB) and molecular techniques, if necessary. On the other hand, the use of CNB allowed classification of tumors into different histological subtypes\textsuperscript{1} and became reproducible among pathologists.

Cytological studies describing HBLs are few and only around 140 cases have been published to date (Table I). However, only four studies deal with larger and representative numbers of patients.\textsuperscript{22–25} A collective analysis of 131 HBL cases with available initial cytological diagnoses showed that FNA is a powerful diagnostic technique in HBL, since 110 (84\%) tumors were accurately diagnosed, 20 (15\%) tumors were diagnosed as malignant

Table III. Cytological and Histological Features of Hepatoblastomas

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Cytology (FNA)</th>
<th>Histology (CNB)</th>
<th>Surgical Specimen</th>
<th>Cytology: Mitosis</th>
<th>Necrosis</th>
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<td></td>
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<tr>
<td>2</td>
<td>E, M</td>
<td>E&gt;F</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>F=E, M</td>
<td>F=E, M</td>
<td>F</td>
<td></td>
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<tr>
<td>4</td>
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<tr>
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<td>20</td>
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</table>

FNA, fine needle aspiration; CNB, core needle biopsy; F, fetal component; E, embryonal component; M, mesenchymal component; SCUD, small cell undifferentiated component; N.A., information not available.

*Patient no. 5: 5a, first diagnosis; 5b, relapse.

*No residual tumor.

*Vital tumor <1%.
Diagnosis of HBL is possible on FNA in the majority of cases where there is resemblance to hepatocytes. Us-Krasovec et al.\textsuperscript{22} described cytological characteristics in 14 primary and one metastatic HBL. Histological confirmation was available in 10 cases. They emphasized the resemblance of tumor cells to immature hepatocytes and described a range of cellular features and different architectural patterns, however, without directly referring to a specific subtype. They concluded that the accurate diagnosis of HBL is possible on FNA in the majority of cases where there is resemblance to hepatocytes.

Parikh et al.\textsuperscript{23} described a series of 20 cases of HBL that were diagnosed by FNA. Based on cytological findings, they proposed classification of HBL into differentiated and undifferentiated groups. In this study, the focus was especially on the cytoarchitectural features such as acinar pattern, papillary pattern and sheets. Presence or absence of mesenchymal components was not described in this study. They emphasized the significance of clinical features, imaging techniques, and serum aFP levels in combination to cytology in the diagnosis of HBL and differential diagnosis to other small round cell tumors. They also pointed out that it is difficult to distinguish well differentiated HBL from hepatocellular carcinoma based on cytological features alone.

Iyer et al.\textsuperscript{24} analyzed a series of 26 cases of HBL diagnosed by FNA. The aim of the study was to define cytomorphological features of HBL and to evaluate the feasibility of FNA in subtyping HBL. The cytological smears were correlated with histological material, which was available in 15 out of 26 cases. Detailed cytomorphological and architectural features were described for the fetal and embryonal components. One case was cytologically described as unclassifiable round cell tumor without differentiation, which corresponded to SCUD subtype on histology. Mesenchymal components were not observed on smears in the eight cases of MEM diagnosed on histology. One showed macrotrabecular pattern on histology, which was not seen on the smear. Moreover, mitoses were observed in embryonal cells and presence of extramedullary hematopoiesis was noted in 20 out of 26 cases. Finally, they proposed a cytology grouping system based on cytological findings. This grouping system was confined to epithelial HBL and comprised fetal and embryonal subtype and the combination thereof with varying proportions (predominantly fetal, predominantly embryonal and equal amounts).

Reported FNA accuracy in diagnosing HBL is very high. Similarly, in our series of 21 tumors, all cytological diagnoses were accurate and revealed HBL. Moreover, all our cytologies were correlated with histological specimens, which were CNB in all cases and postchemotherapy surgical specimens in 19 cases. We observed that smears exhibited only a single component in 8 cases and two or more components in 13 cases. In the comparative analysis with CNB, we observed that in 14 cases identical components were identified on both FNA and histological material from CNB. When comparing the epithelial components only, there was a concordance between FNA and CNB in 17 cases. The majority of postchemotherapy surgical specimens showed only a well differentiated epithelial component, which might be a consequence of the presurgical treatment. Comparative analysis of these three techniques showed that FNA specimens were highly cellular and contained representative material, allowing an accurate diagnosis of HBL. Furthermore, our results showed that the histological subtypes are reproducible on FNA specimens. Importantly, a high-grade component (embryonal, SCUD) was not missed in any cytological case.

Additionally, we observed that fetal subtype is characterized by cytoplasmic vacuolization while necrosis, apoptosis, and mitotic figures are absent. Conversely,
necrosis, apoptosis and mitotic figures are helpful clues to the presence of an embryonal or SCUD component. In some cases, mesenchymal and SCUD components may be present only focally. The cytological detection of components has already been subject of discussion. Us-Krasovec et al. were unable to correctly detect the coexistence of specific subtypes on smears and found some difficulties in differentiating between fetal and embryonal subtypes. Parikh et al. proposed an alternative cytologic classification system for HBL into two groups, differentiated and undifferentiated. However, this grouping system does not precisely reproduce the histological subtypes and differs from the HBL classification most commonly used. Moreover, it appears that mesenchymal components and the MEM type of HBL were not considered in this classification. Finally, Iyer et al. and Barwad et al. reported some difficulties in identification of mesenchymal components. Consequently, a cytological classification limited to epithelial HBL was proposed. In our study, mesenchymal components detected on histological sections were also identified on FNA specimens in all cases. In addition, in four cases, the mesenchymal component was detected only on FNA specimens but was not present on CNB. This may be explained by the larger area that is likely to be sampled by FNA than by CNB.

Differential diagnosis comprises a variety of intra-abdominal tumors occurring in this age group. Epithelial HBL should mainly be differentiated from mesenchymal hamartoma, nephroblastoma/Wilms tumor, neuroblastoma, extrarenal rhabdoid tumor, and hepatocellular carcinoma.

Mesenchymal hamartoma (MH) can clinically and radiologically mimic HBL although morphological features are usually characteristic comprising loose connective tissue, bile duct epithelium and hepatocytes in varying proportions. However, if mainly benign hepatocytes from the periphery of the lesion or enclosed within the lesion are sampled, it may be difficult to distinguish MH from HBL of “well-differentiated” fetal subtype. It is important to note, that serum aFP levels are usually normal to slightly elevated in MH compared to very high levels in patients with HBL.

Nephroblastoma/Wilms tumor, similar to the embryonal or SCUD subtype of HBL, is composed of blastemal, poorly differentiated epithelial cells. The cells are clustered and form tubular structures. Mesenchymal component, if present, is usually composed of spindle-shaped slightly atypical cells. Occasionally, more atypical cells are detected in atypical forms of nephroblastoma. The characteristic renal localization and negative serum aFP levels help in the differential diagnosis.

Hepatic metastases of nephroblastoma may occasionally mimic embryonal or SCUD subtype of HBL. Neuroblastomas are composed of blastemal neuroblasts with round to ovoid to irregularly molded nuclei and coarse chromatin. Neuroblasts are isolated or clustered. Rosette-like formations with central neuropil are usually present. In more differentiated forms, schwannian tissue and ganglion cells are easily identified.

Hepatocellular carcinoma (HCC) is also a major entity to be differentiated from HBL. HCC arises in older children compared to HBL, and is mainly of fibrolamellar (FL HCC) subtype. Classical HCC is composed of polymorphous, malignant hepatocytes, which are large with grayish cytoplasm. Nuclei are large with prominent nucleoli. Usually, a significant population of naked nuclei or giant cells is also observed. Cells in FL HCC are larger than in HBL and polygonal, and have a single prominent nucleolus. Intracytoplasmic hyaline deposits and/or extracellular connective tissue fragments are easily identified on smears. Extramedullary hematopoiesis, which is often present in HBL, is usually absent from HCC.

Extrarenal rhabdoid tumor (ERT) needs to be distinguished from embryonal or SCUD subtype of HBL since this tumor has a dismal prognosis and needs a different treatment. Smears of ERT are usually cellular and contain both single cells and sheets or small clusters. The cells have a polygonal or plasmacytoid shape, abundant cytoplasm and usually eccentrically located nuclei. A characteristic feature of these so-called “rhabdoid” cells is a perinuclear globular cytoplasmic inclusion. Immunohistochemistry can be useful for confirming the diagnosis as the majority of ERT are characterized by alterations of the SMARCB1/INI1 gene resulting in loss of SMARCB1/INI1 protein expression.

Rare cases of HBL with a predominant SCUD component should be differentiated from other pediatric “small blue round cell” tumors (SRCT) such as Ewing sarcoma (ES)/primitive neuroectodermal tumor (PNET), rhabdomyosarcoma (RMS), extrarenal rhabdoid tumor, undifferentiated (embryonal) sarcoma of the liver (UESL), and high-grade lymphoma. Cytologically, this group of tumors is characterized by a predominantly undifferentiated “small round cell” component. Nevertheless, there are some distinctive morphologic features that help to distinguish these lesions from each other. ES/PNET are composed of a double cell population consisting of small dark cells and larger clear cells. Additionally, rosettes can be identified in some ES/PNET. Smears of RMS contain large and highly cellular tissue fragments admixed with a moderate amount of stroma. The tumor cells vary in size and multinucleated giant cells can be present. UESL are composed of sarcomatoid appearing spindle-shaped cells in a myxoid background. Characteristically, the tumor cells can contain cytoplasmic globules that are positive for periodic acid-Schiff (PAS). High-grade lymphomas are composed of a dissociated cell population, in which scattered tangible body microphages and granulocytes can be admixed. Since the clinical and radiological
findings of SRCT are not specific and morphological features overlap, ancillary techniques such as immunohistochemistry or molecular diagnostic techniques are routinely needed for the diagnosis of these tumors. Presence of a better differentiated, cytokeratin-positive component in HBL is helpful to distinguish it from other SRCT.

Conclusion
Diagnosis of HBL is based on clinical presentation, radiological findings, and elevated serum aFP levels. Pathological diagnosis of HBL is possible using FNA and/or CNB techniques. FNA technique was reported to be highly accurate without any example of false-negative diagnoses. Moreover, specific morphological subtypes are easily recognizable on smears making cytology an alternative diagnostic method to CNB.

References
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